Renewal Assessment Report

Dimethenamid-P

Volume 3 – B.6 Toxicology and metabolism data

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Rapporteur Member State: Germany

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When	What
10 August 2016	First version submitted to EFSA

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B Summary of the data and information

B.6 Toxicology and metabolism data

B.6.1 Absorption, distribution, metabolism and excretion in mammals

B.6.1.1 Absorption, distribution, metabolism and excretion by oral route

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.1
Report:	[redacted] 1988 (TOX1999-406) Absorption, distribution and excretion in rats after single and multiple doses of [^{14}C] SAN 582-H [redacted] unpublished, 5 July 1988, BASF RegDoc.# 92/12428 (Appendix I) (Experimental work from 17 March 1987 – 22 May 1987)
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision F, Section 85-1 (not available to the Rapporteur).
Deviations:	In principle, the toxicokinetic part of this study was performed in compliance with the requirements specified in OECD guideline 417. Some additional experiments were included.
GLP:	When the study was performed, GLP was not compulsory. However, according to the study author, GLP was followed although a formal certificate of the laboratory in the report is lacking. Moreover, a Quality Assurance statement is included.
Acceptability:	The study is considered acceptable

Materials and methods:

Test material:
^{14}C SAN 582H [3-thienyl-^{14}C-dimethenamid]: batch no. RA 683-1, specific activity 157 µCi/mg, radiochemical purity: >99 %; Unlabelled SAN 582H: batch no. 6083, purity: not stated in the original report but claimed to be 99.8 % in the applicant's dossier; supplied by [redacted].

Test animals:
Wistar rats [Kfm:WIST]; mean body weight for the males: 215 ± 12 g and 191 ± 6 g for the females; Source: Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland.

The study design is summarised in Table B.6.1-1. The test compound was administered to different groups of rats either by intravenous injection (i.v.) or by oral gavage (p.o.). Group 1 was given a single oral dose of 10 mg/kg bw. Group 2 received an intravenous dose of 10 mg/kg bw. Group 3 was administered a single high oral dose of 1000 mg/kg bw. Group 4 was orally administered unlabelled dimethenamid (10 mg/kg bw/d) over a period of 14 d followed by a final radiolabelled dose. The rats in group 5 were bile duct-cannulated and also received a single low oral dose of 10 mg/kg bw. Groups 1 to 4 consisted of 6 males and 6 females while group 5 consisted of 3 rats per sex. Excreta from each group and bile from group 5 were collected periodically until sacrifice at 168 h after dosing. Three rats per sex from experimental groups 1–4 (i.e. except bile duct-cannulation group) were used for subsequent examination of tissue distribution. Blood was obtained from these animals by cardiac puncture at termination. The three remaining rats per sex and group including all those with cannulated bile duct were subject to radioactive residue determination in the carcasses.

Table B.6.1-1: Study design for the dimethenamid toxicokinetic study in rats (additional experiments not mentioned)

Exp No.	Sex	No. rats	Route	Dose (mg/kg bw)	Time of excreta sampling (h)	Time of bile sampling (h)	Samples analysed
1	M	6	p.o.	10	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
1	F	6	p.o.	10	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
2	M	6	i.v.	10	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
2	F	6	i.v.	10	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
3	M	6	p.o.	1000	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
3	F	6	p.o.	1000	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
4*	M	6	p.o.	15 x 10	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
4*	F	6	p.o.	15 x 10	7, 24, 48, 72, 168	N.P.	Urine, faeces, blood, tissues/ carcass
5**	M	3	p.o.	10	7, 24, 48, 72, 168	7, 24, 48, 72, 168	Bile, urine, faeces, carcass
5**	F	3	p.o.	10	7, 24, 48, 72, 168	7, 24, 48, 72, 168	Bile, urine, faeces, carcass

*: 10 mg/kg bw/d unlabelled dimethenamid for 14 d, followed by a single ^{14}C dose of 10 mg/kg bw.
**: Rats were bile duct cannulated with limited excreta analysis.
N.P.: Not performed.

Generally, rats were sacrificed after 168 h following administration of the radiolabelled test material.
Additional experiments were performed in rats as part of this toxicokinetic study. Blood and tissue samples were collected at different times following dosing. For tissue level monitoring, oral dose levels of 10 and 1000 mg/kg bw were applied. In both dose groups, three rats per sex and time of radioactive residue determination were used. The animals were killed after 1, 4, 24 or 72 h. Furthermore, three rats per sex were examined for blood radiocarbon levels after administration of either a single oral low (10 mg/kg bw) or high (1000 mg/kg bw) dose or an intravenous dose of 10 mg/kg bw. Blood samples were repeatedly drawn from the tail vein at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 24, 48, 72, and 168 h post dosing.
In all experiments, radioactivity was measured by liquid scintillation counting.

Results:

It became obvious that the high oral dose of 1000 mg/kg bw was already acutely toxic to the animals, since 7 rats (5 males and 2 females) in the groups receiving this dose died. However, this rather high mortality rate in the high dose experiments is not considered to have invalidated the toxicokinetic data obtained. In the oral low dose group, a further male animal died in one of the additional experiments probably due to an intubation error.
Recovery and overall material balance:
A general recovery rate of more than 92 % (and in most trials even exceeding 95 %) of the whole radioactivity administered throughout all the experiments is considered satisfactory.
The overall material balance is summarised in Table B.6.1-2.
Absorption:
Absorption of dimethenamid from the gastrointestinal tract was nearly complete in rats, at least for the

low dose. This was suggested by comparing the urinary excretion rates following oral and iv administration of the same low dose and clearly confirmed by summing up radioactivity recovered from urinary and bile duct excretion in the bile duct cannulated animals. These data also show a significant amount of gastrointestinal reabsorption after biliary excretion. Based on the differences in renal elimination between intact and bile duct cannulated animals, reabsorption was estimated to account for 22–35 % of the low dose. The small fraction of radioactivity excreted via the faeces by bile-cannulated rats might represent the minor unabsorbed part of the administered test material.
The occurrence of delayed maximal blood levels (see below) suggests a rather slow absorption from the GIT with half-lives of approx. 4 h in low dose animals.
In the low dose group receiving pre-treatment, absorption was not significantly different from the single dose control group.
For the oral high dose group, an absorption rate of at least 61–63 % was determined in the experiment. However, according to the study author, calculation of the area under curve (AUC) and comparison of the AUC ratio between the high and low oral dose groups revealed a much higher absorption rate amounting to 100 % in male rats. Absorption was yet slower than after the low dose with a half-live of about 13 h.
There were no significant sex differences in absorption.

Table B.6.1-2: Material balance for non-cannulated and bile duct cannulated rats

Group	Route	Dose level (mg/kg bw)	Sex	% of administered dose at 168 h after treatment				
				Urine	Faeces	Bile	Carcass	Total
1	po	10	M	35.3	57.7	N.A.	6.7	99.7
			F	46.9	47.6	N.A.	8.0	102.5
2	iv	10	M	31.2	56.4	N.A.	11.1	98.7
			F	49.4	36.6	N.A.	9.9	95.9
3	po	1000	M	61.6	30.1	N.A.	3.4***	95.1
			F	63.1	26.1	N.A.	3.7	92.9
4*	po	15 x 10	M	34.9	61.6	N.A.	4.4	100.9
			F	53.3	39.9	N.A.	3.6	96.8
5**	po	10	M	7.6	2.2	82.2	4.7	96.7
			F	12.4	3.7	75.1	5.3	96.5

*: Rats received 10 mg/kg bw/d of unlabelled dimethenamid for 14 d followed by a single ^{14}C dose.
**: Bile duct cannulated animals.
*** Individual animal value due to premature death of the other 2 males allocated to this carcass residue determination subgroup.
N.A.: Not applicable.

Distribution:
Radioactivity was widely distributed throughout the body. 1 h after dosing, highest concentrations were measured in the kidneys, liver and blood, in the adrenals, lungs, and spleen. At 4 h after substance administration and afterwards, however, by far the highest levels of radioactivity were consistently found in the blood. Residue levels decreased steadily over time with the exception of blood. Overall, tissue levels were small at 168 h after treatment. For the low dose rats, the concentration at termination was less than 0.5 ppm in all organs and tissues. In general, tissue radioactivity levels were comparable in both sexes and were similar for single dose and multiple dose groups.
Blood levels showed a remarkably high inter-individual variance. After a single oral low dose (10 mg/kg bw) the maximum blood radioactivity was reached at about 72 h after treatment (0.05 µg test material/g blood in the male animal and 0.1 µg test material/g blood in the females). Afterwards, radioactivity decreased slowly. For the oral high dose, the maximum level was also reached at 72 h but did not significantly decline up to study termination after 168 h. Blood radioactivity was mainly associated with red blood cells as radioactivity in plasma was much lower. This assumption is further

supported by relatively high levels of radioactivity found in the spleen as compared to other organs.
Elimination:
Primary elimination of radiolabelled material occurred mainly via liver and bile. Hepatic clearance was rapid since at least 90 % of biliary excretion occurred within the first 24 h after dosing. Due to significant gastrointestinal reabsorption, the amount of renal excretion of radioactivity reached 31-63 % in non-cannulated rats. Renal excretion was rather rapid, too, because approx. 75 % of the total radioactivity excreted in urine were already found within 1 day after treatment. Elimination of the remaining radioactivity was delayed by reabsorption and by the affinity of dimethenamid to the erythrocytes. However, at the end of the investigation period after 168 h post dosing, approx. 94, 87, 90 and 95 % of the administered dose had been eliminated by the renal and faecal route in the experimental groups 1–4.
There were some differences in the excretion pattern between the dose levels. For the low dose (10 mg/kg bw), urinary radiocarbon accounted for 35 - 47 % of the dose as compared to 62 - 63 % for the high dose (1000 mg/kg bw). Accordingly, radioactivity in faeces was 48 - 58 % for low dose groups as compared to 26 - 30 % for the high dose group. These data suggest that biliary excretion might be saturated for the high dose group. Because of the more extensive but up to 7 times slower urinary excretion in the high dose group, the study author assumed that tubular reabsorption might be saturated after a high oral dose, too.
Repeated administration of the low dose tended to increase the elimination rate.
In general, renal elimination was slightly more pronounced in female animals whereas the amount of biliary excretion was higher in males.

Conclusion:

In rats, dimethenamid was almost completely absorbed from the gastrointestinal tract irrespective of dose level, dosage regimen or sex. Absorption was rather slow.
The test substance or its metabolites were mainly excreted via the bile and to a large extent reabsorbed from the gastrointestinal tract. Definitive elimination occurs via the faecal and urinary routes. Apart from blood, tissue residues steadily declined. At study termination 168 h after dosing, approx. 90 % of the radioactivity had been eliminated. The distribution and elimination data suggest that dimethenamid and its metabolites have no tendency to bioaccumulate but do have a certain affinity to red blood cells. The pattern of distribution and elimination was not significantly affected by multiple administration and there were no large sex differences. However, urinary excretion was slightly more pronounced in female rats and generally much more extensive in high dose animals.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.1
Report:	[redacted] 1992 (TOX1999-406) Absorption, distribution, metabolism and excretion of (^{14}C) SAN 582H in rat after single and multiple doses [redacted] unpublished, 6 February 1992, BASF RegDoc.# 92/12428 (Experimental work from 17 March 1987 (in-life phase) – 25 October 1991)
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision F, Section 85-1 (not available to the Rapporteur)
Deviations:	Deviations from OECD Guideline No. 417 (adopted 4 April 1984): None
GLP:	When the study was performed, GLP was not compulsory. However, according to the study author, GLP was followed although a formal

	certificate of the laboratory in the report is lacking. Moreover, a Quality Assurance statement is included.
Acceptability:	The study is considered acceptable.

The in-life phase of this study ([redacted]) is reported above in this RAR, which should be consulted for description of test materials and test animals. In addition, an analytical standard for dimethenamid (unlabelled SAN 582H, purity 99.8 %, analysis no. 20278) was used in the metabolism experiments.

Materials and methods:

Metabolism of dimethenamid was studied by analysis of urine, faeces and bile samples obtained during a previous toxicokinetic study in rats ([redacted]; TOX1999-406). The study design and allocation of animals to the experimental groups is also described in the study summary above. For metabolite analysis, it was chosen to use pooled excreta obtained from 0 to 72 h post dosing for metabolite analysis.
Urine samples were desalted using an XAD-2 column. The methanol eluate collected was concentrated, dissolved in water and extracted with dichloromethane. Non extractable urine samples were further released using β-glucuronidase/arylsulfatase and base hydrolyses followed by acetylation or methylation (derivatisation).
Faeces samples were extracted with methanol and dichloromethane. Non-extractable faeces samples were subjected to base hydrolysis to release additional radioactivity.
Bile was extracted with dichloromethane. Non-extractable bile fractions were further subjected to treatment with β-glucuronidase/arylsulfatase and to base hydrolyses.
Metabolites were mainly isolated from urine samples from the high dose experiment and only in one case from a faeces sample. Separation and purification were performed by means of TLC and HPLC. Isolated metabolites were identified by MS and/or NMR methods. This was confirmed by comparison with synthesised reference standards.
Quantification of metabolites was tried by scanning radioactive TLC spots and by liquid scintillation counting.

Results:

Dimethenamid was rapidly and extensively metabolised. Only 1 - 2 % of unchanged parent compound was detected in the excreta. About 40 metabolites were found in organic extracts and about 20 of these metabolites identified. The metabolite patterns found in rat urine, faeces and in bile are shown in Table B.6.1-3, Table B.6.1-4, Table B.6.1-5, respectively. The structure of the identified metabolites is given in Table B.6.1-6.
Metabolism occurred primarily via the glutathione conjugation pathways. Dimethenamid was rapidly conjugated with glutathione and then further metabolised to form cysteine conjugate (M25) and mercapturate (M17). M25 was further oxidised to form additional metabolites (M1, M2, M10, M13, M14, M16, M18, M19, M21, M22, M26, M27, M30, and M31). Dimethenamid was also metabolised via reductive dechlorination (M3), oxidation (M4, M23), hydroxylation (M5, M11, M15), O-demethylation (M7, M12) and cyclisation (M6, M8, M9, M15, M20). The proposed metabolic pathway scheme of dimethenamid in rats is shown in Figure B.6.1-1.

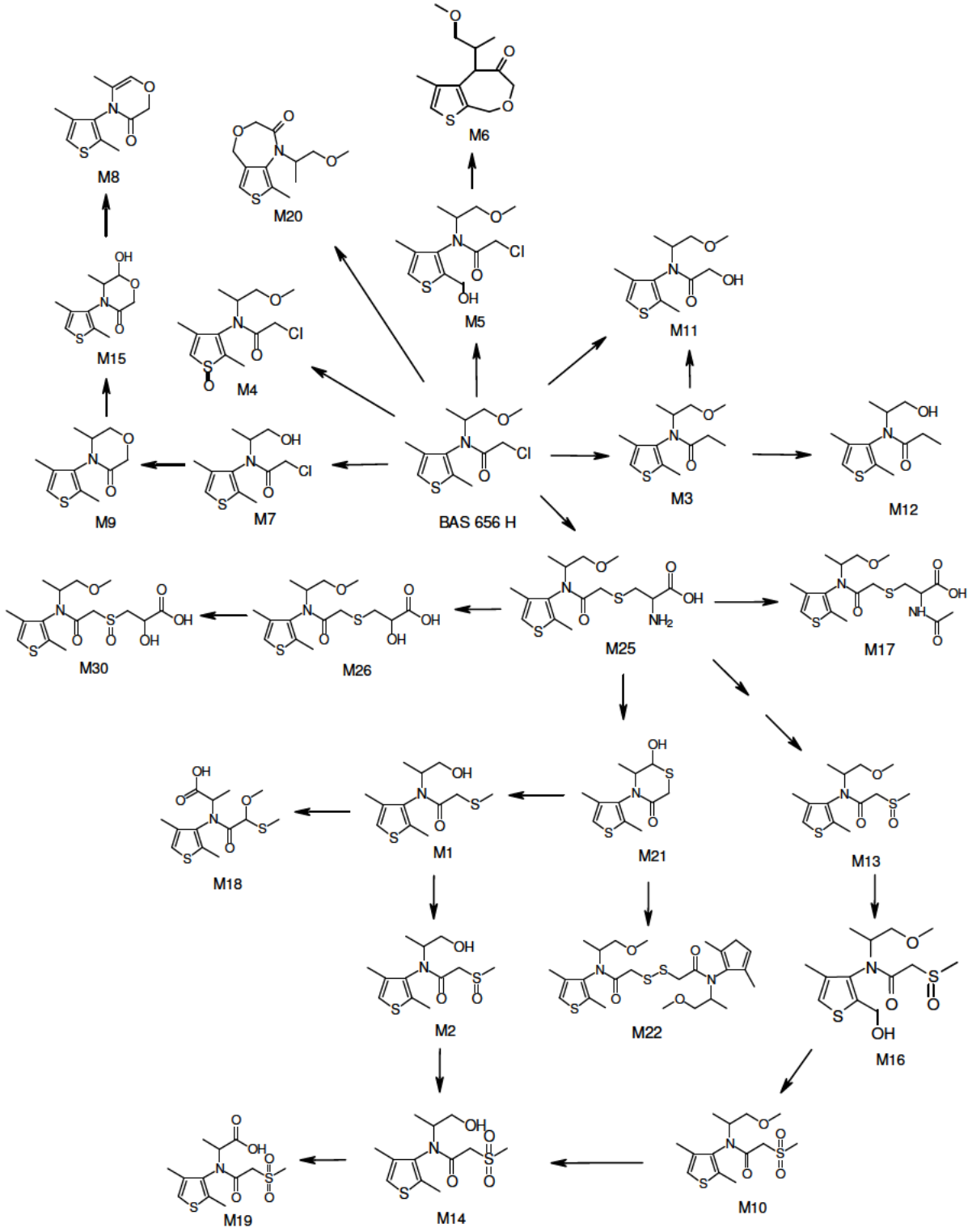


Figure B.6.1-1: Proposed metabolic pathway of dimethenamid (=BAS 656H) in rats

Table B.6.1-3: Dimethenamid metabolite pattern in rat urine

Identity	% of administered dose for the different groups*							
	1M	1F	2M	2F	3M	3F	4M	4F
Dimethenamid	0.2	0.7	0.2	0.5	-	0.2	-	<0.1
M1/M7	0.4	1.6	0.3	3.9	5.3	5.9	0.4	2.7
M2	3.3	6.4	2.4	3.4	5.0	6.8	3.7	9.9
M3	0.2	0.1	0.1	0.2	0.3	0.2	<0.1	0.1
M4	0.2	-	0.5	0.6	-	1.1	-	-
M5	0.3	1.1	0.2	0.6	7.5	5.0	0.2	1.2
M6	0.1	0.3	0.1	0.5	-	-	-	<0.1
M8	-	-	-	-	-	0.3	-	<0.1
M9	<0.1	0.1	<0.1	0.2	-	<0.1	-	-
M10	<0.1	0.2	0.1	0.3	-	0.2	-	<0.1
M11	<0.1	0.2	0.2	0.2	0.4	0.2	0.1	0.2
M12	0.3	0.2	0.3	0.3	0.4	0.5	0.4	0.7
M13	0.9	2.9	0.9	2.3	0.5	1.5	0.3	2.1
M14	1.0	2.2	1.0	2.5	2.7	3.9	0.9	2.4
M15	-	-	-	-	-	-	-	0.1
M16	0.9	1.2	1.1	1.0	2.8	1.7	1.4	2.1
M17	0.3	1.7	0.1	1.9	2.5	3.7	0.2	1.2
M18	0.4	0.6	0.4	0.7	0.9	0.9	0.6	1.1
M19	0.5	0.3	0.2	0.4	0.5	0.6	0.3	0.2
M20	-	-	-	-	-	-	-	-
M21	-	0.5	0.5	0.7	0.9	0.3	-	0.3
M22	-	-	-	-	-	-	-	-
M25	<0.1	0.2	0.2	0.9	0.1	<0.1	<0.1	<0.1
M26	0.1	<0.1	< 0.1	0.1	0.40	<0.1	0.2	0.1
M30	<0.1	0.1	0.2	0.2	0.2	0.2	<0.1	<0.1

*: Pooled 1 - 72 h urine.

Table B.6.1-4: Dimethenamid metabolite pattern in rat faeces

Identity	% of administered dose for the different groups*							
	1M	1F	2M	2F	3M	3F	4M	4F
Dimethenamid	0.9	1.2	2.1	0.8	1.2	1.3	1.4	1.1
M1/M7	2.7	1.8	0.1	2.1	0.6	0.4	2.9	4.5
M2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
M3	0.6	0.3	0.5	0.3	0.1	0.2	0.5	0.2
M4	-	-	-	-	-	-	-	-
M5	0.4	0.4	0.4	0.2	0.3	0.1	0.5	0.2
M6	0.8	0.3	0.2	0.3	0.4	0.3	0.3	0.6
M8	0.2	-	-	-	-	<0.1	-	<0.1
M9	-	-	0.3	-	-	-	-	-
M10	0.7	0.2	0.1	0.1	0.2	0.1	-	0.2
M11	0.3	0.2	1.5	0.5	<0.1	0.3	-	-
M12	-	-	-	-	-	-	<0.1	<0.1
M13	1.5	1.9	-	-	0.3	0.1	<0.1	1.1
M14	1.4	1.8	0.9	0.6	0.3	0.1	2.1	0.9
M15	<0.1	-	-	<0.1	<0.1	<0.1	<0.1	<0.1
M16	2.9	3.3	2.4	1.3	2.0	1.0	4.7	1.7
M17	<0.1	<0.1	0.2	<0.1	<0.1	<0.1	< 0.1	<0.1
M18	0.5	0.5	0.7	0.4	0.3	0.1	0.4	0.3
M19	0.5	0.4	0.2	<0.1	0.5	<0.1	0.8	0.3
M20	0.5	0.3	-	<0.1	<0.1	<0.1	-	-
M21	0.4	0.3	0.2	0.2	0.3	0.2	-	<0.1
M22	0.7	0.1	0.2	0.2	1.0	1.0	-	-
M25	<0.1	<0.1	0.2	<0.1	<0.1	-	<0.1	<0.1
M26	<0.1	<0.1	0.1	< 0.1	<0.1	-	<0.1	<0.1
M30	<0.1	<0.1	0.1	< 0.1	<0.1	-	<0.1	<0.1

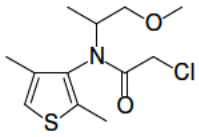
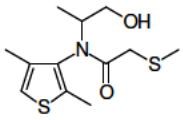
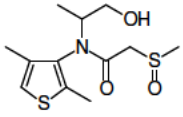
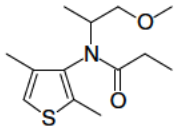
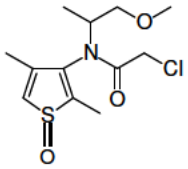
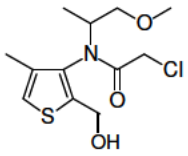
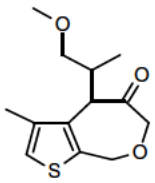
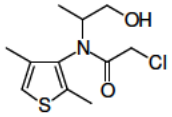
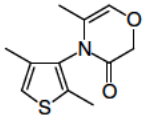
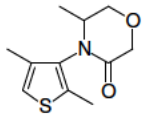
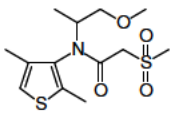
*: Pooled 0 - 72 h faeces

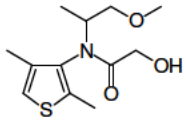
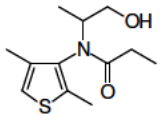
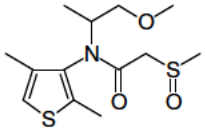
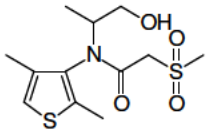
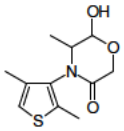
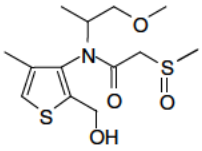
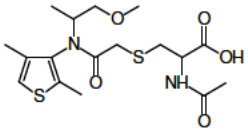
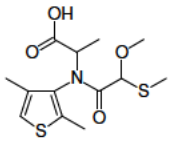
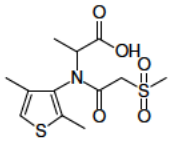
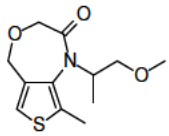
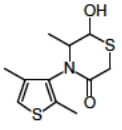
Table B.6.1-5: Dimethenamid metabolite pattern in rat bile

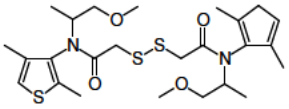
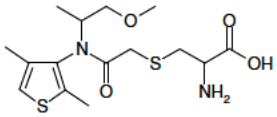
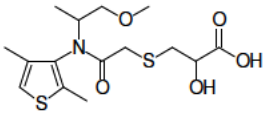
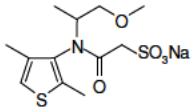
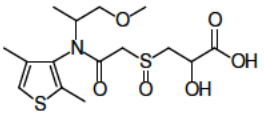
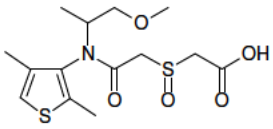
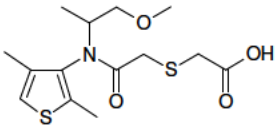
Identity	% of administered dose for the male and female groups*	
	5M	5F
Dimethenamid	<0.1	<0.1
M1/M7	5.0	4.8
M2	0.3	0.7
M3	-	-
M4	1.8	1.3
M5	6.0	2.0
M6	-	-
M8	0.5	0.8
M9	<0.1	-
M10	0.1	<0.1
M11	0.3	0.2
M12	-	-
M13	-	-
M14	0.4	0.1
M15	-	-
M16	0.7	0.3
M17	2.6	3.0
M18	0.2	0.1
M19	0.4	0.1
M20	-	-
M21	2.0	1.8
M22	-	-
M26	0.1	0.1
M30	0.2	0.1

*: Pooled 1 - 72 h bile.

Table B.6.1-6: Structures of identified metabolites found in urine, faeces and bile

Metabolite identity	Structure
Dimethenamid	
M1	
M2	
M3	
M4	
M5	
M6	
M7	
M8	
M9	
M10	

Metabolite identity	Structure
M11	
M12	
M13	
M14	
M15	
M16	
M17	
M18	
M19	
M20	
M21	

Metabolite identity	Structure
M22	
M25	
M26	
M27	
M30	
M31	
M32	

Conclusion:

Dimethenamid was rapidly and extensively metabolised. Only 1 - 2 % of unchanged parent compound was detected in the excreta. Over 40 metabolites were detected of which about 20 could be structurally identified. Metabolism occurred primarily via glutathione conjugation pathways. Dimethenamid was also metabolised via reductive dechlorination, oxidation, hydroxylation, O-demethylation, and cyclisation. There was no significant sex difference in dimethenamid metabolism.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point: KCA 5.1

Report: [redacted] 1992 (TOX1999-407)
SAN 582H: Determination of the presence of sulfonate metabolite in mice
[redacted]
unpublished
BASF RegDoc.# 92/12445

Guideline(s): EPA Pesticide Assessment Guidelines, Subdivision F, Section 85-1

Deviations: No relevant deviations

GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.1
Report:	[redacted]., 1992 (TOX1999-408) SAN 582H: Addendum to determine sulfoxide of thiogylcolic acid conjugate in mouse excreta [redacted] unpublished BASF RegDoc.# 92/12446
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision F, Section 85-1
Deviations:	No relevant deviations
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

The objectives of these studies are to determine the presence of the sulfonate and sulfoxide of thioglycolic acid metabolites in urine and faeces of mice. These metabolites were identified as terminal residues in corn.

Materials and methods:

The mice metabolism study was conducted using [3-thienyl-^{14}C] dimethenamid. The radiochemical purity was 98.6 %. Specific activity was 50.5 mCi/mole. The study was conducted in 2 groups. Each group (A and B) consisted of 5 males and 5 females mice. Groups A and B were administered a single oral dose at 1 and 100 mg/kg bw, respectively.
Urine and faeces samples were separately collected daily for 4 days and the animals were sacrificed at 96 hours after dose administration. Urine and faeces samples collected over the experimental period were separately collected, then combined per group, freeze-dried (sulfonate only) and extracted with organic solvents. Isolation and purification of sulfonate and sulfoxide of thioglycolic acid metabolites required multiple TLC and HPLC systems. The identification of these metabolites was based on cochromatography with reference standards in two-dimensional TLC.

Results:

A summary of radiocarbon in urine and faeces is presented in Table B.6.1-7. Dimethenamid was rapidly excreted in urine and faeces. There was no significant difference between the sexes of the same group in the route of excretion. For the male mice in group A (1 mg/kg), the % amount of radiocarbon in urine and faeces were 43.99 and 47.26, respectively. For the female mice in group A, the % amount of radiocarbon in urine and faeces were 46.25 and 42.12, respectively. The % amount of radiocarbon in urine (male; group B, 100 mg/kg) and faeces were 59.60 and 33.64, respectively. The % amount of radiocarbon in urine and faeces were 59.89 and 28.30, respectively, for the female mice (group B). The material balance from urine, faeces and cage wash varied from 88.81 % to 94.23 %. Cage wash only contributed a small percentage of radiocarbon, 0.62 % to 2.92 % in both dose groups and sexes.
Sulfonate metabolite:
In urine, methanol extract of the freeze-dried sample had a recovery of 97 % of the radiocarbon. The sulfonate metabolite was isolated and purified using multiple TLC and HPLC methods. The presence of metabolite sulfonate was confirmed with the reference standard in two-dimensional TLC. The % of sulfonate in urine for groups A and B were 0.060 and 0.069, respectively. The results are summarised in Table B.6.1-8 and the metabolite structures are reproduced in Table B.6.1-9.

In faeces, methanol extract of the freeze-dried sample also had a recovery of 97 % of the radiocarbon. The sulfonate metabolite was isolated, purified and identified similarly to the urine samples. The amount of sulfonate in faeces for groups A and B were 0.25 % each.
Sulfoxide of thioglycolic acid:
In urine, combined ethyl acetate extract had a recovery of approximately 80 % of the radiocarbon. The fraction containing the highest radiocarbon (46.4 %) was used for isolation and identification. The sulfoxide of thioglycolic acid metabolite was isolated and purified using multiple TLC and HPLC methods. The presence of sulfoxide of thioglycolic acid metabolite was confirmed with the reference standard in two-dimensional TLC. The % of sulfoxide of thioglycolic acid in urine for groups A and B were 0.25 and 0.24, respectively (see Table B.6.1-8).
For faeces, similar techniques as urine were used for the metabolite identification. The % of sulfoxide of thioglycolic acid in faeces for groups A and B were 0.25 and 0.40, respectively.

Table B.6.1-7: Summary of ^{14}C-Dimethenamid Material Balance for Dose Groups A (1 mg/kg) and B (100 mg/kg) in Mice Administered Orally

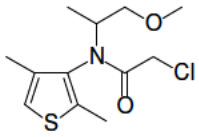
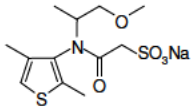
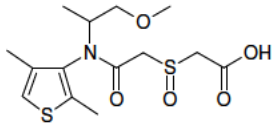
Dose Group	Dose Level	Sample	% of Administered Dose at 96 hr after treatment*	
			Male	Female
A	1 mg/kg	Urine	43.99	46.25
		Faeces	47.26	42.12
		Cage Wash	1.68	2.92
		Total	92.93	91.29
B	100 mg/kg	Urine	59.60	59.89
		Faeces	33.64	28.30
		Cage Wash	0.99	0.62
		Total	94.23	88.81

*: Data are the average of 5 animals per group and sex, except Group B with 4 animals. Animals were sacrificed 96 hr after ^{14}C dimethenamid administration.

Table B.6.1-8: Percentage of Sulfonate in Urine and Faeces for Groups A (1 mg/kg) and B (100 mg/kg) Mice Administered with ^{14}C Dimethenamid Orally

Dose Group	**Urine**	**Faeces**	**Total**
	% of Sulfonate in Urine and Faeces		
A	0.060	0.25	0.31
B	0.069	0.25	0.319
	% of Sulfoxide of Thioglycolic Acid in Urine and Faeces		
A	0.25	0.25	0.50
B	0.24	0.40	0.64

Table B.6.1-9: Structures of Dimethenamid, Sulfonate and Sulfoxide of Thioglycolic Acid

Metabolite Identity	Structure
Dimethenamid	
Sulfonate (M27)	
Sulfoxide of thioglycolic acid (M31)	

Conclusion:

Dimethenamid was readily excreted by mice. Urinary radiocarbon accounted for approximately 44 % to 60 % while faeces accounted for approximately 28 % to 47 %. Total recovery varied from 88.81 to 94.23 %. Sulfonate was found to be 0.06 % (group A) and 0.069 % (group B) in urine and 0.25 % (both groups) in faeces. Sulfoxide of thioglycolic acid in urine accounted for 0.25 % (both groups). In faeces, this metabolite accounted for 0.25 % at a dose level of 1 mg/kg bw and 0.40 % at dose level of 100 mg/kg bw.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.1
Report:	[redacted]., 1992 (TOX1999-409) SAN 582H: Determination of the presence of plant metabolites in rat [redacted] unpublished BASF RegDoc.# 92/12448
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision F, Section 85-1
Deviations:	No relevant deviations
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

The objectives of these studies are to determine the presence of the sulfonate, thioglycolic acid and sulfoxide of thioglycolic acid metabolites in the excreta of rats. These metabolites were identified as terminal residues in corn.

Materials and methods:

The rat metabolism study was conducted using [3-thienyl-^{14}C] dimethenamid. The radiochemical purity was 98 % (Batch Number: RA 683-2). Specific activity was 50.56 mCi/mole. The study was conducted in 2 groups. Each group (A and B) consisted of 10 rats - 5 males and 5 females. Groups A

and B were administered a single oral dose at 1 and 100 mg/kg bw, respectively.
Urine and faeces samples were separately collected daily for 3 days and the animals were sacrificed at 72 hours after dose administration. Representative urine sample was prepared by pooling portions of the urine sample from the same group collected each day in proportion to their total radioactivity. A portion of the combined urine was first cleaned up using SPE method, followed by TLC and/or HPLC methods and/or mass spectrometry for metabolite identification. For the extraction and analysis of faeces sample, approximately equal proportions of faeces from each animal of the same group were combined, thoroughly mixed and freeze-dried. The faeces sample was extracted with a mixture of methanol and acetone. This extract was further cleaned up with multiple TLC methods. Presence of metabolites was confirmed similarly to the urinary metabolites as described above.

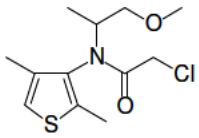
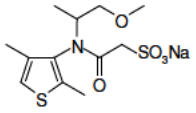
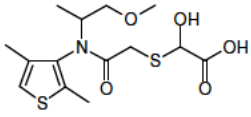
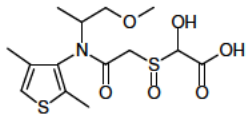
Results:

A summary of the metabolite findings in rat excreta is shown in Table B.6.1-10 and the structures are shown in Table B.6.1-11.
Sulfonate metabolite:
In urine, the sulfonate metabolite was isolated and purified using multiple TLC methods. The presence of metabolite sulfonate was confirmed with the reference standard in two-dimensional TLC and HPLC methods. The confirmation of sulfonate identification was further supported by mass spectrometry. The amount of sulfonate in urine for groups A and B were 0.025 % and 0.030 %, respectively. In faeces, the % of sulfonate for groups A and B were 0.16 and 0.02, respectively.
Sulfoxide of thioglycolic acid:
Again, this metabolite was isolated, purified and identified using multiple TLC and HPLC methods. The % amount of sulfoxide of thioglycolic acid in urine were 0.007 (group A) and 0.002 (group B), respectively. However, the presence of this metabolite was not confirmed in faeces.
Thioglycolic acid:
The presence of this metabolite was not confirmed in rat urine or faeces.

Table B.6.1-10: Percentage of Sulfonate in Urine and Faeces for Groups A (1 mg/kg) and B (100 mg/kg) Rat Administered with ^{14}C Dimethenamid Orally

Dose Group	Urine	Faeces	Total
	% of Sulfonate in Urine and Faeces		
A	0.025	0.016	0.041
B	0.030	0.02	0.050
	% of Sulfoxide of Thioglycolic Acid in Urine and Faeces		
A	0.007	Not Confirmed	0.007
B	0.002	Not Confirmed	0.002

Table B.6.1-11: Structures of Dimethenamid, Sulfonate, Thioglycolic Acid and Sulfoxide of Thioglycolic Acid

Metabolite Identity	Structure
Dimethenamid	
Sulfonate (M27)	
Thioglycolic Acid (M32)	
Sulfoxide of Thioglycolic Acid (M31)	

Conclusion:

Sulfonate metabolite was isolated and identified in both rat urine and faeces (0.025 % in group A and 0.030 % in group B). In faeces, the sulfonate metabolite accounted for 0.016 % in group A and 0.02 % in group B. Sulfoxide of thioglycolic acid was present in urine (0.007 % in group A and 0.002 % in group B) but its presence is not confirmed in faeces. The presence of thioglycolic acid was also not confirmed in rat urine and faeces.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.1
Report:	[redacted] 2014a (ASB2014-8383) Excretion and metabolism of ^{14}C -dimethenamid-P (BAS 656 H) after oral administration in rats 2012/1194996 unpublished
Guideline(s):	EPA 870.7485, EPA 860.1000, OECD 417, EEC 87/302
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.1
Report:	[redacted]., 2012a (ASB2014-8384) ^{14}C -BAS 656 H - Study on bile excretion in rats

	2012/1021081 unpublished
Guideline(s):	OECD 417 (April 1984), Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to regulation (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, EPA 870.7485, JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on *In vivo* Fate in Animals (2001)
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

The studies described above were performed with racemic dimethenamid and metabolite identification was not performed using recent techniques. To augment these studies, a new rat metabolism study (2012) was performed using dimethenamid-P and using state of the art identification. It was conducted with the isomer dimethenamid-P to further elucidate the detailed metabolism and excretion in male and female rats after oral administration.

Materials and methods:

Description:	Dimethenamid-p (BAS 656 PH)		
Batch # / purity:	Radiolabelled (^{14}C):	824-6027	99.0 %
	Radiolabelled (^{13}C):	1030-1004	100 %
	Nonlabelled:	L74-120	96.5 %
Stability of test compound:	Stable during dosing period		
Vehicle and/or positive control:	Aqueos solution of carboymethylcellulose (0.5 %) and Cremophore (1 %)		
Test animals:			
Species:	Rat		
Strain:	Sprague Dawley rats (Crl:CD(SD)) (Charles River Laboratories, Germany)		
Age:	About 8 weeks at start of acclimatisation		
Sex:	Male and female		
Number of animals:	16 (10 males+10 females, 6 males for bile excretion)		
Weight at dosing:	223 - 456 g (prior to dosing)		
Acclimation period:	Not reported		
Diet:	Kliba lab diet for mouse and rat, *ad libitum*		
Water:	Tap water *ad libitum*		
Housing:	During acclimatisation in groups in Macrolon cages, then individually in plexi-glass metabolism cages		
Husbandry:			
Environmental conditions:			
Temperature:	21 - 24 °C		
Humidity:	45 - 75 %		
Photoperiod:	Alternating 12-hour light and dark cycles		

Preparation of dosing solutions:
For oral administration of the test item to animals of dose groups DXM and DXF, mixtures of ^{14}C labelled, ^{13}C labelled and unlabelled dimethenamid-P (in a ratio of 2:33:65) were prepared in 0.5 % aqueous solution of carboxymethylcellulose, and 1 % Cremophore was added.
In order to demonstrate the stability of the test item in the application formulations, confirm the identity and determine the isotope pattern, HPLC and FIA-MS analyses were performed. For both dose groups, diluted application solutions were LSC measured and the measured values were taken for calculation.

Aliquots of the formulated test item were administered to the rats by gavage. The actual dose applied was 251.9 mg/kg bw and day for dose group DXM and 250.2 mg/kg bw and day for dose group DXF, respectively.
For oral dosing of the RM and SM groups, mixtures of ^{14}C-labelled, ^{13}C labelled and unlabelled BAS 656 H were prepared in 0.5 % aqueous solution of carboxymethylcellulose, and 1 % Cremophor EL was added. About 10 mL/kg body weight of the respective preparation was administered to the rats by gavage.
Study design and methods: Dates of work: August 30, 2011 – January 9, 2013
The treated rats consisted of an oral single dose group (10 rats/sex, 250 mg/kg bw) and for bile excretion two oral single dose groups (10 mg/kg bw and 250 mg/kg bw male animals). All animals received the oral dose administered via gavage.
In the case of dose group DXM, urine was collected 6 h, 12 h and 24 h after administration and in further time intervals of 24 h up to 168 h, and faeces were sampled 12 h and 24 h after administration and in further time intervals of 24 h up to 168 h (combined samples for 10 animals). After seven days, the animals were anesthetised and sacrificed.
In the case of dose group DXF, urine was collected 6 h, 12 h and 24 h after administration and in further time intervals of 24 h up to six days (last sampling 148 h after dosing; combined samples for 10 animals). A pooled urine sample (12 h to 48 h) was prepared for metabolite identification. Faeces of dose group DXF were sampled 12 h and 24 h after administration and in further time intervals of 24 h up to six days (last sampling 148 h after dosing). The animals were sacrificed after six days.
For both dose groups DXM and DXF, blood was collected from the sacrificed animals in tubes containing the anticoagulant EDTA, and approximately half of each sample was stored in a Nalgene bottle, the remaining half was centrifuged to obtain plasma. Moreover, liver, kidney, spleen and carcass were taken from the sacrificed rats and stored in a freezer. Plasma, liver, kidney, spleen and carcass were not further investigated because the mass balance was acceptable. In addition, the cages were cleaned with water and methanol, and the cage wash samples were also stored frozen.
In the cases of dose groups RM and SM, bile was collected from each animal in three-hour time intervals, and urine and faeces were sampled in time intervals of 24 h up to 72 h. The remaining samples of bile, urine and faeces after determination of the radioactivity as well as aliquots of the application preparations were stored at 20 °C prior to analyses.
Equal portions of the bile samples of the animals No 2, 5 and 6 (dose group RM) were combined from the time periods of 0-3 h, 3-6 h, 6-9 h, 9-12 h and 12-18 h, respectively.
Samples were analysed for total ^{14}C-radioactivity by samples combustion and/or liquid scintillation counting (LSC).

Table B.6.1-12: Summary of experimental set-up

Dose Group	Origin	Nominal Dose [mg/kg bw]	Use	Comments
DXM	Excretion and Metabolism	1 × 250	- Identification of metabolites in faeces - Metabolite patterns in urine and faeces, quantification	Male animals
DXF	Excretion and Metabolism	1 × 250	- Identification of metabolites in urine and faeces - Metabolite patterns in urine and faeces, quantification	Female animals
RM	Bile excretion	1 × 10	- Identification of metabolites in bile - Metabolite patterns in bile, quantification (single low dose)	Male animals
SM	Bile excretion	1 × 250	- Metabolite patterns in bile, quantification (single high dose)	Male animals

Results:

Excretion
The results on excreted radioactive residues after single oral administration of ^{14}C-dimethenamid-P to rats at a dose level of 250 mg/kg body weight (dose groups DXM and DXF) are summarised in

Table B.6.1-13. The mean recovery of radioactive residues in urine, faeces and cage wash was found to be 89.35 % and 89.44 % of the administered dose for male (dose group DXM) and female rats (DXF), respectively. Within the observation period of seven days or 148 h (dose group DXM or DXF, respectively), total excretion of radioactive residues via urine was 40.89 % of the dose for dose group DXM and 54.87 % of the dose for dose group DXF. The portions of radioactive residues excreted via faeces accounted for 46.41 % of the dose for dose group DXM and 32.20 % of the dose for dose group DXF. In the cage wash, 2.05 % of the dose (dose group DXM) and 2.37 % of the dose (DXF) were recovered. Excretion via urine was nearly complete after 120 h after dosing for both dose groups, and excretion via faeces was nearly complete within 72 to 96 h after dosing.

A summary of the excretion of radioactive residues via bile by rats of these dose groups is presented in

Table B.6.1-14.

The bile excretion study showed high absorption of ^{14}C-dimethenamid-P after single oral administration of the test item to male, bile catheterised rats. Mean excretion of radioactive residues via bile within 72 h was 79.62 % and 50.34 % of the administered dose (individual values ranging from 21.61 to 72.57 % of the dose) for the dose levels of 10 and 250 mg/kg bw, respectively (see

Table B.6.1-14). Excretion via urine in the same observation period accounted for mean values of 13.12 % and 30.29 % of the dose for the low and the high dose, respectively. Smaller portions of radioactive residues were recovered in faeces (4.36 % of the dose for the low dose and 3.76 % of the dose for the high dose).
In bile, excretion was almost complete after 9 to 12 h in the case of the low dose group RM or after 24 to 30 h in the case of the high dose group SM (besides the slower decrease, the % dose values in bile of dose group SM showed a second relative maximum at the end of Day 1). In urine and faeces, excretion was nearly complete within 24 to 48 h in both dose groups RM and SM.
The absorbed dose was calculated as sum of the excreted amount of administered activity in urine (with cage wash) and bile as well as the residual activity in carcass and yielded mean values of 94.03 and 84.64 % of dose for the tested dose levels of 10 and 250 mg/kg bw, respectively. The absorption decreased with increasing dose.

Table B.6.1-13: Route of excretion and total recovery of dimethenamid-P in rat (percent of radioactive dose)

Group	Target dose [mg/kg bw]	Route of administration	Sex of animal*	Urine [%]	Faeces [%]	Total [%]
Treated	250	Single oral	M	40.89	46.41	89.35
			F	54.87	32.20	89.44

Table B.6.1-14: Excretion via bile in rat (percent of radioactive dose)

Group	Target dose [mg/kg bw]	Route of administration	Sex of animal	Bile [%]
Treated	10	Single oral	M*	79.62
	250		F1	21.61
			F2	56.84
			F3	72.57

* Mean of three animals

Table B.6.1-15: Excretion and retention of radioactivity via urine and faeces after single oral administration of ^{14}C -BAS 656 PH to male and female rats at a dose levels of nominal 250 mg/kg bw (group mean values, in percent of radioactive dose)

	[% of the administered radioactivity]	
	Male	**Female**
Urine (h)		
0-6	2.36	3.53
6-12	5.00	6.14
12-24	10.00	17.12
24-48	14.02	19.15
48-72	4.66	4.70
72-96	2.54	2.04
96-120	1.29	1.32
120-144	0.55	0.87
144-168	0.46	-
Subtotal Urine	40.89	54.87
Faeces (h)		
6-12	0.45	0.09
12-24	18.56	9.63
24-48	18.33	17.38
48-72	5.46	3.65
72-96	1.81	0.74
96-120	0.93	0.46
120-144	0.54	0.26
144-168	0.32	-
Subtotal Faeces	46.41	32.20
Other sources		
Cage wash	2.05	2.37
Total	89.35	89.44

Table B.6.1-16: Excretion and retention of radioactivity via bile after single oral administration of ^{14}C -BAS 656 PH to male and female rats at a dose levels of nominal 250 mg/kg bw (group mean values, in percent of radioactive dose)

h	[% of the administered radioactivity]			
	Male	Female		
Bile				
0-3	68.36	2.71	9.79	17.44
3-6	6.94	6.27	7.83	8.18
6-9	2.66[1]	1.95	7.94	4.55
9-12	0.93	0.85	4.45	4.37
12-15	0.19	0.45	5.14	4.71
15-18	0.15	1.10	4.46	8.08
18-21	0.06	4.81	3.10	7.34
21-24	0.06	1.98	7.61	7.95
24-27	0.03	0.96	4.03	7.02
27-30	0.03	0.18	0.96	1.49
30-33	0.02	0.11	0.43	0.53
33-36	0.02	0.09	0.21	0.39
36-39	0.02	0.04	0.15	0.13
39-42	0.02	0.02	0.11	0.07
42-45	0.02	0.03	0.09	0.04
45-48	0.02	0.01	0.11	0.04
48-51	0.02	0.01	0.12	0.03
51-54	0.02	0.00	0.06	0.03
54-57	0.01[1]	n. r.	0.05	0.03
57-60	0.01[1]	n. r.	0.06	0.03
60-63	0.01[1]	0.00	0.03	0.03
63-66	0.01[1]	0.00	0.04	0.02
66-69	0.01[1]	0.01	0.05	0.02
69-72	0.01[1]	0.03	0.02	0.03
Total	79.63	21.61	56.84	72.57

n.r. not reported

[1] For some time intervals, only bile samples of two animals were obtained (6-9 h: animals 5 and 6, 54-72 h: animals 2 and 6), and the mean values of two animals are given for the respective individual time intervals; the total was calculated as the mean value of the sums for the three animals of dose group RM

Table B.6.1-17: Excretion and retention of radioactivity via urine, faeces and bile after single oral administration of ^{14}C-BAS 656 PH to male rats at a dose levels of nominal 10 and 250 mg/kg bw (group mean values, in percent of radioactive dose)

Dose	10 mg/kg bw	250 mg/kg bw
Urine 0-72 h	13,12	30.29
Faeces 0-72 h	4.36	3.76
Cage wash	0.25	1.10
Stomach content	0.00	0.01
Stomach	0.02	0.01
Gut content	0.02	0.16
Gut	0.02	0.03
Carcass	1.93	2.91
Bile	78.73	50.34
Total	98.45	88.60
Bioavalability	94.03	84.64

Metabolic pathway:
Dimethenamid-P is extensively metabolised in the rat mainly by initial glutathione conjugation, enzymatic cleavage of the tripeptide intermediate (M656PH024, not detected in the investigated rat samples) and subsequent metabolic reactions on the resulting cysteine conjugate M656PH025 (iso), the predominant component identified in bile. Smaller portions of radioactive residues were identified as metabolites generated by direct transformation of the parent compound. The parent molecule dimethenamid-P was detected only in faeces samples in portions below 2 % of the applied dose after single oral administration. (The numbering in the codes “M656PHxyz” is equivalent to that in the former “Mx” codes except for the addition of “zero” figures to obtain always a code with nine characters and the PH indicates the subject was tested with dimethenamid-P.)
One main route of further conversion of the cysteine conjugate M656PH025 (iso) is N-acetylation forming the mercapturic acid M656PH017 (iso) and its O-demethylated or oxidised derivatives M656PH097 (iso) and M656PH100 (iso).
Another main route of further transformation after conjugation with glutathione is hydrolysis of the S-conjugates to the mercaptan M656PH080 (rota) followed by S-methylation to the metabolite M656PH067 (rota), both basic intermediates for the formation of various further derivatives.
O-demethylation of the parent compound or of the S-methylated intermediate M656PH067 (rota) forms the metabolites M656PH007 and M656PH001 (rota), respectively, which are subsequently conjugated with glucuronic acid to form the prominent metabolites M656PH034 (iso) and M656PH095 (iso). The analogous reactions produce metabolite M656PH086 (iso) from metabolite M656PH003.
An additional metabolic reaction of metabolite M656PH067 (rota) is oxidation to the sulphoxide M656PH013 (iso) (analogous reactions convert M656PH001 (rota) to metabolite M656PH002 (iso), M656PH095 (iso) to M656PH098 (iso) (at least four isomers found) or M656PH107 (iso) to M656PH101 (iso)). Further oxidation of the sulphoxides results in the formation of the sulphone metabolites M656PH010, M656PH014 (rota), M656PH096 (iso) and M656PH092 (iso).
O-demethylation also occurs with the metabolites M656PH013 (iso), M656PH010 and M656PH080 (rota) to form metabolite M656PH002 (iso), metabolite M656PH014 (rota) and the putative precursor of the glucuronic acid conjugate M656PH091 (iso) and of the derivative M656PH082 (iso) (derived after oxidation of the sulphur in the thiophene ring to the sulphoxide), respectively. Conjugation of metabolites M656PH002 (iso) and M656PH014 (rota) leads to the glucuronides M656PH098 (iso) and M656PH096 (iso), respectively. Conjugation of M656PH002 (iso) with sulphuric acid produces the sulphate metabolite M656PH088 (iso).

The chlorine in dimethenamid-P can also be replaced by hydrogen through reductive dechlorination to the metabolite M656PH003 (and the analogous reaction leading from M656PH034 (iso) to metabolite M656PH086 (iso) or from M656PH036 (iso) to metabolite M656PH106 (iso) or by a hydroxyl group through hydrolysis to the intermediate M656PH011 (not detected).
Oxidation of the 2-methyl group on the thiophene ring to the hydroxyl methyl and subsequent conjugation with glucuronic acid yields metabolite M656PH036 (iso) (via the intermediate M656PH005 which was not detected), and analogous reactions produce metabolite M656PH101 (iso) from metabolite M656PH013 (iso) (via M656PH016 (iso)), metabolite M656PH107 (iso) from M656PH067 (rota) and metabolite M656PH092 (iso) from M656PH010. The methyl group on the methylethyl moiety of M656PH011 and M656PH086 (iso) is also oxidised to the hydroxyl methyl to form metabolites M656PH087 (rota) and M656PH108 (iso), respectively. The same reaction followed by conjugation with glucuronic acid produces metabolite M656PH099 (iso) from M656PH010. Two oxidation steps at the same methyl group of M656PH080 (rota) produce the carboxyl metabolite M656PH093 (rota).
Metabolite M656PH080 (rota) is also transformed to metabolite M656PH022 (iso) by dimerisation, to the S-glucuronide M656PH105 (iso) by conjugation, or to metabolite M656PH102 (rota) by oxidation.
Metabolite M656PH103 (iso) is formed from M656PH002 (iso) by oxidation of the 2-hydroxy-1-methylethyl moiety and conjugation with glucuronic acid or from M656PH098 (iso) by oxidation of the conjugated 2-hydroxy-1-methylethyl moiety. Metabolite M656PH085 (iso) is formed from M656PH034 (iso) (or M656PH086 (iso)) by cleavage of the amide bond.
The amine derivative M656PH083 (rota) (two rotamers detected by HPLC MS) observed as main component in the solubilisates released from the residual radioactive residues after solvent extraction of faeces by reflux with water probably originated from dimethenamid-P by reaction with amines in faeces.

The proposed metabolic pathway of dimethenamid-P in the rat is shown in Figure B.6.1-2, Figure B.6.1-3 and

Figure B.**6.1-4**. A summary of the identified components is given in Table B.6.1-18.

Table B.6.1-18: Summary of identified metabolites in urine, faeces and bile of rats after oral administration of dimethenamid-P

Metabolite Designation (Code)	Molecular Mass	Urine	Faeces	Bile
Dimethenamid-P (BAS 656 PH)	275.799		+	
M656PH001 (rota)	273.42		+	
M656PH002 (iso)	289.419	+		
M656PH003	241.354	+	(+)	
M656PH007	261.772			+ (n. q.)
M656PH010 (two rotamers)	319.446	+ (n. q.)		
M656PH013 (iso)	303.446	+ (n. q.)		
M656PH014 (rota) – 52.7 min	305.419		+	
M656PH014 (rota) – 54.0 min			+	
M656PH016 (iso)	319.446	+ (n. q.)		
M656PH017 (iso) – 73.0 min	402.535	+		+
M656PH017 (iso) – 73.5 min		+		+
M656PH022 (iso)	544.824		+	
M656PH025 (iso) – 47.9 min	360.498			+
M656PH025 (iso) – 48.9 min				+
M656PH034 (iso)	437.898	+		+
M656PH036 (iso)	467.924	+		
M656PH067 (rota) (former code PL 36-88)	287.447		+	
M656PH080 (rota) (formerly mercaptan)	273.42	+ (n. q.)		
M656PH082 (iso)	275.393		+	
M656PH083 (rota) (two rotamers)	256.369		+	
M656PH085 (iso)	361.372	+ (n. q.)		
M656PH086 (iso)	403.453	+ (n. q.)		
M656PH087 (rota)	273.353	+ (n. q.)		
M656PH088 (iso)	369.484	+ (n. q.)		
M656PH091 (iso)	451.518	+		
M656PH092 (iso) – 26.3 min	511.571	+ (n. q.)		
M656PH092 (iso) – 27.9 min		+ (n. q.)		
M656PH093 (rota) – 40.7 min	303.403	+ (n. q.)		
M656PH093 (rota) – 42.7 min		+		
M656PH095 (iso)	449.546	+		(+)
M656PH096 (iso) – 37.0 min	481.544	+		
M656PH096 (iso) – 38.9 min		+		
M656PH097 (iso) – 52.0 min	388.509	+ (n. q.)		
M656PH097 (iso) – 53.0 min		+ (n. q.)		
M656PH098 (iso) – 22.8 min	465.545	+		
M656PH098 (iso) – 23.4 min, 23.8 min		+		

Metabolite Designation (Code)	Molecular Mass	Urine	Faeces	Bile
(two isomers)				
M656PH098 (iso) – 24.2 min		+		+
M656PH098 (iso) – 25.4 min		+		+
M656PH099 (iso)	511.571	+ (n. q.)		
M656PH100 (iso)	418.535	+		
M656PH101 (iso) – 20.9 min (two isomers)	495.571	+ (n. q.)		
M656PH101 (iso) – 21.8 min (two isomers)		+ (n. q.)		
M656PH102 (rota) (two rotamers)	289.419	+ (n. q.)		
M656PH103 (iso) (two isomers)	479.529	+ (n. q.)		
M656PH105 (iso)	449.546	+ (n. q.)		
M656PH106 (iso) (two isomers)	433.479	+ (n. q.)		
M656PH107 (iso)	479.572	+ (n. q.)		
M656PH108 (iso)	419.452	+ (n. q.)		

n. q. not quantified;
(+) tentatively assigned in faeces or bile

Conclusion:

The bile excretion study showed high absorption of dimethenamid-P after single oral administration of the test item to male, bile catheterised rats. Absorption was slightly higher after administration of the low dose (approximately 94 % of the administered dose in the case of dose group RM) compared to the high dose (approximately 85 % dose in the case of dose group SM).
In the cases of the high dose groups DXM and DXF, similar portions were eliminated via urine and faeces. Excretion via urine was nearly complete after 120 h after dosing, and excretion via faeces was nearly complete within 72 to 96 h after dosing. In the cases of the dose groups RM and SM, mean excretion of radioactive residues via bile within 72 h was 79.62 % and 50.34 % dose for the dose levels of 10 and 250 mg/kg bw, respectively. Biliary excretion was almost complete after 9 to 12 h in the case of the low dose group RM or after 24 to 30 h in the case of the high dose group SM.
Dimethenamid-P was extensively metabolised in the rat. Transformation mainly proceeded via initial glutathione conjugation, enzymatic cleavage of the tripeptide intermediate and subsequent metabolic reactions on the resulting cysteine conjugate M656PH025 (iso), the predominant component identified in bile (20.69 % dose in the low dose group RM, 3.22 % to 17.91 % dose in the high dose group SM). Important routes of further conversion were hydrolysis of the S-conjugates to the mercaptan M656PH080 (rota) followed by S-methylation to the metabolite M656PH067 (rota), both intermediates for the formation of various further derivatives (e.g. the dimer M656PH022 (iso) and the demethylated metabolite M656PH001 (rota), the two main metabolites in faeces), or N-acetylation of the cysteine conjugate forming the mercapturic acid M656PH017 (iso) and its derivatives. Conjugation with glucuronic acid occurred at several positions, mainly after O-demethylation or hydroxylation, and the main components in urine were glucuronides. O-demethylation of the parent compound or of the S-methylated intermediate M656PH067 (rota) forms the metabolites M656PH007 and M656PH001 (rota), respectively, which were subsequently conjugated with glucuronic acid to form the prominent metabolites M656PH034 (iso) and M656PH095 (iso). Oxidation of the sulphur atom in metabolite M656PH067 (rota) (and M656PH001 (rota)) formed sulphoxides and sulphones, e.g. M656PH098 (iso), M656PH014 (rota) and M656PH096 (iso), the latter represented the main component in urine. The unchanged parent molecule dimethenamid-P was detected only in faeces samples in low portions in both dose groups DXM and DXF. The metabolite patterns were qualitatively similar for both sexes, with some variations in the relative portions and the exceptions that M656PH017 (iso) was detected in bile as well as in urine of female rats but not in urine of male rats and that metabolite M656PH082 (iso) was only detected in faeces of male rats.

The main biotransformation steps of dimethenamid-P in rats are:

- Conjugation with glutathione and enzymatic cleavage of the tripeptide to the cysteine conjugate
- N-acetylation of the cysteine moiety
- Hydrolysis of S-conjugates to the mercaptan (followed by S-methylation)
- Oxidation of the sulphur atom to form sulphoxides and sulphones
- O-demethylation
- Hydroxylation
- Conjugation with glucuronic acid
- Replacement of the chlorine atom by hydrogen (reduction) or by a hydroxyl group (hydrolysis)
- Dimerisation of a mercaptan

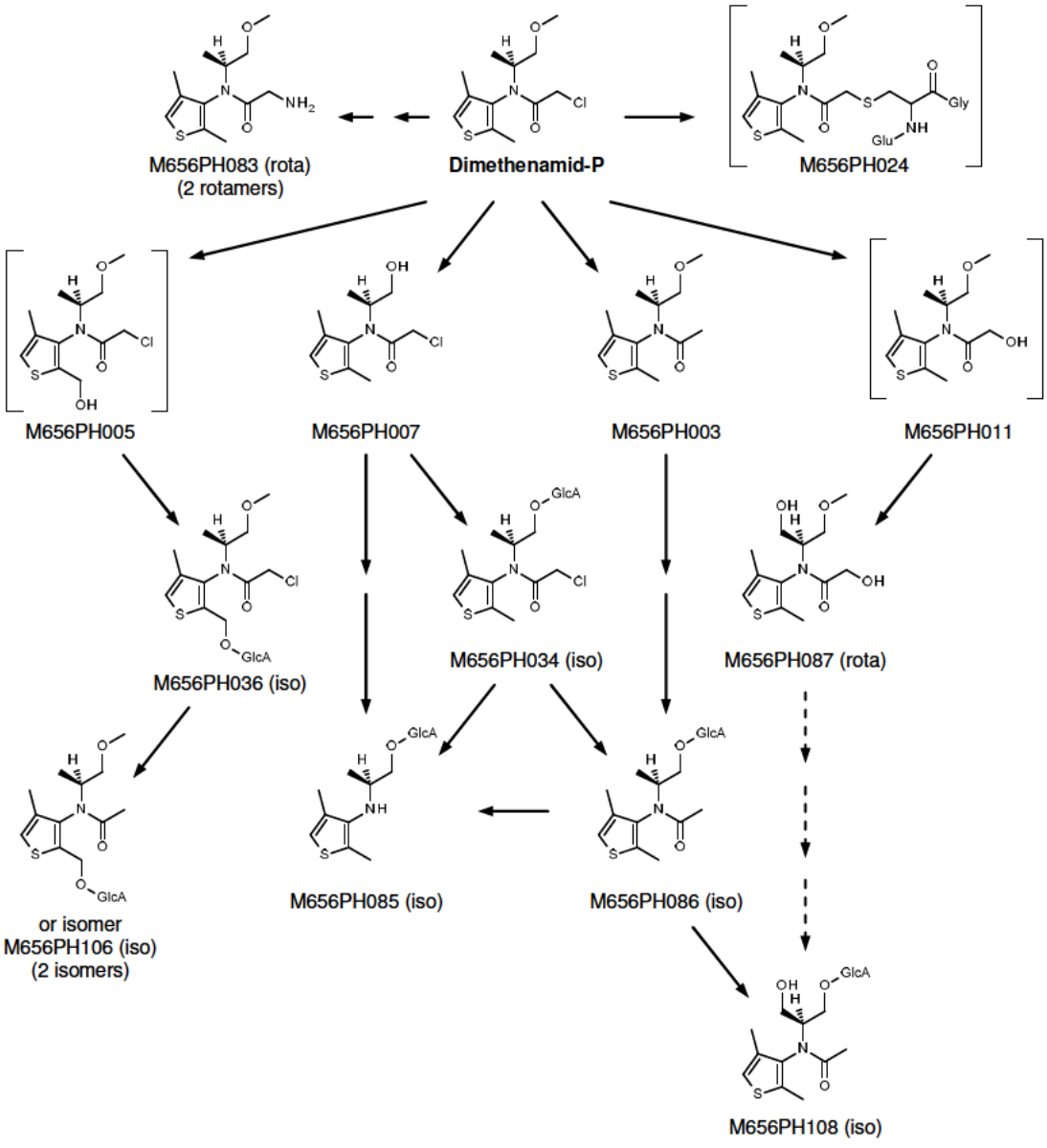


Figure B.6.1-2: Proposed metabolic pathway of dimethenamid-P in rats (part 1)

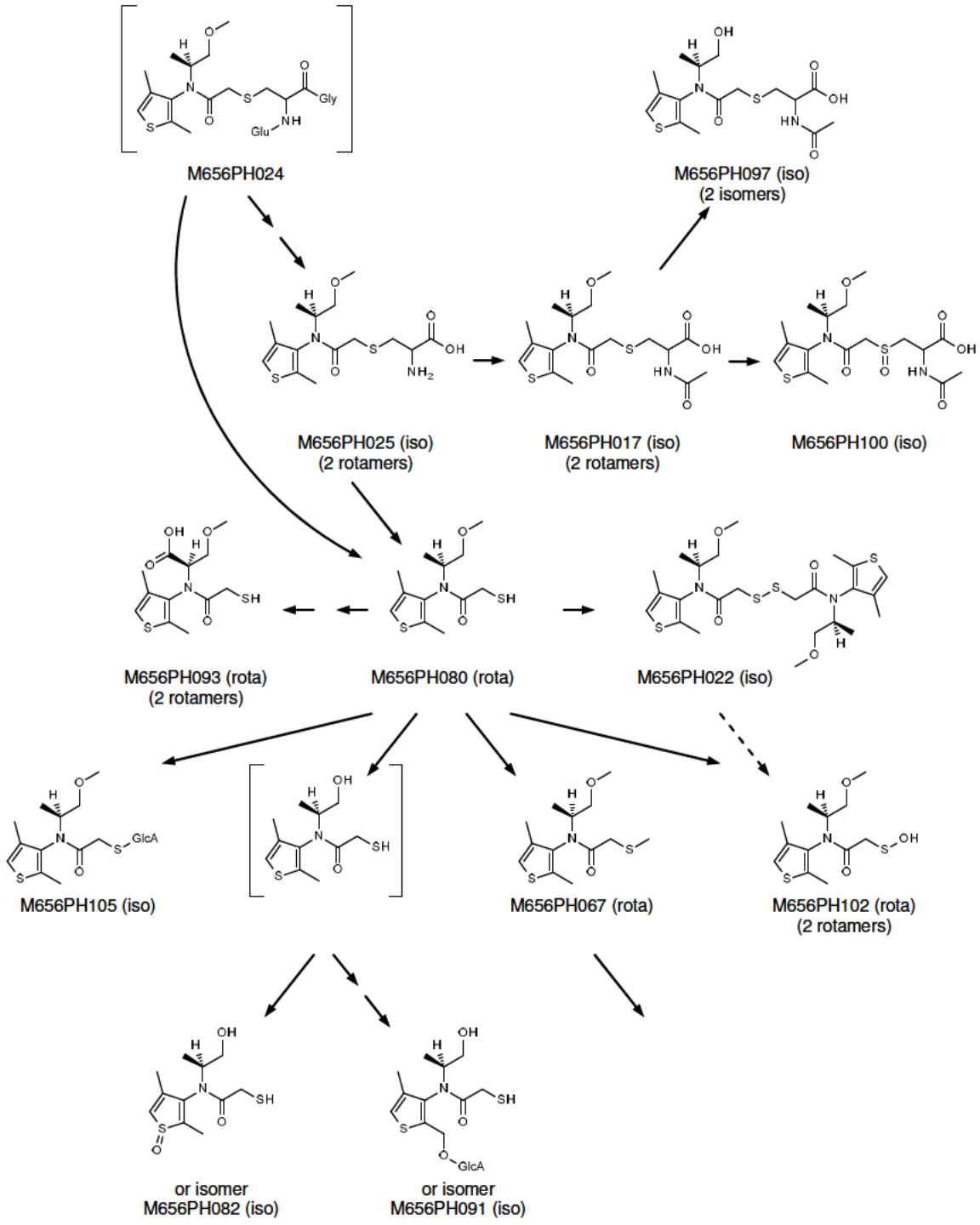


Figure B.6.1-3: Proposed metabolic pathway of dimethenamid-P (BAS 656 H) in rats (Part 2: derivatives after conjugation with glutathione)

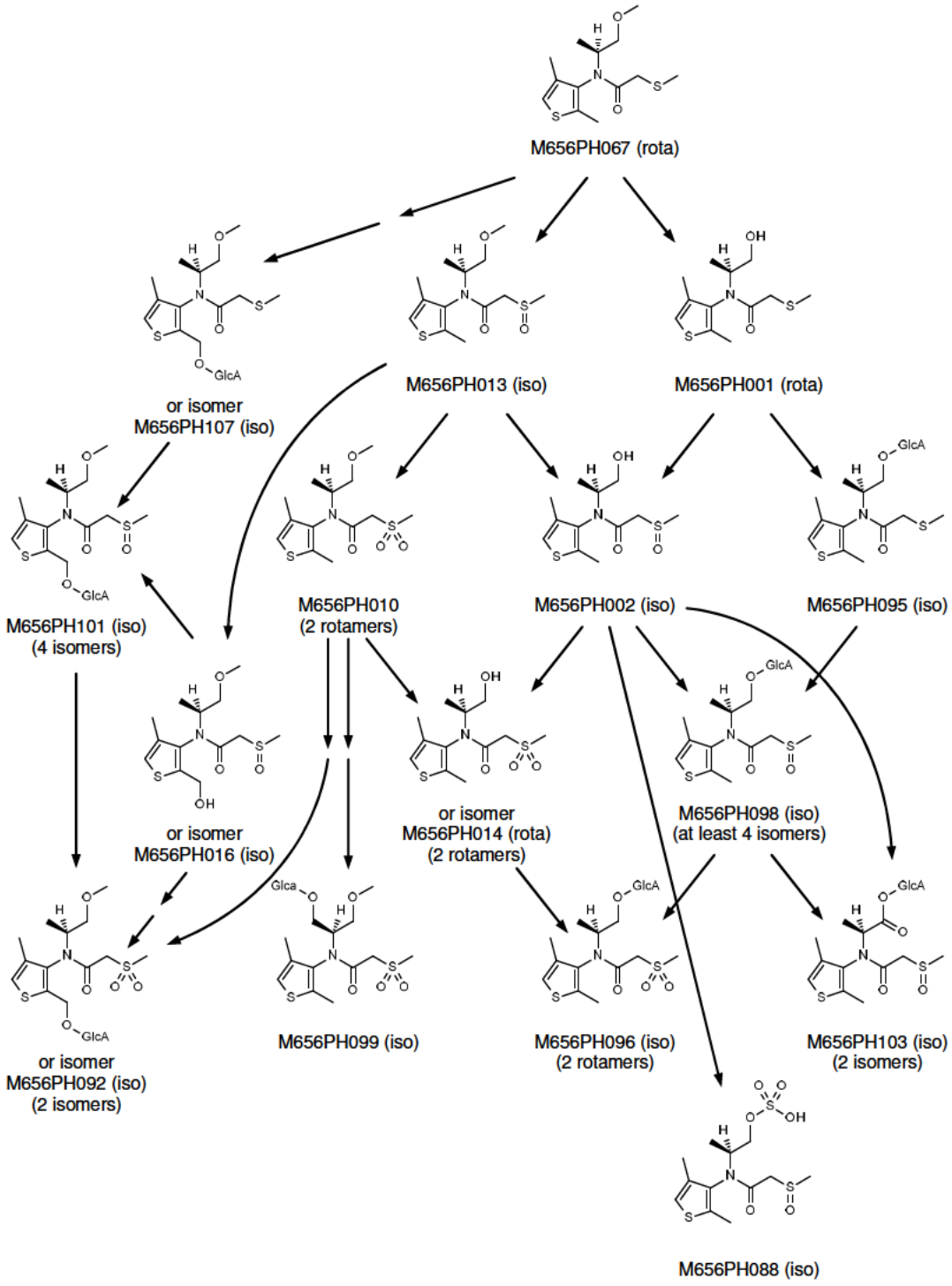


Figure B.6.1-4: Proposed metabolic pathway of dimethenamid-P (BAS 656 H) in rats (Part 3: Derivatives of the S-methyl metabolite M656PH067 (rota))

The study is considered to be acceptable and can be used for the evaluation of the absorption, distribution, metabolism and excretion of dimethenamid-P. The study results of the new study confirm to a certain extent the results of the older studies.

B.6.1.2 Absorption, distribution, metabolism and excretion by other routes

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.1
Report:	[redacted]., 1993 (TOX1999-410) Qualitative investigations of the in-vitro (liver and kidney) metabolism of dimethenamid (SAN 582 H) [redacted] unpublished BASF RegDoc.# 93/11765
Guideline(s):	None, for investigation purposes
Deviations:	No relevant deviations
GLP:	Yes (laboratory certified by Eidgenössisches Departement des Innern, Berne, Switzerland)
Acceptability:	The study is considered to be acceptable.

In this *in-vitro* metabolism study, ^{14}C-dimethenamid was incubated with rat liver cytosol, liver microsomes and kidney S9, with and without glutathione and in the presence of different cofactors.

Materials and methods:

This *in-vitro* metabolism study was conducted using [3-thienyl-^{14}C]-labelled test material. The batch Number was 901101 and the specific activity was 183.3 µChi/mg. Untreated male Sprague-Dawley and Han-Wistar rats were used in this *in-vitro* study. Liver microsomal and cytosolic fractions were freshly prepared at the beginning of the study. Incubations were made using liver microsomal protein and/or liver cytosolic protein and/or kidney S9 protein in Tris-HCl buffer (pH 7.4, 100 mM, 3 mM $MgCl_2$) and the substrate dimethenamid. In addition, depending on the experiment, the incubation mixtures were supplemented with nicotinamide-adenine dinucleotide (NADPH), reduced glutathione (GSH), flavin-adenine dinucleotide (FAD) and pyridoxal-5-phosphate as cofactors. The incubation temperature was controlled at 37 °C in a shaking water bath. Samples were taken at intervals of 5, 30, 60 and 120 min from each incubation. The samples were analysed using HPLC methods with reference standards.

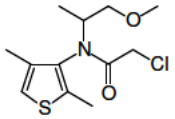
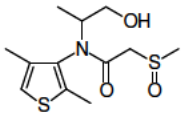
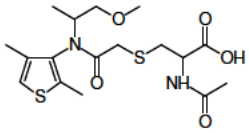
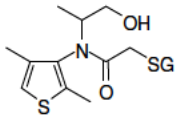
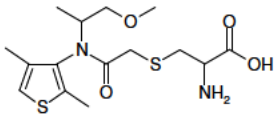
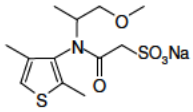
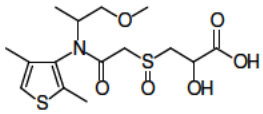
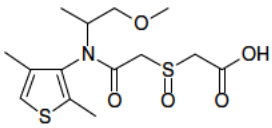
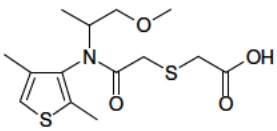
Results:

Dimethenamid was readily and extensively metabolised by liver and kidney enzymes. HPLC analysis indicated that approximately 98 % of dimethenamid was metabolised within the 120 min incubation. With the exception of the cytosolic incubations, the metabolites M27 and M31 were detected in all cases. Methyl sulfinyl conjugate (M2), mercapturic acid conjugate/glutathione conjugate (M17/M24), cysteine conjugate (M25), sulfonate (M27), sulfoxide of thiolactic acid (M30), sulfoxide of thioglycolic acid (M31) and thioglycolic acid (M32) were found in liver and liver/kidney incubations. The metabolic profiles for liver (microsomes/cytosol) as well as combined liver (microsomes/cytosol) and kidney S9 were similar. Dimethenamid is mainly metabolised to its glutathione conjugate (M24) with liver cytosol. The results are summarised in Table B.6.1-19 and the metabolite structures are shown in
Table B.6.1-20. The proposed *in-vitro* rat liver and kidney metabolic pathway is shown in
Figure B.6.1-5.

Table B.6.1-19: Summary of results in the *in-vitro* studies of ^{14}C-dimethenamid

Incubation	Sampling Time (Min)	% of Dimethenamid metabolised	Metabolites
Liver Cytosol, NADPH and Glutathione (GSH)	30		M24
Liver Microsomes, No GSH Added	5	15	M27, M31, M17/M24 and M25
	30	52	M27, M30, M31, M17/M24, M25 and M32
Liver Microsomes, GSH Added	5		M27, M31, M17/M24 and M25
	30		M27, M30, M31, M17/M24, M25 and M32
Liver Microsomes/Cytosol and Kidney S9 with Cofactors (NADPH, GSH, FAD and Pyridoxal Phosphate)	5	41	M27, M30, M31, M17/M24 and M25
	30	93	M27, M30, M31, M17/M24 and M25
	60	97	M27, M2, M30, M31, M17/M24, M25 and M32
	120	98	M27, M2, M30, M31, M17/M24, M25 and M32
Liver Microsomes and Cytosol with Cofactors (NADPH, GSH, FAD and Pyridoxal Phosphate)	5	36	M24/M17, M30, M31 and M2
	30	90	M27, M24/M17, M30, M31 and M2
	60	95	M27, M24/M17, M30, M31 and M2
	120	97	M2, M30, M31, M17/M24, M25 and M32

Table B.6.1-20: Structures of identified metabolites in the *in-vitro* metabolism studies

Metabolite Identity	Structure
Dimethenamid	
M2	
M17	
M24	
M25	
M27	
M30	
M31	
M32	

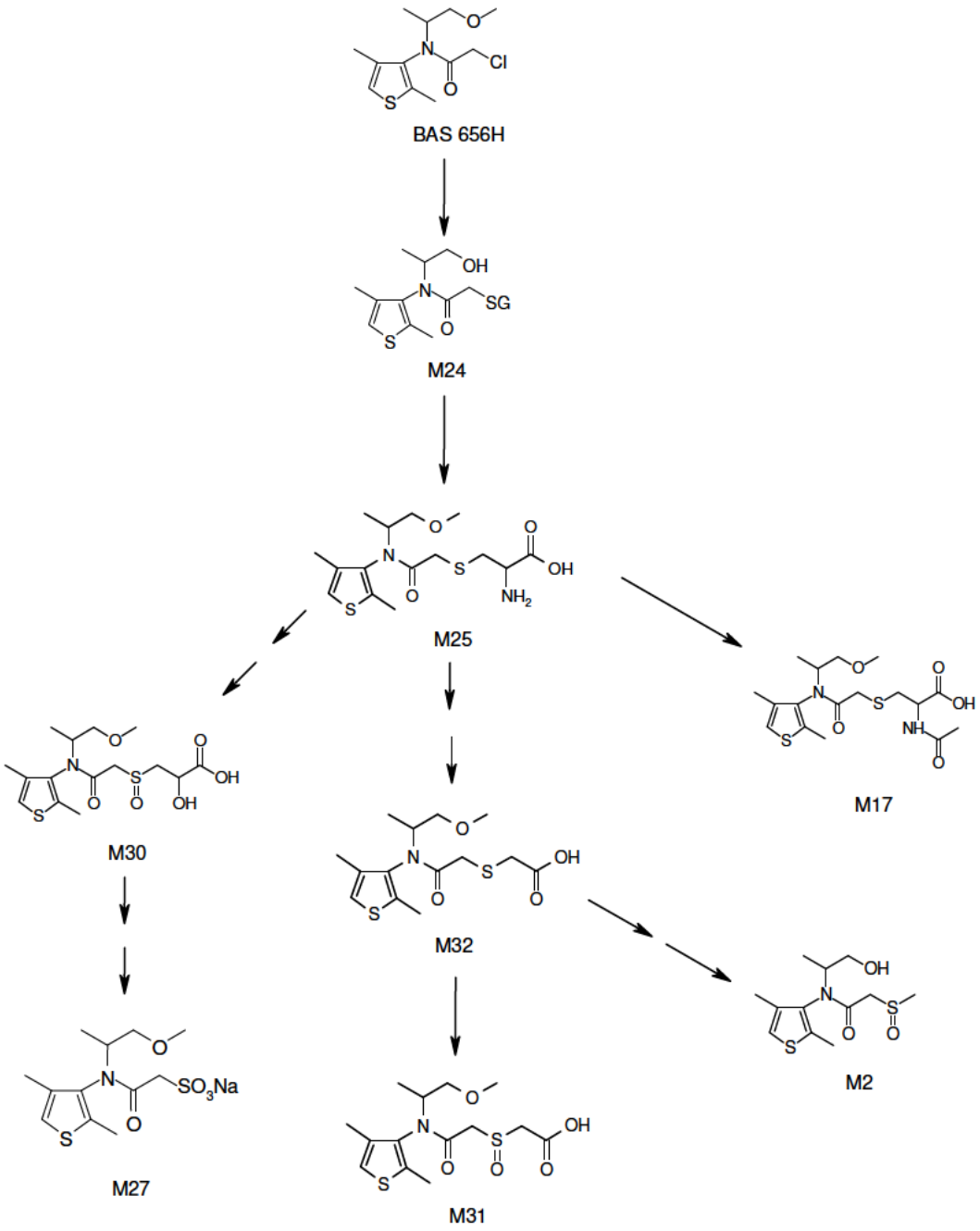


Figure B.6.1-5: Proposed *in-vitro* metabolic pathway of dimethenamid using rat liver and kidney

Conclusion:

Dimethenamid was readily and extensively metabolised by rat liver and rat liver/kidney enzymes *in vitro*. Glutathione conjugation is the major route of metabolism. Other sulfur-containing metabolites (e.g. M2, M17/M24, M25, M27, M30, M31 and M32) were formed mainly by enzymatic oxidations. The findings in this *in-vitro* study are similar to the *in-vivo* rat metabolism study.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.1
Report:	[redacted], 2002a (ASB2014-8385) Comparison of *in vitro* metabolism of enantiomers of BAS 656 H (dimethenamid) 2002/1004042 unpublished
Guideline(s):	None, for investigation purposes
Deviations:	Not applicable
GLP:	Yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:

Description:	Racemic dimethenamid (BAS 656 H) Dimethenamid-P (BAS 656 PH)
Batch # / purity:	Radiolabelled (^{14}C): Racemic dimethenamid: 1338-48G >97 % 7.464 MBq/mg Dimethenamid-P: 1338-48H >98 % 6.783 MBq/mg Nonlabelled: Racemic dimethenamid: CP029795 Dimethenamid-P: 01311-220
Stability of test compound:	Stable during testing
Vehicle and/or positive control:	Vehicle: liver slices Positive control: liver slices were incubated with testosterone instead of the active substance to prove the metabolic activity of the liver slices
Test animals:	Mammals
Species:	Rat
Strain:	Male Wistar rats (CrlGlxBrlHan:WI) (Charles River Laboratories, Germany)

Study design and methods:

The study was carried out at the [redacted]

Test substance preparation:

For the racemate and the S-isomer of BAS 656 H, the following test substance solutions were prepared separately: BAS 656 H was dissolved in DMSO at concentrations of about 50, 25, 18.75, 12.5 and 6.25 mM. Into each application, a solution of pure ^{14}C-labelled test substance (50000 dpm/µL) was added. A total of 10 µL of test substance solution was added into 5 mL incubation medium (with a dilution factor of 500) resulting in a nominal concentration in the incubate of about 100, 50, 37.5, 25 and 12.5 µM. The radioactivity added to each incubation mixture is thus 500,000 dpm/5 mL.

Preparation and incubation of liver slices:

For the preparation of tissue slices, the livers from freshly sacrificed rats were resected and were stored in ice cold Krebs Henseleit buffer, saturated with carbogen (95 % oxygen, 5 % nitrogen) for immediate use. For slicing with the Krumdieck slicer, tissue cores were prepared (diameter 8 mm),

placed into and sliced in ice-cold, carbogen saturated Krebs Henseleit buffer. Slice thickness was adjusted to approx. 200 µm.
The incubation was performed at approx. 37 °C in 6 well tissue plates, each well containing 5 mL Williams E Medium and two liver tissue slices. For heat denaturated control samples, the tissue slices were heated for about 10 min in boiling water. The plates were bidirectionally shaken and continuously gassed with 95 % oxygen and 5 % nitrogen. After a pre-incubation period of 30 min, the medium was substituted by test compound containing medium (10 µL DMSO test compound solution in 5 mL medium) and was incubated for approx. 24 h. After the incubation period the tissue slices were removed and samples were frozen in Falcon vials at -20 °C until analysis.
HPLC-MS and NMR analysis:
After the incubations the liver slices were removed from the plate. Incubates could be used directly for HPLC analysis and were injected without further purification. For LC-MS analysis the 100 µM incubation of racemate was used due to higher amounts of test compound for measurements. For LC-MS, purification was carried out by HPLC and collecting fractions during the time window between 11 and 25 min. For ^{1}H-NMR analysis approx. 100 µg of M4 were purified and concentrated by HPLC and a fraction collector. For the purification, different incubation media were used to generate a pooled sample of M4. The eluent was dissolved and the sample was diluted in $CDCl_3$ for analysis.

Results:

Metabolic pathway of BAS 656H in rat liver slices:
The present study was designed to investigate possible differences between the metabolism of stereoisomers of dimethenamid-P and racemic dimethenamid. For this purpose liver slices of male Wistar rats were prepared and incubated with the S-isomer and the racemate of ^{14}C-dimethenamid. First trials with dimethenamid were carried out to optimise the incubation conditions, mainly the concentration of the test compounds in the incubation medium. Thus, the S-isomer and the racemate of dimethenamid were incubated for 24 h in an atmosphere of 95 % oxygen and 5 % nitrogen at 37 °C with nominal concentrations of 12.5, 25, 50, and 100 µM (concentrations of the test substances in the incubation medium). In addition, one control incubation was performed for each test concentration using heat denaturated rat liver slices.
In comparison with the active incubation, these control incubations allow the differentiation between enzymatic and non-enzymatic processes (abiotic processes could also occur in control incubations) and allow the calculation of the turn-over (by calculating the relative decrease of parent compound in the active versus the inactive incubation using heat denaturated liver slices). For further series of incubations, the incubation concentration was selected by the turn-over of the metabolic reactions and by the received metabolite profile. Derived from these range finder studies, a nominal concentration of 37.5 µM was chosen (between 25 µM and 50 µM). This concentration was interpolated to guarantee a significant turn-over to make sure that the major part of the test substance will be metabolised but on the other hand to still have parent compound present after the incubation to allow the calculation of the turn-over of the enzymatic reactions.
Dimethenamid was extensively metabolised in liver slices: At a nominal concentration of 37.5 µM dimethenamid in the incubation medium, the turn-over was >50 %. Up to 20 metabolites could be separated by HPLC. All metabolites are more polar than the parent compound and have retention times between 11 min and 35 min. The main metabolite M4 has a retention time of about 22.5 min and has a relative amount of the total peak area of about 17 - 18 % (37.5 µM incubation). The parent compound eluates at about 41 min.
To identify the major metabolic pathway of dimethenamid in rat liver slices, the 7 most intense peaks were analysed by LC-MS and NMR. The identified metabolites are summarised in Table B.6.1-21. The metabolism of BAS 656 H in rat liver slices occurred via various oxidation and conjugation reactions: It could be demonstrated by LC-MS and NMR-techniques that the main *in vitro* metabolite is identical to the *in vivo* metabolite M4. In the rat metabolism study, this metabolite was identified to be the sulfoxide of dimethenamid. Further metabolic reactions in rat liver slices are hydroxylations of the dimethylthiophene system with consequent glucuronidations to form the glucuronic acid conjugates M36. Oxidative desalkylation of the methoxy group results in the hydroxyl metabolite M7 that also undergoes a glucuronidation to form M34. Dimethenamid was conjugated with glutathione resulting in the formation of the GSH-adduct M33 and the cysteine-adduct M25 that was formed after

a substitution of chlorine with GSH by consequent degradation of the tripeptide.
The *in vivo* situation in the rat is in analogy to the findings in this *in vitro*-study: dimethenamid was rapidly and extensively metabolised in rats. The key steps of the proposed metabolic pathway of BAS 656 H in rats occurred via glutathione conjugation, oxidation, hydroxylation, O-demethylation, reductive dechlorination, and cyclisation. With respect to these results it has to be concluded that the *in vitro* metabolism of dimethenamid in rat liver slices follows in principle the same metabolic pathways that could be observed in rats *in vivo*. As described before, it could be demonstrated that the main *in vitro* metabolite is identical to the *in vivo* metabolite M4. In the rat metabolism study this metabolite was identified to be the sulfoxide of dimethenamid. The *in vitro* metabolites M7 and M25 are also identified rat metabolites resulting from oxidative demethylation and glutathione conjugation, respectively. Due to the applied MS technique in the rat *in vivo* study it can be postulated that it was not possible to identify glucuronic acid *in vivo*-conjugates. This explains why all glucuronic acid conjugates in the present study were not described in the rat study. In any case, the glucuronidation of Phase I hydroxy metabolites is a well-known metabolic reaction and is a consequent conjugation following the key step of hydroxylation. The glucuronidation is, therefore, a part of the same metabolic pathway.

Table B.6.1-21: Turn-over of the metabolic reactions of dimethenamid-P and racemic dimethenamid[1]

Sample	**Turn-over (%)**	
	Dimethenamid-P	Racemic dimethenamid
1	76.00	88.22
2	52.27	87.66
3	55.37	92.99
4	59.00	96.50
5	70.50	67.42
6	53.40	90.20
7	72.99	87.11
8	46.03	56.75
9	76.54	88.06
10	70.30	88.87
Mean value	63.24 ± 11.21	84.38 ± 12.35

1 The turn-over was calculated as relative decrease of parent compound in the active incubation versus parent compound in the heat denaturated control incubation.

Metabolic pathway:
The main steps of the metabolic pathway of BAS 656 H in rat liver slices are glutathion conjugation, oxidation reactions of the dimethylthiophene system, demethylation of the methoxy group, oxidation of the sulfur atom to form a sulfoxide, and consequent glucuronidation reactions of the hydroxy metabolites to form glucuronic acid conjugates. In principle these are the same metabolic reactions that are observed and described for the *in vivo* situation in the rat. Therefore the applied *in vitro* system is an appropriate model to investigate possible differences in the metabolism of the enantiomers of BAS 656 H. A summary of the identified components is given in Table B.6.1-22.

Table B.6.1-22: Summary of identified metabolites rat liver slices

Percentage of metabolites expressed as relative area [%][1]					
Racemic dimethenamid			Dimethenamid-P		
Metabolite designation (New)	Mean value	Standard deviation	Metabolite designation (New)	Mean value	Standard deviation
N/A	9.9	1.8	N/A	8.5	2.0
M656H004	28.4	6.0	M656PH004	31.9	3.0
M656H025	8.8	1.0	M656PH025	9.3	1.0
M656H033	23.0	4.7	M656PH033	15.7	4.5
M656H035 (iso)	6.7	1.0	M656PH035 (iso)	6.7	1.7
M656H035 (iso)	15.6	1.4	M656PH035 (iso)	20.3	1.8
M656H036	7.5	1.6	M656PH036	7.8	2.8

[1] Calculated as the quotient of the peak area of the metabolite divided by the sum of peak areas of all regarded metabolites.

Conclusion:

The study is considered to be acceptable. Methods used did not allow distinguishing between chiral structures. However, the conclusion and the results can be used for the evaluation of the absorption, distribution, metabolism and excretion of dimethenamid-P.
In vitro incubations of racemic and dimethenamid-P in rat liver slices showed that the metabolism of the racemate and the S-isomer of dimethenamid is qualitatively and quantitatively comparable.

Data point: KCA 5.1

Report: [redacted] 2014b (ASB2014-8386)
Comparative *in-vitro*-metabolism with ^{14}C -BAS 656-PH
2013/1337274
unpublished

Guideline(s): None, for investigation purposes

Deviations: Not applicable
No sex differences, inter-individual variability and the stability of metabolites under the conditions of the study were investigated.

GLP: Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material:
Description: Dimethenamid (BAS 656 PH)
Batch # / purity: Radiolabelled (^{14}C):
Dimethenamid-P: 824-7101
>99.8 %
7.58 MBq/mg
Nonlabelled:
Dimethenamid-P: BEAU201204
Stability of test compound: Stable during testing
Vehicle and/or positive control:
Vehicle: hepatocytes

	Positive control: hepatocytes were incubated with ethoxy coumarin and testosterone instead of the active substance to validate the metabolic activity of the hepatocytes
Test animals:	Mammals
Species:	Dog, rat and human
Strain:	Female and male Sprague Dawley rats (SD), male and female Beagle Dog, human (Xenotech, Germany)

Study design and methods:
The study was carried out at the [redacted]
Test substance preparation:
The radiolabelled and non-radiolabelled test materials were prepared with the following specifications. The radiolabelled test item (solution in acetonitrile) was evaporated under nitrogen and taken up in an appropriate volume of DMSO. For this stock solution, a concentration of 0.236 mg/mL was determined. For the preparation of the stock solution of the unlabelled test item, 250 mg dimethenamid-P was dissolved in 20 mL acetonitrile to yield a concentration of 12.5 mg/mL.
The different application solutions were prepared by using specific amounts of unlabelled test item were concentrated to dryness, mixed with the desired amounts of radiolabelled test item and diluted with DMSO. The ratio of radiolabelled to unlabelled test item was approximately 8:92 for the application solutions for experiments with 10 µM dimethenamid-P, and approximately 0.8:99.2 for the application solutions for assays with 100 µM dimethenamid-P, respectively. The purity of each application solution (and the retention time of dimethenamid-P) was confirmed by HPLC analysis.
Hepatocytes:
Cryopreserved hepatocytes from dog, rat and human were stored in liquid nitrogen. On each incubation day, the cells were thawed according to a protocol provided by the supplier using appropriate kits. Aliquots of the resulting cell suspensions in hepatocyte incubation medium were diluted with phosphate-buffered saline (PBS), and the number of viable cells was measured using an automated cell counter. The cell suspensions were then adjusted to the desired cell density of 2×10^6 viable cells per mL with incubation medium. In the case of dog and rat hepatocytes, male and female cells were combined in this final step in a ratio of 1:1. The human hepatocytes were purchased as a mixture of male and female cells.
In vitro assays
On each incubation day, the application solutions in DMSO (mix of ^{14}C-labelled and unlabelled test item in the case of dimethenamid-P) were diluted with hepatocyte incubation medium by a factor of 100 to prepare the respective application media. The application media were incubated at a final concentration of approximately 10 µM with rat, dog or human hepatocytes. In the case of dimethenamid-P, additional incubations were performed at approximately 100 µM for high dose conditions.
Each sample comprised of 0.3 mL of application medium and 0.3 mL of hepatocyte cell suspension in one of the wells of a 24 well cell culture plate. The reactions were performed for 180 minutes at approximately 37 °C. In some cases, shorter incubations for 30 min or 60 min were also conducted. Incubation was terminated by pipetting the incubation mixture into a weighed tube containing cold ethanol and cell lysis was assisted by ultrasonication. In this stage, the samples were stored frozen prior to concentration.
In addition, two negative controls, two positive controls and a blank control (application medium with DMSO instead of test item) were performed for each species. Under these conditions no metabolisation should occur. For the “stability control”, the application medium was mixed only with incubation medium instead of cell suspension. For the “zero incubation control” (t=0 min), the reaction was stopped immediately after addition of the cell suspension. The stability control was only performed with the high dose of 100 µM and within the second experimental series with dog hepatocytes also with 10 µM.
In the positive controls, testosterone or ethoxycoumarin instead of the active substance was incubated with hepatocytes from the different species to validate the metabolic activity of the different hepatocytes.
In each experimental setup, the incubation of the substrates as well as all control assays was performed in triplicate.

Results:

Control Experiments:
The blank controls performed for each species without test item showed no significant amounts of radioactivity (LSC measurements), and no radioactive peaks were detected by HPLC analysis.
The triplicates of each negative control (stability control without cells and zero incubation control) were comparable and only showed unchanged dimethenamid-P. Hence, no metabolisation or degradation of dimethenamid-P occurred without hepatocytes.
The positive controls showed that the metabolic activity of the hepatocytes with respect to Phase I metabolic reactions was sufficiently high. The portions of metabolised testosterone reached values above 70 % of the radioactive residues recovered.
HPLC analysis of the positive controls with ethoxycoumarin revealed no unchanged ethoxycoumarin after incubation with dog and rat hepatocytes and portions of approximately 6 % to 8 % of the applied radioactivity (% AR) for ethoxycoumarin after incubation with human hepatocytes for 180 min. Low portions of 7-hydroxycoumarin were detected after incubation with dog and rat hepatocytes (2 % to 7 % AR). The portions of 7-hydroxycoumarin after incubation with human hepatocytes accounted for approximately 20 % to 23 % AR. All samples contained considerable portions of 7-hydroxycoumarin-β-D-glucuronide and 7-hydroxycoumarin sulphate which indicates that 7-hydroxycoumarin was formed as a relevant O-dealkylated intermediate in hepatocytes of each species tested. The formation of the conjugated metabolites 7-hydroxycoumarin-β-D-glucuronide and 7-hydroxycoumarin sulphate was a measure for the metabolic activity of the hepatocytes also with respect to Phase II reactions (conjugation). The concentrations of 7-hydroxycoumarin-β-D-glucuronide accounted for approximately 18 % to 37 % AR after incubation with dog hepatocytes, 29 % to 44 % AR after incubation with rat hepatocytes and 17 % to 23 % AR after incubation with human hepatocytes. The portions of 7-hydroxycoumarin sulphate (indicating sulphotransferase activity) amounted to approximately 24 % to 35 % AR in the case of dog, 24 % to 30 % AR in the case of rat and 2 % to 5 % AR in the case of human hepatocytes.
Viability of the Hepatocytes:
The hepatocyte suspensions were adjusted to a cell density of 2 x 10^6 viable cells per mL to achieve a final cell density of approximately 106 cells per mL in the incubation assays. After incubation for 180 min, the viability of the cells was determined using a luminescent cell viability assay.
The viability of the cells incubated with dimethenamid-P (measured in Relative Luminescence Units, RLU) was in the range of 77 % to 109 % of the viability of the cells incubated without test item.
Metabolites formed after incubation of dimethenamid-P with hepatocytes:
Since the first experiments with 10 µM dimethenamid-P and dog hepatocytes (series 1) and all incubations with rat hepatocytes showed complete conversion of the active substance to more polar biotransformation products within 180 min; additional incubations were performed with 10 µM dimethenamid-P and dog hepatocytes for 30, 60 and 180 min (series 2).
HPLC MS analysis of the samples after incubation with human hepatocytes allowed the assignment of four m/z-values to the relevant ^{14}C-peaks corresponding to the test item or its main conversion products.
After incubation with animal hepatocytes, dimethenamid-P was detected in all control samples with 0 min incubation and in the samples after incubation of 100 µM dimethenamid-P with dog hepatocytes for 180 min (19.96 % AR). In the other samples after incubation with animal hepatocytes, no dimethenamid-P was detected any more. The transformation of dimethenamid-P thus proceeded faster in animal hepatocytes.
Based on the retention time (RTHPLC) and the m/z-value of 276.082, the ^{14}C-peak at 31.1 min is assigned to dimethenamid-P. The respective peak representing the unchanged active substance is present in most of the samples after incubation with human hepatocytes (except for 10 µM dimethenamid-P, 180 min). The portion of dimethenamid-P in the concentrated supernatants continuously decreased from 81.27 % AR to 22.73 % AR after 60 min of incubation with 10 µM dimethenamid-P. At the high concentration of 100 µM dimethenamid-P, the portion of the active substance showed a slower decrease (Table B.6.1-22). This lower conversion rate at the high concentration possibly indicates saturation of the metabolic capacity.
The peak at a retention time of approximately 23 min with a corresponding m/z-value of 262.066 was only detected in human hepatocyte samples (10 µM and 100 µM dimethenamid-P).

Therefore, HPLC MS/MS spectra were evaluated for this peak which was identified as metabolite M656PH007 a derivative of the parent compound with the methyl ether cleft. This Phase I metabolite was also detected along with its glucuronic acid conjugate in a study on the metabolism of ^{14}C-dimethenamid-P in rats. HPLC MS/MS analysis revealed that this component eluted as a double peak which results from the occurrence of two rotamers with hindered rotation. Therefore the ARs are presented as a sum of both peaks. In the 10 µM incubation, the AR for M656PH007 decreased over time from 27.33 % AR to 11.98 % AR. However, in the 100 µM incubation, the AR for M656PH007 increased over time from 7.63 % to 25.30 %.
The peak at a retention time of approximately 18 min corresponds to the m/z-value of 438.098. Since this peak occurred in dog hepatocyte samples only in single replicates and in minor concentrations, HPLC MS/MS spectra were evaluated for this peak as well. The MS/MS data allowed the identification of metabolite M656PH034 (iso). As expected, since it is a conjugated form of M656PH007, HPLC MS/MS analysis revealed that this component also eluted as a double peak which results from the occurrence of two rotamers. It was detected after incubation of 10 µM dimethenamid-P with human hepatocytes for 180 min, as well as in dog hepatocytes. This M656PH034 was also detected in considerable concentrations in the study on the metabolism of ^{14}C-dimethenamid-P in rats [redacted], 2014, ASB2014-8383). M656PH034 was detected ranging from 9.03 % AR in dog hepatocytes at 10 µM to 22.99 % AR in human hepatocytes at 100 µM (see Table B.6.1-23). The decrease of the portion of M656PH007 after 180 min goes parallel to the concomitant occurrence of the M656PH034.
The peak at a retention time of approximately 10 min with a corresponding m/z-value of 292.077 was detected after incubation of 10 µM dimethenamid-P with human hepatocytes for 60 min, (12.13 % AR) for 180 min (20.72 % AR), after incubation of 100 µM dimethenamid-P with human hepatocytes for 180 min (21.56 % AR). With dog hepatocytes, it was detected after incubation of 100 µM dimethenamid-P for 180 min (15.63 % AR) and after incubation of 10 µM dimethenamid-P for 30 min (29.34 % AR) and for 60 min (19.98 % AR). Dogs are thus capable of forming the metabolite represented by the m/z-value of 292.077 as well.
A highly polar fraction with ^{14}C-peaks at retention times of 1.4 to 1.9 min occurred in many ^{14}C chromatograms and represented the only or predominant radiosignals observed after incubation with rat hepatocytes and after incubation of 10 µM dimethenamid-P with dog hepatocytes for 180 min. In the other samples after incubation with dog hepatocytes, these highly polar peaks belonged to the most abundant ^{14}C-peaks. In the experiments with 10 µM dimethenamid-P and human hepatocytes, a highly polar fraction was detected after incubation times of 60 and 180 min. HPLC MS analysis yielded no significant ions corresponding to these highly polar ^{14}C-peaks. Since the portions of those highly polar fractions are higher in the animal samples compared to the human hepatocyte samples, these results are sufficiently covered by the incubations with animal hepatocytes (see Table B.6.1-23).
Nevertheless, an additional HPLC method was applied for further investigation of this highly polar fraction. The HILIC chromatograms of the concentrated supernatants of the terminated incubation mixtures with human hepatocytes (10 µM, 180 min) show that the highly polar fraction was separated into several peaks with retention times (RTHILIC) of 8 to 10 min (each below 10 % AR). The highly polar fraction in the rat samples was separated into five to eight peaks with retention times of 8 to 21 min (RTHILIC, each below 21 % AR).
A minor ^{14}C-peak at approximately 35 min (RTHPLC) was only detected in one single replicate of the incubation of 100 µM dimethenamid-P with human hepatocytes for 180 min and not detected again in the ^{14}C-chromatogram of the HPLC MS analysis of the same sample. This minor peak was therefore considered not relevant.

Table B.6.1-23: Comparison of metabolites of dimethenamid-P (10 µM or 100 µM) formed with human hepatocytes and formed with rat and dog hepatocytes

RT_{HPLC} [min]	***m/z***	**Human Mean % AR**		**Rat Mean % AR**		**Dog (Series 1) Mean % AR**		**Dog (Series 2) Mean % AR**
		10 µM	100 µM	10 µM	100 µM	10 µM	100 µM	10 µM
0 min (Control)								
31.1	276.082	81.27	84.21	79.86	79.31	81.59	76.12	76.01
Incubation time: 30 min								
1.4		-	-	Not applied		Not applied		59.49
9.5	292.077	-	-					12.05*
9.9	292.077	-	-					29.34*
23.6	262.066	22.97	-					-
23.9	262.066	13.06*	7.63*					-
*23 min (sum)***		*27.33***	*7.63**					-
31.1	276.082	53.35	83.89					-
Incubation time: 60 min								
1.6		13.48	-	Not applied		Not applied		60.87
1.8		-	-					17.58*
10.0	292.077	12.13 *	-					19.98*
18.2	438.098	-	-					9.03*
23.5	262.066	21.37	-					-
23.7	262.066	15.26	12.18*					-
*23 min (sum)***		*36.63*	*12.18**					-
31.1	276.082	22.73	80.70					-
Incubation time: 180 min								
1.5		26.53	-	75.13	82.09	78.68	23.09	72.36
1.8		-	10.41*	-	-	-	-	11.90*
10.0	292.077	20.72	21.56*	-	-	-	15.63	-
18.6	438.098	22.99	-	-	-	-	6.71*	-
23.3	262.066	-	18.34*	-	-	-	-	-
23.6	262.066	11.98*	16.13*	-	-	-	-	-
*23 min (sum)***		11.98*	25.30**	-	-	-	-	-
31.1	276.082	-	54.86	-	-	-	19.96	-

* In the case of components which were only detected in one or two of the three replicate samples, the mean value of those samples in which the peak was detected is given to indicate a worst case situation; therefore, the sum of the components may exceed 100 % of the applied radioactivity

** For the double peak at approximately 23 min representing rotamers of M656PH007 (*m/z* 262.066), a composite value (sum) is given in addition; if a double peak was not detected in each of the three replicate samples, the sum was calculated for the respective replicate prior to calculating the mean value of those samples in which the peak was detected.

[1] Calculated as the quotient of the peak area of the metabolite divided by the sum of peak areas of all regarded metabolites.

Conclusion:

In summary the present study shows that ^{14}C-dimethenamid-P (Reg. No. 363581), which is the pure S-enantiomeric form of BAS 656 H, is extensively metabolised by hepatocytes from dogs, rats and humans under the investigated conditions.
Four ^{14}C-peaks and a highly polar fraction are present in human hepatocyte samples at a level above 5 % AR (based on average of triplicates). For these four peaks, a retention time and m/z value could be assigned. Three of these peaks with an assigned m/z value, including the peak representing the unchanged active substance dimethenamid-P, also appear after incubation with animal hepatocytes, particularly in dog hepatocyte samples.
One of these components was determined as M656PH007, m/z-value of 262.066, and is a derivative of the parent compound with the methyl ether cleft. This Phase I metabolite was also detected in a study on the metabolism of ^{14}C-dimethenamid-p in rats.
Another of these components was determined as M656PH034, m/z value of 438.098, is the glucuronic acid conjugate of metabolite M656PH007. Both metabolites M656PH007 and M656PH034 (iso), representing phase I and phase II of metabolic transformation, were also detected in a study on the metabolism of ^{14}C-dimethenamid-P in rats. As M656PH007 and M656PH034 are rotamers, they represented double peaks in the chromatograms.
No significant ions were detected by HPLC MS analysis of the highly polar ^{14}C-fraction. These peaks were therefore further characterised using an additional HPLC method to separate the highly polar fraction in the samples of human hepatocytes into several peaks (each below 10 % AR). Since the portion of this highly polar fraction is higher in the animal samples compared to the human hepatocyte samples, these results are sufficiently covered by the incubations with animal hepatocytes.
Thus, all metabolites detected after incubation with human hepatocytes were also present in animal hepatocyte samples, except for the metabolite M656PH007 which has already been described in the *in vivo* rat metabolism study with dimethenamid-P.
The study is considered to be acceptable. The conclusion and the results can be used for the evaluation of the *in vitro* metabolism of dimethenamid-P in cultured hepatocytes.

B.6.2 Acute toxicity

B.6.2.1 Oral

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.2.1
Report:	[redacted] 1996 (TOX1999-413) Acute oral toxicity study with SAN 1289 H technical in rats. [redacted] unpublished, 17 July 1996, BASF RegDoc.#96/11087 (Experimental work from 22 February 1996 to 15 March 1996)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 81-1 (November 1984)
Deviations:	Deviations from OECD Guideline No. 401 (adopted 24.02.1987): Due to technician error, terminal body weight for three animals found dead in the 500 mg/kg dose group were not recorded.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material: Dimethenamid-P; SAN 1289 H Technical; batch No. 6663-50-1; purity: 96.3 % dimethenamid (91.1 % S-dimethenamid)

Test Animals: [Crl: CD®(SD)BR] rats; weights (Day 0): males 355 g (315-390 g), females 246 g (227-277 g), Source: Charles River Laboratories, Kingston, New York

The undiluted test material was administered to groups of ten fasted CD rats (5/sex) by single oral gavage at dose levels of 350, 400 or 500 mg/kg bw. The dosing volume was 0.29, 0.34 or 0.42 mL/kg at 350, 400 or 500 mg/kg, respectively. The animals were observed for 14 d after dosing. Body weights were determined pretest, on the day of dosing, and weekly thereafter. All animals received a gross necropsy.

Results:

Clinical observations observed in all animals on the day of dosing consisted of lacrimation and excessive salivation. All high-dose animals exhibited decreased activity. Signs seen on the day after dosing included: yellow ano-genital staining, black and/or brown staining on the snout, oral area, buccal area and/or extremities, lethargy, decreased food consumption, and decreased faecal volume or no stool. All surviving animals were free of clinical signs by Day 5 after dosing. Overall, the animals gained body weight as expected for their age. Macroscopic changes were observed only in animals found dead during the study, and consisted of red thymic region, fluid in thoracic cavity, red lungs, black mucosa or brown fluid in stomach and red testes. For animals killed at the end of the post-treatment observation period, no evidence of test article-related gross organ lesions were found.

Table B.6.2-1: Results of acute oral testing in rats

Mortality	Dose level [mg/kg bw]			LD_{50} (95 % C.I.)*
	350	400	500	[mg/kg bw]
Males	0/5	1/5	5/5	429 (398-463)
Females	0/5	0/5	2/5	531 (453-622)
Combined Sexes	0/10	1/10	7/10	466 (435-500)

* Calculation performed according to Litchfield and Wilcoxon, 1949

Conclusion

The test material was harmful by oral administration to rats and has to be classified accordingly.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point: KCA 5.2.1

Report: [redacted] 1991 (TOX1999-414)
Acute oral toxicity study of SAN 582H technical (K/E) in rats
[redacted]
unpublished, 19 June 1991, BASF RegDoc.# 91/11940
(Experimental work from 27 March 1991 – April 10 1991)

Guideline(s): U.S. EPA FIFRA, Subdivision F, Para. 81-1 (November 1984)

Deviations: Deviations from OECD Guideline No. 401 (adopted 24.02.1987): None that were considered to have compromised the validity of the study.

GLP: Yes (laboratory certified by United States EPA Code of Federal

Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material: Racemic dimethenamid; SAN 282 H Technical, batch no. 9021, purity 97.1 %
Test Animals: CD® (Sprague-Dawley derived) rats; age: 9 - 12 week at study initiation, body weights: males 308 - 360 g, females 249 - 270 g), Source: Charles River Labora tories, Kingston, New York

The acute oral toxicity of racemic-dimethenamid was investigated using Charles River Sprague-Dawley rats. Five rats per sex and dose received the test substance undiluted at dose levels of 150, 300, and 600 mg/kg bw. The animals were observed for 14 d following the single administration. Body weight was recorded prior to the study and on Days 7 and 14. Clinical observations were recorded 1, 2 and 4 h after dosing, and daily thereafter for 14 d. A check for moribund or dead animals was performed twice daily. A gross necropsy was performed on all animals.

Results:

Dose levels, mortality and estimated LD_{50} with 95 % confidence limits are summarised in Table B.6.2-2.

Table B.6.2-2: Racemic dimethenamid – Results of acute oral testing in rats

Mortality	Dose level [mg/kg bw]			LD_{50} (95 % C.I.)*
	150	300	600	[mg/kg bw]
Males	0/5	1/5	5/5	371 (275 – 502)
Females	0/5	0/5	4/5	427 (300 – 609)
Combined Sexes	0/10	1/10	9/10	397 (320 – 492)

* Calculation performed according to Litchfield and Wilcoxon, 1949

At the upper dose of 600 mg/kg, all male animals died and 4/5 females died within 1 d following treatment, and at 300 mg/kg bw, 1/5 males died at Day 2. No mortality occurred at 150 mg/kg.
Clinical observations on the day of dosing included oral and ocular discharges and hypoactivity in all three dose groups, and in addition at the mid and high doses, some animals had nasal discharge, wet rales, faecal staining, soft stool and abdominal griping. Signs seen only at the high dose were irregular gait, coarse and fine tremors, hypopnea, irregular breathing, urinary staining and prostration. Signs seen after the day of dosing included hypoactivity and decreased food consumption, and in 1 low dose animal a red ocular discharge. All symptoms in surviving rats were reversible by Day 4. Most surviving animals gained weight by Day 7, and all animals gained weight by Day 14 after dosing.
In animals that died, macroscopic pathology observed included primarily changes in the gastrointestinal tract. In the stomach and intestine, discoloration or thickening of walls, and the presence of red fluid indicated suggested an irritating effect of the test substance. In one rat apparent residual test substance was found. In addition, some animals that died at the mid and high doses had discolored lungs and/or lungs with red foci. No macroscopic findings were noted in animals sacrificed at study termination that could be related to treatment.

Conclusion:

The oral LD_{50} for racemic dimethenamid in Sprague-Dawley rats was calculated to be 371 mg/kg bw in males, 427 mg/kg bw in females and 397 mg/kg bw for the combined sexes.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

B.6.2.2 Dermal

Data point:	KCA 5.2.2
Report:	[redacted] 1996 (TOX1999-451) Acute dermal toxicity study with SAN 1289 H technical in rabbits. [redacted] unpublished, 17 July 1996, BASF RegDoc.#96/5395 (Experimental work from February 1996 – March 1996)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para 81-2 (November 1984)
Deviations:	Deviations from OECD Guideline No. 402 (adopted 24.02.1987): None that were considered to have compromised the validity of the study.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid-P; SAN 1289 H Technical; batch No. 6663-50-1; purity: 96.3 % dimethenamid (91.1 % S-dimethenamid)
Test Animals:	New Zealand White, Hra: (NZW)SPF; weights (Day 0): males: 2.2-2.4 kg, females: 2.3-2.5 kg, Source: HRP, Inc. Denver, Pennsylvania

In a limit test, the test material was applied undiluted for 24 h to the clipped epidermis (dorsal trunk surface) of 5 male and 5 female rabbits at a dose of 2000 mg/kg bw. The application site was then covered by a gauze dressing which in turn was covered with a piece of impervious plastic. Following the exposure period, the wrappings were removed and the test site wiped free of excess material with 0.9 % saline. The exposure surface was approx. 12 x 14 cm² which is at least 10 % of the body area. The animals were observed for 14 d after dosing. Body weights were determined on the day before testing, on the day of dosing and on Days 8 and 15 after dosing. A gross necropsy was performed on all animals.

Results:

All animals survived throughout the study and were free of pharmacological and toxicological signs. 4 of 10 animals exhibited slight body weight losses by Day 8, but gained weight thereafter. The remaining 6 animals exhibited body weight gains or no weight change during the study. There were no internal findings at necropsy. 3 of 10 animals exhibited a red subcutaneous discoloration/foci at the dose site.

Conclusion:

The dermal LD_{50} of the test article in rabbits is greater than 2000 mg/kg bw under the conditions of this study.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.2.2
Report:	[redacted] 1991 (TOX1999-452) Acute dermal toxicity study of SAN 582 H technical (K/E) in rabbits. [redacted] unpublished, 19 June 1991, BASF RegDoc.# 91/11942 (Experimental work from 21 March 1991 – 4 April 1991)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para 81-2 (November 1984)
Deviations:	Deviations from OECD Guideline No. 402 (adopted 24.02.1987 None that were considered to have compromised the validity of the study.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid; SAN 282 H Technical, batch no. 9021, purity 97.1 %
Test Animals:	New Zealand White; age: at least 8 week old at study initiation, body weights (Day-1): males: 2.2-2.4 kg, females: 2.2-2.5 kg, Source: Haz leton Res. Animals, Inc. Denver, Pennsylvania, U.S.A.

The acute dermal toxicity of racemic dimethenamid was investigated using New Zealand white rabbits 5 rabbits per sex were treated dermally with undiluted test substance at a single dose level of 2000 mg/kg bw (limit test).

Results:

All animals survived throughout the observation period. There were no clinical observations and no gross necropsy findings considered related to treatment. No severe dermal effects were observed.

Conclusion:

The dermal LD_{50} in rabbits for racemic dimethenamid was determined to be >2000 mg/kg bw under the conditions of this study.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

B.6.2.3 Inhalation

Data point:	KCA 5.2.3
Report:	[redacted] 1996 (TOX1999-415) An acute (4-hour) inhalation toxicity study of SAN 1289 H technical in the rat via nose-only exposure. [redacted] unpublished, 28 June 1996, BASF RegDoc.# 96/5397 (Experimental work from March 1996 – April 1996)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para 81-3 (November 1984)
Deviations:	Deviations from OECD Guideline No. 403 (adopted 12.05.1981): With 2.2 mg/L, the inhalative exposure concentration tested was below the concentration of 5 mg/L required for limit tests.

	According to EPA Guidelines, the exposure concentration required for a limit test amounts to > 2 mg/L. This limit differs from the respective OECD and EU requirement (> 5 mg/L).
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid-P; SAN 1289 H Technical; batch No. 6663-50-1; purity: 96.3 % active substance
Test Animals:	[Crl: CD®(SD)BR] rats; weights (Day 0): males 325 g (311 - 332 g), females 239 g (233 - 246 g), Source: Charles River Laboratories, King ston, New York

The acute inhalation toxicity of dimethenamid-P was determined in Sprague-Dawley rats in a limit test. A single group of five male and five female rats were exposed to the test material (undiluted) as a liquid aerosol for 4 h using a head-nose inhalation system. The target atmospheric concentration was >2.0 mg/L. Actual chamber concentrations were determined every hour during the exposure using a gas chromatographic method. Particle size was also determined once each hour, and the temperature and relative humidity were recorded every half hour following treatment. The animals were observed for 14 d after the exposure. Body weights were determined prior to the beginning of the test and on Days 7 and 14 after exposure. All animals were sacrificed at Day 15 and a gross necropsy was performed.

Results:

The average concentration (measured by gravimetric and GC analysis) was 2.2 mg/L, while the nominal concentration amounted to 9.3 mg/L. The mass median aerodynamic diameter (MMAD) was determined to be 3.4 µm with a geometric standard deviation (GSD) of 2.0. Approx. 4 % of the aerosol particles were ≤1.0 µm in size, approx. 59 % were ≤4.0 µm and approx. 94 % were ≤10.0 µm in size.
All animals survived through the observation period. Laboured breathing was noted in the last 2 h of the exposure for 2 of the ten animals. Signs exhibited upon removal from the chamber and during the first 2 h after exposure included secretory (lacrimation, chromodacryorrhea, red and clear nasal discharge and dried red facial material) and respiratory responses. Similar signs were observed in some animals for up to 2 d after exposure. No clinical signs were observed after Day 2. Body weights increased normally and no abnormalities were noted at necropsy.
Note:
With 2.2 mg/L, the inhalative exposure concentration tested was below the concentration of 5 mg/L required in OECD Guideline No. 403 for limit tests. However, at 2.2 mg/L no mortality and only transient clinical signs clearly indicated low inhalation toxicity. On these grounds, and because the level tested were well above predicted human exposure levels, the RMS considered the study was acceptable for risk assessment purposes.

Conclusion:

Under the conditions of this study the inhalation LC_{50} (4 h) for dimethenamid-P was determined to be >2.2 mg/L for male and female Sprague-Dawley rats. Taking into consideration the test results obtained with racemic dimethenamid, labelling of dimethenamid-P is not considered to be required.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.2.3
Report:	[redacted] 1986 (TOX1999-453) 4-hour acute inhalation toxicity study with SAN 582 H in rats [redacted] unpublished, 19 September 1986, BASF RegDoc.# 86/11166 (Experimental work from 5 September 1986 – 19 September 1986)
Guideline(s):	OECD Guideline No. 403 (adopted 12 May, 1981)
Deviations:	Deviations from OECD Guideline No. 403 (adopted 12.05.1981): low proportion of inhalable particles in the exposure atmosphere.
GLP:	Yes (laboratory certified by Eidgenössisches Departement des Innern, Berne, Switzerland)
Acceptability:	The study is considered to be supplementary.

Materials and methods:

Test Material:	Racemic dimethenamid; SAN 582 H Technical; batch no. 8605; purity: 91.43 %
Test Animals:	KFM-HAN. Wistar rat (outbred, SPF-quality); age at start of exposure 12 - 14 week, body weights: males 259 - 286 g, females 215 - 243 g, Source: Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland

The acute inhalation toxicity of racemic dimethenamid was evaluated using five Wistar rats per sex in a nose-only inhalation system. The animals were exposed to a liquid aerosol of the test substance for four hours at a gravimetrically determined concentration of 4.99 mg/L of air, which represented the maximum attainable concentration under the exposure conditions. Particle size distribution was determined twice during the exposure and the exposure concentration measured gravimetrically five times during the exposure. The animals were monitored for mortality and clinical signs four times during the first day and daily thereafter. Body weights were recorded prior to exposure and on Days 8 and 15. A gross necropsy was performed on all animals.

Results:

Particle size distribution measurements showed that only 17.5 % of the exposed particles were within an inhalable range of 0.4 - 5.8 µm. The highest percentage was found in the size range of 9 - 10 µm (78.7 %); approx. 6 % of the aerosol particles had a size of less than 1 µm. All animals survived the exposure and the 14 d observation period. Clinical signs observed were sedation, dyspnoea, curved body position and ruffled fur during the exposure in several animals and through Day 4 with one animal. All animals showed generally normal body weight gain throughout the study period. No macroscopic pathology findings related to the test substance were noted at sacrifice.

Conclusion:

The acute inhalation LC_{50} (4 h) for racemic dimethenamid in Wistar rats was determined to be >4.99 mg/L air (maximum attainable concentration).

Re-evaluation by the RMS (2015):

The study is still considered to be supplementary due to the low proportion of inhalable particles in the exposure atmosphere.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.2.3
Report:	[redacted] 2012a (ASB2014-8387) BAS 656-P H - Acute inhalation toxicity (nose only) study in the rat 2011/1171036 [redacted] Dates of experimental work: 04-Jan-2012 - 28-Feb-2012
Guideline(s):	OECD 403 (2009), EPA 870.1300, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.2
Deviations:	Deviations from OECD Guideline No. 403: None that were considered to have compromised the validity of the study.
GLP:	Yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	BAS 656-PH
Description:	Brown, liquid
Lot/Batch #:	COD-001509
Purity/content:	95.9 % (tolerance ± 1.0 %)
Stability of test compound:	Stable (Expiry date: 01-Oct-2013)
Vehicle:	Test substance was applied unchanged.
Test animals:	
Species:	Rat
Strain:	Wistar / RccHanTM:WIST
Sex:	male and female
Age:	approx. 8 - 12 weeks
Weight at dosing (mean):	Males: 249 - 278 g; females: 223 - 244 g
Source:	Harlan Laboratories UK Ltd, Oxon, UK
Acclimation period:	at least 5 days
Diet:	Harlan 2014C Rodent Diet, Harlan Laboratories UK Ltd, Oxon, UK, *ad libitum*
Water:	Drinking water, *ad libitum*
Housing:	In groups of five by sex in solid-floor polypropylene cages with stainless steel lids, furnished with softwood flakes.
Environmental conditions:	
Temperature:	19 - 25 °C
Humidity:	30 - 70 %
Air changes:	at least 15/hour
Photo period:	Alternating 12-hour light and dark cycles

Animal assignment and treatment:
For determination of the acute inhalation toxicity (nose-only inhalation, 4-hour-exposure) groups of five male and five female rats were exposed to 5.16 mg/L of the test substance dimethenamid-P that was applied as an aerosol. After exposure, animals were observed for at least 14 days. Individual body weights were recorded on arrival, shortly before exposure (day 0) and on days 1, 3, 7 and 14. Detailed clinical observations were recorded for each animal separately hourly during exposure and immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for fourteen days. At the end of the fourteen day observation period all animals were killed by intravenous overdose of sodium pentobarbitone. All animals were

subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.
Statistics/calculations:
No statistics were performed. The quantity of the test item collected by the filter was calculated as follows:

$$TI = \frac{A_{spl} \times N_{std} \times D_{spl}}{A_{std}}$$

Where: TI = amount of test item collected by the filter (mg), Aspl = mean peak area for sample solution, Astd = mean peak area of standard solution, corrected to nominal standard concentration, Nstd = nominal standard concentration (mg/mL), Dspl = dilution factor for sample solution.
The concentration of the test item in the atmosphere was calculated as follows:

$$C_{atm} = \frac{TI_{ret}}{V}$$

Where: Catm = concentration of the test item in test atmosphere (mg/L), TIret = amount of test item collected by the filter (mg), V = volume of test atmosphere sampled through the filter (L)
Generation of the test atmosphere and exposure:
The test item was aerosolised using a glass concentric jet nebuliser (Radleys, Saffron Waiden, Essex, UK) located at the top of the exposure chamber. The nebuliser was connected to a glass syringe attached to an infusion pump, which provided a continuous supply of test item formulation under pressure, and to a metered compressed air supply. Compressed air was supplied by means of an oil free compressor and passed through a water trap and respiratory quality filters before it was introduced to the nebuliser. The cylindrical exposure chamber had a volume of approximately 30 litres (dimensions: 28 cm diameter x 50 cm high). The concentration within the exposure chamber was controlled by adjusting the rate of the infusion pump. The extract from the exposure chamber passed through a 'scrubber' trap and was connected with a high efficiency filter to a metered exhaust system. The chamber was maintained under negative pressure. Homogeneity of the test atmosphere within the chamber was not specifically determined during the study.
Prior to the day of exposure each rat was acclimatised (for approximately 2 hours) to a tapered polycarbonate restraining tube. During the day of exposure, each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber and sealed by means of a rubber 'O' ring to achieve nose-only conditions for each animal to the test atmosphere during the exposure period of four hours. A target concentration of 5.0 mg/L was used for the exposure. As the mean achieved (analytical) concentration was 103 % of target and no deaths occurred, no further levels were required.
Analytical investigation:
The test atmosphere was sampled nine times during the exposure period. The sampling procedure involved two litres of test atmosphere being drawn through a glass fibre filter. The actual chamber concentration was also measured gravimetrically seventeen times during the exposure period.
Each filter was weighed before and after sampling in order to calculate the weight of collected test item. The difference in the two weights, divided by the volume of atmosphere sampled, gave the actual chamber concentration. The nominal chamber concentration was calculated by dividing the mass of test item used by the total volume of air passed through the chamber.
Particle Size Analysis:
The particle size of the generated atmosphere inside the exposure chamber was determined three times during the exposure period using a Marple Personal Cascade lmpactor (Westech IS Ltd, Beds., UK). This device consisted of six impactor stages (8.8, 5.8, 3.6, 1.9, 0.79 and 0.33 µm cut points) with stainless steel collection substrates and a backup glass fibre filter, housed in an aluminium sampler. The sampler was temporarily sealed in a sampling port in the animals' breathing zone and 0.67 L of exposure chamber air was drawn through it using a vacuum pump set at a flow rate of 2 L/min. The collection substrates and backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by difference. The mean amount for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 8.8, 5.8, 3.6, 1.9, 0.79 and 0.33 µm was calculated. The resulting values were

converted to probits and plotted against Log10 cut-point size. From this plot, the Mass Median Aerodynamic Diameter (MMAD) was determined (as the 50 % point) and the geometric standard deviation was calculated. In addition the proportion (%) of aerosol less than 4 µm (considered to be the inhalable fraction) was determined.

Results:

No lethality occurred at the tested concentration of 5.16 mg/L during the study period of 14 days. Therefore, the study satisfies the criteria of a limit test.
Based on the absence of mortality the following LC_{50} value was determined:

LC_{50} (male and female rats): >5.16 mg/L

Wet fur, hunched posture, piloerection and increased respiratory rate was noted in all animals during exposure, on removal from the chamber and one hour post-exposure. One day after exposure, all animals exhibited increased respiratory rate and hunched posture, two male and two female animals also exhibited piloerection. These observations were considered to be associated with the restraint procedure and not indicative of toxicity. All animals recovered quickly to appear normal on day 4 post-exposure. The nature and duration of the observations are indicated in Table B.6.2-3.

Table B.6.2-3: Nature and duration of clinical signs observed in rats exposed for 4 hours to dimethenamid-P as an aerosol

Test group 1 (5.16 mg/L)	Males	Females
Wet fur	h0-h1	h0-h1
Hunched posture	h4-d2	h4-d2
Piloerection	h4-d1	h4-d1
Increased respiratory rate	h3-d3	d3-d3

hn: hour n of exposure; d0: post-exposure on the day of exposure; dn: day n after exposure

All males and four female animals exhibited slight body weight losses on the first day post-exposure. Reasonable body weight development was noted in all animals during the remainder of the recovery period, with the exception of one female animal which exhibited a slight body weight loss from days 3 to 7 post-exposure.
No gross pathological abnormalities were detected in all animals during necropsy at termination of the study.
The exposure conditions are summarised in Table B.6.2-4.

Table B.6.2-4: Exposure conditions

Air flow (L/min)	Temperature (°C)	Relative humidity (%)	Oxygen concentration (%)
40	19-20	73-86	20.8

Test atmosphere concentrations are presented in Table B.6.2-5.

Table B.6.2-5: Atmosphere concentrations

Mean achieved (mg/L)	Standard deviation	Nominal (mg/L)
5.16	0.25	34.7

The measurements of particle-size distribution revealed mass median aerodynamic diameters (MMAD) of 3.59 µm with a geometric standard deviation of 2.39 respectively (see Table B.6.2-6).

Table B.6.2-6: Particle size distribution

Mean achieved (analytical) atmosphere concentration (mg/L)	Mean mass median aerodynamic diameter (µm)	Inhalable fraction (% <4 µm)	Standard deviation
5.16	3.59	54.9	2.39

Conclusion:

The study is considered to be acceptable.
No death occurred in a group of 10 rats exposed to a mean analytical atmosphere concentration of 5.16 mg/L (4 h). In conclusion, the 4 hour inhalation LC_{50} of dimethenamid-P for male and female rats is estimated to be >5.16 mg/L.

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

B.6.2.4 Skin irritation

Data point: KCA 5.2.4

Report: [redacted] 1996 (TOX1999-416)
Primary dermal irritation study with SAN 1289 H technical in rabbits.
[redacted]
unpublished, 17 July 1996, BASF Reg.Doc.#96/5406
(Experimental work in February 1996)

Guideline(s): U.S. EPA FIFRA, Subdivision F, Para. 81-5 (November 1984)

Deviations: Deviations from OECD Guideline No. 404 (adopted 17.07.1992):
None that were considered to have compromised the validity of the study.

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material: Dimethenamid-P; SAN 1289 H Technical; batch No. 6663-50-1; purity: 96.3 % dimethenamid (91.1 % S-dimethenamid)

Test Animals: New Zealand White, Hra: (NZW)SPF; weights: 2.0-2.4 kg, Source: HRP, Inc. Denver, Pennsylvania

A single group of 6 New Zealand White rabbits (3 males and 3 females) were treated topically with approx. 0.5 mL of the undiluted test substance. The application site (6 cm^2) was covered with a gauze patch and secured with tape (semiocclusive dressing). Elizabethan collars were used to prevent disruption of the wrappings and ingestion of the test material. After a 4-h exposure period, the application sites were gently wiped free of excess test material with 0.9 % saline and gauze. The skin irritation was scored at 30 minutes and 24, 48 and 72 h after removal of the test material.

Results:

Results from the individual animals are presented in Table B.6.2-7.

Table B.6.2-7: Dimethenamid-P – Skin irritation values (erythema/oedema)

Animal	**Time after Patch Removal**				**Mean Scores (24h–48h–72h)**
Number	30 min	24 h	48 h	72 h	
1	0/0	1/0	1/0	0/0	0.7/0.0
2	1/0	1/0	1/0	0/0	0.7/0.0
3	1/0	2/0	2/0	0/0	1.3/0.0
4	0/0	0/0	0/0	0/0	0.0/0.0
5	1/0	2/0	1/0	0/0	1.0/0.0
6	1/0	2/0	1/0	0/0	1.0/0.0

Three animals exhibited slight erythema with no oedema and 2 animals exhibited very slight (barely perceptible) erythema with no oedema. These animals were free of all dermal irritation by 72 h after test material removal.

Conclusion:

Under the conditions of this study, the test material is a slight irritant to rabbit skin following a single 4-h application. The mean erythema and oedema scores over the first three days were calculated to be 0.8 and 0.0, respectively. Thus, according to EU criteria, classification and labelling is not required.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point: KCA 5.2.4

Report: [redacted]., 1988 (TOX1999-454)
Primary dermal irritation study in rabbits with SAN 582 H technical.
[redacted]
unpublished, 30 November 1988, BASF Reg.Doc.# 88/11363
(Experimental work in July 1988)

Guideline(s): U.S. EPA FIFRA, Subdivision F, Para. 81-5 (November 1984)

Deviations: Deviations from OECD Guideline No. 404 (adopted 17.07.1992): None that were considered to have compromised the validity of the study.

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material: Racemic dimethenamid; SAN 582 H Technical, batch no. 8605, purity 91.4 %

Test Animals: New Zealand White Rabbits – Hra: (NZW) SPF, Source: Hazleton Res. Products, Inc., Denver, Pennsylvania, U.S.A.

The skin irritation potential of racemic dimethenamid was determined using 6 male New Zealand White rabbits. The hair on the back was closely clipped, and 0.5 mL of the test material applied undiluted beneath a gauze square and covered with a semi-occlusive dressing for four hours. Following exposure, the test sites were wiped free of excess test material with water and gauze. Observations for dermal irritation were made at 30 to 60 min and 24, 48 and 72 h after removal of the

patches.

Results:

Very slight erythema was observed in 5 of 6 rabbits at the 30 - 60 minute period, and very slight oedema occurred in one animal during the same period (see Table B.6.2-8). All animals were free of dermal irritation by 24 h post treatment. The mean erythema and oedema scores over the first 3 days and all animals were calculated to be 0.2 and 0.0, respectively.

Table B.6.2-8: Racemic dimethenamid – Skin irritation values (erythema/oedema)

Animal	Time after Patch Removal				Mean
Number	30 – 60 min	24 h	48 h	72 h	Scores
E47095	1/0	0/0	0/0	0/0	0.25 / 0.0
E47096	1/0	0/0	0/0	0/0	0.25 / 0.0
E47098	0/0	0/0	0/0	0/0	0.0 / 0.0
E47099	1/0	0/0	0/0	0/0	0.25 / 0.0
E47100	1/0	0/0	0/0	0/0	0.25 / 0.0
E47101	1/0	0/0	0/0	0/0	0.25 / 0.0

Conclusion:

Racemic dimethenamid produced mild transient dermal irritation that cleared within 24 h. According to EU criteria, classification and labelling would not be required.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

B.6.2.5 Eye irritation

Data point: KCA 5.2.5

Report: [redacted] 1996 (TOX1999-417)
Primary eye irritation study with SAN 1289 H technical in rabbits
[redacted]
unpublished, 17 July 1996, BASF RegDoc.#96/5396
(Experimental work in February 1996)

Guideline(s): U.S. EPA FIFRA, Subdivision F, Para. 81-4 (November 1984)

Deviations: Deviations from OECD Guideline No. 405 (adopted 24.02.1987): None that were considered to have compromised the validity of the study.

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material: S-dimethenamid Technical (SAN 1289 H Technical); batch No. 6663-50-1; purity: 96.3 % dimethenamid (91.1 % S-dimethenamid), pH: 4.2 (dimethenamid in aqueous suspension)

Test Animals: New Zealand White, Hra: (NZW)SPF; weights: 2.0 - 2.3 kg, Source:

HRP, Inc. Denver, Pennsylvania

Dimethenamid-P was tested for its eye irritating potential in 6 New Zealand White rabbits. A volume of 0.1 mL of the undiluted test substance was applied once to one eye of each rabbit. Examination and scoring of the eyes were carried out 1, 24, 48 and 72 h after the application of the test substance. Starting with the 24-h observation, fluorescein dye was used at each examination time-point to confirm the presence or absence of corneal ulceration. At the time the fluorescein dye was rinsed from the eyes during the 24-h examination, attempts were made to remove any residual test material from the eye.

Results:

The following eye irritation values were obtained over the 72 h observation Table B.6.2-9.

Table B.6.2-9: Individual eye irritation scores and means

Eye effect	Corneal Opacity				Iris				Conjunctival Redness				Conjunctival Swelling			
Examination (h)	1	24	48	72	1	24	48	72	1	24	48	72	1	24	48	72
Animal No.																
4076 (female)	0	0	0	0	0	0	0	0	1*	0	0	0	1	0	0	0
4082 (female)	0	0	0	0	0	0	0	0	1*	0	0	0	1	0	0	0
4084 (female)	0	0	0	0	0	0	0	0	1**	1	0	0	1	0	0	0
4086 (female)	0	0	0	0	0	0	0	0	1*	0	0	0	0	0	0	0
4083 (male)	0	0	0	0	0	0	0	0	1**	1	0	0	0	0	0	0
4085 (male)	0	0	0	0	0	0	0	0	1**	0	0	0	0	0	0	0
Mean Score		0								0.11				0		

*Discharge (moderate) **Discharge (severe)

All 6 rabbits exhibited slight conjunctival redness and/or chemosis and moderate to severe conjunctival discharge at 1 h after exposure. The discharge and chemosis were not observed at 24 h after treatment. Four animals were free of conjunctival redness by 24 h and the remaining 2 animals were free by 48 h. There were no corneal or iridial effects observed.

Conclusion:

The test substance is not considered to have produced eye irritation according to EU criteria. Thus, classification and labelling is not required.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point: KCA 5.2.5

Report: [redacted]., 1988 (TOX1999-455)
Primary eye irritation study in rabbits with SAN 582H technical.
[redacted].
unpublished, 30 November 1988, BASF Reg.Doc.# 88/11364
(Experimental work in August 1988)

Guideline(s): U.S. EPA FIFRA, Subdivision F, Para. 81-4 (November 1984)

Deviations: Deviations from OECD Guideline No. 405 (adopted 24.02.1987): None that were considered to have compromised the validity of the study.

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material: Racemic dimethenamid; SAN 582 H Technical, batch no. 8605, purity 91.4 %

Test Animals: New Zealand White Rabbits – Hra: (NZW) SPF, Source: Hazleton Res. Products, Inc., Denver, Pennsylvania, U.S.A.

The eye irritation of racemic dimethenamid was determined using 6 New Zealand White rabbits. An amount of 0.1 mL of test material was instilled undiluted into the lower conjunctival sac of 1 eye in each rabbit. No wash was performed. Observations for irritation were made at 1, 24, 48 and 72 h after treatment.

Results:

At 1 h after dosing, all six animals had conjunctival redness (score 2) and discharge, and four animals had chemosis (see Table B.6.2-10). All animals were free of conjunctival irritation by 72 h. No iridial or corneal effects were observed at any time-point of investigation. For conjunctival redness and chemosis, mean scores of 0.72 and 0.33 were calculated, respectively.

Table B.6.2-10: Individual eye irritation scores and means

Eye effect	Corneal Opacity				Iris				Conjunctival Redness				Conjunctival Swelling			
Examination (h)	1	24	48	72	1	24	48	72	1	24	48	72	1	24	48	72
Animal No.																
E47040 (male)	0	0	0	0	0	0	0	0	2**	2*	2*	0	1	1	0	0
E47053 (male)	0	0	0	0	0	0	0	0	2**	2*	1*	0	1	1	1	0
E47112 (male)	0	0	0	0	0	0	0	0	2**	1*	0	0	0	0	0	0
E47066 (female)	0	0	0	0	0	0	0	0	2*	1*	1	0	1	1	1	0
E47072 (female)	0	0	0	0	0	0	0	0	2*	1*	1	0	1	1	0	0
E47080 (female)	0	0	0	0	0	0	0	0	2*	1	0	0	0	0	0	0
Mean Score		0								0.72				0.33		

*Discharge (slight) **Discharge (moderate)

Conclusion:

The test substance is not considered to have produced eye irritation according to EU criteria. Thus, classification and labelling is not required.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

B.6.2.6 Skin sensitisation

Data point:	KCA 5.2.6
Report:	[redacted], 1996 (TOX1999-418) Closed-patch repeated insult dermal sensitisation study with SAN 1289 H technical in guinea pigs (Buehler Method) [redacted] unpublished, 17 July 1996, BASF RegDoc.#96/11088 (Experimental work from February 1996 – March 1996)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 81-6 (November 1984)
Deviations:	Deviations from OECD Guideline No. 406 – Buehler Test (adopted 17.07.1992): None that were considered to have compromised the validity of the study.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid-P; SAN 1289 H Technical; batch No. 6663-50-1; purity: 96.3 % dimethenamid (91.1 % S-dimethenamid), pH: 4.2 (dimethenamid in aqueous suspension)
Test Animals:	Dunkin Hartley Guinea Pigs Crl:(HA)BR, weights, males: 343 - 408 g; females: 339 - 387 g, Source: Charles River Laboratories, Kingston, New York

The skin sensitising potential was assessed using the Buehler test. For induction, 20 Dunkin-Hartley Guinea pigs (10/sex) received topical applications of 0.3 mL of the undiluted (100 %) test substance on one flank for 6 h under occlusive dressing. Treatments were once weekly for 3 weeks. Ten untreated animals served as controls. A topical challenge application of 0.5 mL of undiluted (100 %) test substance preparation was carried out 14 d after the third induction by treatment of the untreated, opposite flank using the same procedure as that for induction. The control animals were also treated during the challenge phase to differentiate dermal irritation scores from sensitisation reactions. Readings for dermal changes were performed 24 and 48 h after patch removal. According to the report, the reliability and sensitivity of the method is tested every 4 – 6 months with a positive control agent, dinitrochlorobenzene.

Results:

Irritation increased in incidence and severity during the induction phase. At challenge, 17/20 test animals exhibited clear dermal responses compared to 0/10 in the controls. These results are presented in

Table B.6.2-11.

Table B.6.2-11: Buehler Test – Incidence of Dermal Responses at Challenge

		Dermal scores										
Group	Hrs	0	0.5	1	2	3	Ed	E	B	P1	N	IIS2
Treatment	24	0	4	16	0	0	4	0	0	17	20	85 %
	48	2	7	10	1	0	2	0	0		20	
Irritation Control3	24	9	1	0	0	0	0	0	0	0	10	0 %
	48	10	0	0	0	0	0	0	0		10	

1 P = Positive response; number of animals with a score of 1 or greater at 24 and/or 48 h, out of the 10 or 20 animals per group
2 Incidence Index of Sensitisation = P/N x 100, where N = total number of animals
3 Irritation control groups were treated at Challenge only
Ed=Edema; E=Eschar; B=Black/dark tissue

Conclusion:

Dimethenamid-P is considered to be a skin sensitiser in the Buehler test and has to be classified accordingly.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point: KCA 5.2.6

Report: [redacted] 1987 (TOX1999-456)
SAN 582 H: Skin Sensitisation Test in Guinea Pigs (Maximisation Method of Magnusson and Kligman)
[redacted]
unpublished, 26 March 1987, BASF RegDoc.#87/11222
(Experimental work from 22 April 1987 – 16 May 1987)

Guideline(s): OECD-Guideline No. 406 – GPMT (adopted 1981)

Deviations: Deviations from OECD Guideline No. 406 – GPMT (adopted 17.07.1992):
(1) The undiluted adjuvant (FCA) was used (instead of required: 1:1 mixture (v/v) FCA / water or physiological saline)
(2) For induction, negative controls received intradermal injections of FCA at both sites A and C. However, according to current OECD Guidelines, the 1:1 mixture specified above should have been administered at injection site A, while at site C, a 50 % w/v formulation of the vehicle (DMSO) in the above mentioned 1:1 mixture should have been applied. At site C of test group animals, 50 % w/v formulation of the vehicle (DMSO) in the above mentioned 1:1 mixture should have been used as basis for the 5 % dimethenamid dilution; instead, 5 % dimethenamid dilution in FCA was applied.
(3) The report fails to mention whether the application site was cleaned and shaved prior to observation and grading.
The deviations (1) and (2) may have compromised the validity of the study.

GLP: No (Not subject of GLP regulations), not specified, quality assurance audit was performed

Acceptability: The study report is considered to be unreliable. Due to many deviations

from the guideline and the unreliable report. The study is considered to be not acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; SAN 582 H technical; lot no. 8502; purity: 91 %, solubility: DMSO and ethanol
Test Animals:	Dunkin Hartley Guinea Pigs (males), age: approx. 4 weeks, mean body weight (Day 1): 243 g (200 – 280 g), Source: Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland

Racemic dimethenamid was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test according to Magnusson and Kligman. In a pre-test, moderate to severe scale induction was observed after exposure to either a 1 or 5 % solution in DMSO. Slight redness was induced in 1 of 2 guinea pigs administered the 5 % solution, therefore, the main test was performed using the 5 % dilution. In the main test, 20 animals were used in each of the negative control, test and positive control groups. The first phase of induction was conducted by intracutaneous injections of adjuvant alone, 5 % test substance solution in DMSO, or 5 % test substance in adjuvant. After 7 d, the application site of both test and control groups were shaved and topically treated with a 10 % sodium laurylsulfate aqueous solution to induce skin irritation. 24 h later, the second phase of induction followed with a 48 h topical application of DMSO only (controls) or of 5 % test substance solution in DMSO. The challenge performed 2 weeks after the dermal induction consisted of 24-h topical exposure of both control and treatment groups to 5 % test substance solution in DMSO. Skin reactions were scored immediately, 24 and 48 h after patch removal.

Results:

The challenge results are summarised in Table B.6.2-12.

Table B.6.2-12: Racemic dimethenamid – Results of Maximisation Test

		Dermal scores[1]						
Group	h	0	1	2	3	P[2]	N	IIS[3]
Treatment	24	18	1	0	0	15	19	79 %
	48	4	15	0	0			
Irritation Control[4]	24	20	0	0	0	9	20	45 %
	48	11	9	0	0			
Positive Control (DNCB)	24	1	4	5	10	20	20	100 %
	48	0	0	3	17			

[1] 0 = No reaction; 1 = Scattered mild redness; 2 = Moderate to diffuse redness; 3 = Intense redness and swelling
[2] P = Positive response; number of animals with a score of 1 or greater at 24 and/or 48 h, out of the 10 or 20 animals per group
[3] Incidence Index of Sensitisation = P/N x 100, where N = total number of animals
[4] Irritation control groups were treated with the test susbtance at challenge only

One test group guinea pig died on day 10 prior to completion of the study. Between the non-sensitised animals (negative controls) and the sensitised guinea pigs, there were no differences in the intensity of the skin reaction. A mild redness was observed 48 h after removal of the challenge patch in both groups (9/20 and 15/19, respectively). The treated areas were blue-grey coloured and after 72 h scaly. Necropsy of this animal did not reveal any special lesions. It appears that the test substance concentration of 5 % that was chosen for challenge was too high since 45 % of the irritation controls developed erythema.

Conclusion:

The study does not fully comply with the requirements of currently accepted guidelines. Furthermore, the challenge with 5 % test substance solution in DMSO caused skin irritation in 45 % of the negative controls, which makes it difficult to assess the skin sensitisation potential of the test substance. Although the severity of skin lesions observed in the treatment group was not increased compared to controls, the incidence of animals with dermal irritation was in fact increased (positive scores in 79 % vs. 45 % of the animals). According to guidelines, a substance is considered to possess skin sensitisation potential if at least 30 % of the animals show a positive response. The result of the study is judged to be equivocal.

Re-evaluation by the RMS (2015):

The study is still considered to be not acceptable.

Data point:	KCA 5.2.6
Report:	[redacted] 1995 (TOX2000-1560) Contact Hypersensitivity to Dimethenamid Technical – in Albino Guinea Pigs – Maximisation-Test [redacted] Sponsor: Sandoz Agro Ltd., Toxicol. Dep., Muttenz, Switzerland unpublished, 14 November 1995, BASF RegDoc.#95/11324 (Experimental work from 09 October 1987 – 03 November 1987)
Guideline(s):	OECD Guideline No. 406 (adopted July 17, 1992)
Deviations:	None that compromised the validity of the study
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; batch no. AR 31190; purity: 99.0 %
Test Animals:	Ibm: GOHI (Himalayan spotted) Guinea pigs (SPF quality); age: 6-8 weeks; mean body weight: 441.5 g, Source: Biological Research Lab. Ltd., Fuellinsdorf, Switzerland.

Racemic dimethenamid was tested for its sensitising effect on the skin of the guinea pig in the Maximisation test according to Magnusson and Kligman. Twenty animals were used in the test group and ten animals were used in the negative control group. The first phase of induction was conducted by intracutaneous injections of 1:1 (v/v) mixture of FCA and physiological saline, 5 % test substance in PEG 400 or 5 % test substance emulsion in 1:1 (v/v) mixture of FCA and physiological saline.
The second phase of induction was a 48-h topical application on 10 % sodium-lauryl-sulfate-pretreated skin one week later using undiluted test substance. The challenge was performed two weeks after the dermal induction, once again using undiluted test substance. Skin reactions were scored at 24 and 48 h after patch removal.

Results:

The challenge results are summarised in the following table. One test group animal was found dead on test day 21.

Table B.6.2-13: Incidences of positive dermal responses following the challenge exposure with racemic dimethenamid

		Erythema scores (Draize)[1]						
Group	h	0	1	2	3	P[2]	N	IIS[3]
Treatment	24	0	3	16	0	19	19	100 %
	48	4	11	4	0			
Irritation Control[4]	24	10	0	0	0	0	10	0 %
	48	10	0	0	0			
Positive Control (4-aminobenzoic acide ethyl ester)	24	14	4	2	0	7	20	35 %
	48	13	6	1	0			
Positive Control (2-mercapto-benzothiazol)	24	1	6	9	4	20	20	100 %
	48	0	6	8	6			

[1] 0 = No reaction; 1 = Very slight erythema; 2 = Well-defined erythema; 3 = Moderate to severe erythema; 4 Severe erythema (beet redness) to slight eschar formation (injuries in depth)
[2] P = Positive response; number of animals with a score of 1 or greater at 24 and/or 48 h, out of the 10 animals per group
[3] Incidence Index of Sensitisation = P/N x 100, where N = total number of animals
[4] Irritation control groups were treated with the test susbtance at challenge only

No positive reactions were observed in the control group. All treatment animals had very slight to well defined erythema at the 24 hour reading, and 15/19 still showed a skin reaction at 48 hours.

Conclusion:

Racemic dimethenamid was shown to produce dermal sensitisation in guinea pigs.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

B.6.2.7 Phototoxicity

Studies submitted with the dossier for the Renewal Assessment Report:

Data point: KCA 5.2.6

Report: [redacted]., 2013a (ASB2014-8388)
BAS 656-P H - *In vitro* 3T3 NRU phototoxicity test
BASF DocID 2013/1110119
[redacted]
Dates of experimental work: 18-Mar-2013 - 13-May-2013

Guideline(s): OECD 432 (2004) *In vitro* 3T3 NRU Phototoxicity test, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142

Deviations: UV/VIS absorption spectrum and photostability of the test material were not reported.

GLP: Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test material	BAS 656-PH
Description:	Liquid; brown, clear
Lot/Batch #:	COD-001509
Purity:	95.9 % (tolerance ± 1.0 %) (see Certificate of Analysis, study code 346279_31)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Oct 2013 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by the high purity and by mixing prior to preparation of test substance solutions. The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was determined analytically.
Solvent used:	Dimethylsulfoxide (DMSO)
Control materials:	
Vehicle control:	The vehicle control cultures with and without irradiation only contained the vehicle used for the test substance at the same concentration and volume as used for the test substance and the positive control.
Solvent/final concentration:	DMSO 1 % (v/v) in PBS
Positive control compounds:	Chlorpromazine (CPU) was dissolved in DMSO A complete 96-well plate containing 8 concentrations was performed in parallel to demonstrate sensitivity of the test method.
Without irradiation	1.9-3.8-7.5-15-30-60-90-180 µg/mL
With irradiation	0.03-0.05-0.1-0.2-0.4-0.8-1.6-3.2 µg/mL
Test organisms:	The BaIb/c 3T3, clone A31, cell line was isolated from the muscle tissue of mouse embryo. This fibroblast cell line has a high proliferation rate (doubling time 16-20 hours) and a high plating efficiency (>70 %) of untreated cells both necessary for the appropriate performance of the study. The Balb/c 3T3 cell line which was used in this experiment was obtained from the "European Collection of Cell Cultures" Salisbury, Wiltshire SP4 OJG, UK (date 09 Aug 2006) and is stored at -196 °C (liquid nitrogen).
Culture media and reagents:	
Culture medium:	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with - 10 % (v/v) newborn calf serum (NCBS) - 4 mM L-glutamine - 100 IU penicillin - 100 µg/mL streptomycin
Neutral Red solution:	- 0.4 g Neutral Red powder (NR; Sigma N4638) - 100 mL deionised water
Neutral Red medium:	- 1 mL Neutral Red solution - 79 mL culture medium (DMEM incl. supplements) Incubated overnight at 37 °C with 5 % CO_2 and filtered with a 0.22 µm filter prior to use.
Other solutions and reagents:	- phosphate buffered saline (PBS) without Ca/Mg - trypsin/EDTA solution (0.05 %; 0.02 %) - Neutral Red desorb solution (1 mL acetic acid, 50 mL ethanol, 49 mL deionised water)
Irradiation source:	The Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfelfing,

Germany) used with filter H1 produced wavelength > 320 nm. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

Test concentrations:

Pre-test: Up to 1050 µg/mL with and without irradiation.

NRU test conditions: An appropriate amount of test article substance was taken up in the vehicle, shaken thoroughly and diluted in accordance with the planned doses under light protection conditions immediately before administration.
The experiment was performed in 96 well plates in one experiments (6 replicates per concentration with and without irradiation; two plates per substance (test substance or positive control) were prepared.) The test substance concentrations were:
Without: 4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1050.0 µg/mL
With: 4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1050.0 µg/mL

Treatment and NRU Phototoxicity test:
Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.5 x 10^5 cells/well). After an attachment period of about 24 hours the cells were washed once with 100 µL PBS and subsequently treated with the respective substance (8 concentrations each with 6 replicates of the test substance or the positive control) and the vehicle control in parallel for 1 hour in the dark (5 % (v/v) CO_2, ≥90 % humidity; 37 °C). Then, one microtiterplate per substance was irradiated for 50 minutes with UVA (UV intensity underneath the lid 1.5-2.1 mW/cm² = 5 J/cm²) whereas the respective reference plate was kept in the dark for the same period. After test substance removal and washing step (100 µL PBS) the cells were incubated in culture medium overnight. The medium was removed after 24 hours, the cells washed again, 100 µL medium containing 50 µg/mL Neutral red was added and the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Afterwards, the cells were washed and the dye was extracted by Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake using a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multilabel counter) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.
Evaluation/Assessment:
For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor Prediction model for substances which allow the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the presence and absence of light. This model includes the special case of absence of cytotoxicity in the presence and absence of light for substances obviously showing no phototoxic potential (see below).
- The Mean Photo Effect prediction model which is used if no equi-effective concentrations (EC_{50}) are obtained in the absence and presence of UV light.

Cytotoxicity:
The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrariliy set at 100 %.

$$Cytotoxicity\,[\%] = \frac{\text{Absorbance}_{mean}\ \text{of the test group}}{\text{Absorbance}_{mean}\ \text{of the vehicle control}} \times 100$$

In case of cytotoxicity, an EC_{50} value (Inhibition concentration 50 % relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve).
Photo-Irritancy-Factor:
For substances which induce a 50 % cytotoxicity (EC_{50}) in the presence and absence of light the

Photo-Irritancy-Factor (PIF) is calculated based on comparison of the EC_{50} values in the absence (-UVA) and presence (+UVA) of UVA irradiation

$$PIF = \frac{EC50\ (-UVA)}{EC50\ (+UVA)}$$

resulting in the following classification rules:

PIF ≥5	phototoxic potential predicted
If 2 < PIF < 5:	probable phototoxic potential predicted
If PIF ≤2:	no phototoxic potential predicted

If cytotoxicity occurs only after irradiation a C PIF has to be calculated using the highest test concentration (Cmax) applied in the experimental part in the absence of UV light (-UVA)

$$C\ \ PIF = \frac{C\max\ (-UVA)}{EC50\ (+UVA)}$$

resulting in the following classification rules:

C PIF >1	probable phototoxic potential predicted
If C PIF ≤1	no phototoxic potential predicted

If no cytotoxicity occurs in the concurrently performed experiments in the absence and presence of UV light up to the highest applied test concentration it has to be considered that the test substance has no phototoxic potential.
In this case, a formal PIF = *1 is used to characterise the result

$$PIF = *1 = \frac{C\max\ (-UVA)}{C\max\ (+UVA)}$$

resulting in the following classification rule:

PIF = *1	no phototoxic potential predicted

Mean Photo Effect:
The Mean Photo Effect is calculated based on a comparison of the +UVA and -UVA concentration response curves on a grid of concentrations ci (i=1, ..., N) chosen from the common concentration range of the (-UVA) and (+UVA) experiments. The photo effect (PEi) at concentration ci is calculated as the product of the concentration effect (CEi) and the response effect (REi). The mean photo effect (MPE) is defined as a weighted averaging across all PEi values, with a weighting factor defined by the highest response value.
The resulting classification rules are:

If MPE ≥ 0.1	phototoxic potential predicted
If MPE < 0.1	no phototoxic potential predicted

Other parameters:
The pH was measured at least for the two top doses and for the vehicle controls with and without irradiation. Osmolarity was measured at least for the two top doses and for the vehicle controls with and without irradiation. Test substance precipitation was checked immediately after treatment and at the end of treatment. Test cultures of all test groups were examined microscopically before staining with NRU, which allows conclusions to be drawn about attachment of the cells.
Statistics:
No special statistical tests were performed.

Mean absorbance values and standard deviations were calculated from the single values using calculation software (e.g. MS Excel). The calculations were made using the unedited values. For the report the values were rounded, therefore there may be deviations in the given relative values. If technical errors occurred in single wells (outlier) at least 4 single values per test group were sufficient for calculating reliable mean values. Outliers are defined as values that have half or double the value of the respective mean.
Acceptance criteria:
The assay has to be considered valid if the following criteria are met:

- The vehicle control needs to fulfil the following criteria:
  - The mean OD550 value (with and without UVA irradiation) should be > 0.3.
  - Cell viability after irradiation should be at least 80 % of the concurrent non-irradiated vehicle control.
  - The standard deviation of the mean values of both vehicle control rows should not exceed ± 15 %.
- The positive control chlorpromazine needs to fulfil the following criteria:
  - the EC_{50} value should be in the ranges:
    With irradiation (+UVA): 0.1 - 2.0 µg/mL
    Without irradiation (-UVA): 7.0 - 90.0 µg/mL
  - and the PIF≥6.

Results:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was determined analytically.
Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UVA irradiation precipitation in culture medium was observed at test substance concentrations of 1050 µg/mL.
After treatment with the test substance, clear cytotoxic effects indicated by Neutral Red absorbance values of below 50 % of control were observed in the Main Experiment in the absence and the presence of UVA irradiation at least in the highest applied concentrations (see Table B.6.2-14). Thus an EC_{50} was calculated for all experimental parts.
Without UVA irradiation, there was a decrease in the cell number from 215.4 µg/mL (EC_{50}: 206.3 µg/mL) onward.
With UVA irradiation, there was a decrease in the cell number at 464.2 µg/mL (EC_{50}: 311.9 µg/mL) and above.
Based on the EC_{50} values a PIF of 0.7 (no phototoxic potential) was obtained.

Table B.6.2-14: Mean relative cytotoxicity of dimethenamid-P with and without UVA irradiation in Balb 3T3 cells

Test group	UVA irradiation*	Precipitation**	Mean ODcorr. ***	Cytotoxicity [% of control]
Vehicle control (1 % DMSO)	-	-	0.413	100.0
Dimethenamid-P				
4.6 µg/mL	-	-	0.416	100.6
10.0 µg/mL	-	-	0.433	104.7
21.5 µg/mL	-	-	0.462	111.8
46.4 µg/mL	-	-	0.433	104.7
100.0 µg/mL	-	-	0.411	99.3
215.4 µg/mL	-	-	0.189	45.8
464.2 µg/mL	-	-	0.181	43.9
1050.0 µg/mL	-	+	0.034	8.2
Vehicle control (1 % DMSO)	-	-	0.489	100.0
Dimethenamid-P				
4.6 µg/mL	+	-	0.456	93.3
10.0 µg/mL	+	-	0.495	101.3
21.5 µg/mL	+	-	0.493	100.9
46.4 µg/mL	+	-	0.492	100.6
100.0 µg/mL	+	-	0.455	93.2
215.4 µg/mL	+	-	0.371	75.9
464.2 µg/mL	+	-	0.045	9.1
1050.0 µg/mL	+	+	0.049	10.0

*: Irradiation with Sol 500 solar simulator for 50 minutes (approx.. 5 J/cm²)
**: Precipitation in PBS at the end of exposure period
***: Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

After treatment with the positive control chlorpromazine clear cytotoxic effects indicated by Neutral Red absorbance values of below 50 % of control were observed in the absence and the presence of UVA irradiation at least in the highest applied concentrations.
Without UVA irradiation, there was a decrease in the cell number from 30.0 µg/mL (EC_{50}: 21.2 µg/mL) onward.
With UVA irradiation, there was a decrease in the cell number at 0.8 µg/mL (EC_{50}: 0.7 µg/mL) and above.
Based on the EC_{50} values a PIF of 29.8 (phototoxic potential) was obtained (see Table B.6.2-15).

Table B.6.2-15: Mean relative cytotoxicity of chlorpromazine with and without UVA irradiation in Balb/c 3T3 cells

Test group	UVA irradiation	Mean OD *	Mean ODcorr. **	Relative Cytotoxicity [% of control]	Standard deviation [%]
Blank	-	0.038	-	-	-
Vehicle control 1	-	0.429	0.392	-	9.6
Vehicle control 2	-	0.524	0.486	-	3.5
Vehicle control mean (1 % DMSO)	-	0.476	0.439	100.0	12.9
Chlorpromazine					
1.9 µg/mL	-	0.476	0.438	99.8	3.1
3.8 µg/mL	-	0.490	0.452	103.1	2.9
7.5 µg/mL	-	0.466	0.428	97.6	3.9
15.0 µg/mL	-	0.367	0.330	75.1	4.4
30.0 µg/mL	-	0.101	0.064	14.5	3.1
60.0 µg/mL	-	0.062	0.025	5.7	14.1
90.0 µg/mL	-	0.040	0.002	0.5	1.1
180.0 µg/mL	-	0.038	0.000	0.1	0.1
Blank	+	0.038	-	-	-
Vehicle control 1	+	0.501	0.463	-	3.4
Vehicle control 2	+	0.567	0.529	-	6.4
Vehicle control mean (1 % DMSO)	+	0.534	0.496	100.0	8.6
Chlorpromazine					
0.03 µg/mL	+	0.504	0.466	94.1	2.5
0.05 µg/mL	+	0.509	0.471	95.1	3.1
0.10 µg/mL	+	0.509	0.472	95.1	4.7
0.20 µg/mL	+	0.502	0.464	93.6	5.5
0.40 µg/mL	+	0.495	0.458	92.3	6.2
0.80 µg/mL	+	0.226	0.188	38.0	12.9
1.60 µg/mL	+	0.042	0.004	0.8	0.6
3.20 µg/mL	+	0.040	0.002	0.3	0.3

*: Mean absorbance at 550 nm of 6 wells, in general
**: Mean absorbance (test group) minus mean absorbance (blank)

Conclusion:

According to the results of the present study, the test substance dimethenamid-P is considered not to be a phototoxic substance in the *in vitro* 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells under the experimental conditions.
According to the OECD Test Guideline 432 a photoreaction requires sufficient absorption of light quanta. Thus, before biological testing is considered, a UV/vis absorption spectrum of the test chemical must be determined according to OECD Test Guideline 101. Chemicals being unlikely to be photoreactive may not need to be tested in the *in vitro* 3T3 NRU phototoxicity test. The UV/VIS absorption spectrum and the photostability of the test material were not reported. However, this does not limit the acceptability of the study.

B.6.3 Short-term toxicity

B.6.3.1 Oral 28-day study

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.3.1
Report:	[redacted] 1996 (TOX1999-419) A 4-week range-finding study of SAN 1289 H in rat via dietary administration [redacted] unpublished, 30 January 1996, BASF RegDoc.#96/11147 (Experimental work from August 1995 – September 1995
Guideline(s):	Range-finding study; method was determined by the sponsor (guideline was not specified)
Deviations:	Deviations from OECD Guideline No. 407 (adopted 27.07.1995): (1) Cholesterol was not determined in clinical-chemistry investigations, (2) The weight of the following organs were not determined: adrenals, spleen, thymus, and heart; (3) The following organs/tissues were not preserved: spinal chord, large and small intestines, thymus, thyroid, accessory sex organs, urinary bladder, lymph nodes, peripheral nerve, and bone marrow section; (4) No histopathology was performed
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be supplementary as a range-finding study only.

Materials and methods:

Test Material:	Dimethenamid-p; SAN 1289 H; batch No. 6663-25-6; purity: 94.7 %
Test Animals:	[Crl: CD®(SD)BR] rats; weights (Day 0): males 182 g (168 - 199 g), females 156 g (143-171 g), Source: Charles River Laboratories, King ston, New York

The test substance was administered to groups of 5 male and 5 female Sprague-Dawley CD rats at dietary concentrations of 0, 500, 1500 and 3000 ppm over a period of 4 weeks. In addition, a low-dose group received 50 ppm for 1 week and 150 ppm for 3 weeks. Since the majority of exposure duration was at 150 ppm, this group will be referred to as the “150 ppm” group hereafter.

Food consumption and body weights were determined each week. The animals were examined for evident signs of toxicity or mortality at least once a day. During the weekly weighing, the animals were subjected to an additional comprehensive clinical examination.
Clinicochemical and haematological examinations were carried out during the last week of dosing.
Following 4-week treatment all animals were sacrificed, selected organs were weighed and a gross pathological examination (but no histopathology) was performed.
The test substance intake is given in the following Table B.6.3-1.

Table B.6.3-1: Test substance intake

Dietary dose level	**Test substance intake (mg/kg bw/d)**	
(ppm)	Males	Females
"150"	12	12
500	50	52
1500	155	143
3000	306	290

Results:

All animals survived for the duration of the study, and there were no clinical signs observed which were considered related to treatment. Also, there were no treatment-related effects on haematological parameters.
The clinical and clinicochemical findings, as well as organ weight changes are summarised in the following Table B.6.3-2.

Table B.6.3-2: Clinical, haematological, clinicochemical and organ weight findings

	Sex	Dose level (ppm)				
		0	"150"	500	1500	3000
Clinical findings:						
Body weight (wk 4) [g]	m	390	385	380	384	359 n.s.
	f	232	234	232	223 n.s.	227 n.s.
Body weight change (wk 0–4) [g]	m	+207	+202	+197	+202	+176 n.s. (-15 %)
	f	+76	+77	+75	+67 n.s. (-12 %)	+71 n.s. (-7 %)
Clinicochemical findings:						
γ-Glutamyltransferase [IU/L]	m	0 ± 0	1 ± 1	0 ± 1	1 ± 1	7 ± 2 NT
	f	1 ± 0	0 ± 1	2 ± 3	2 ± 1	5 ± 1 NT
Organ weights:						
Abs. liver wt [g]	m	12.5	12.3	12.4	13.4	15.2 n.s. (+22 %)
	f	6.7	7.4	7.7	7.5	9.2** (+37 %)
Rel. liver wt [mean % bw]	m	3.5	3.5	3.6	3.9 n..s. (+11 %)	4.7** (+34 %)
	f	3.2	3.5	3.7** (+15 %)	3.7** (+15 %)	4.7** (+44 %)

NT Not tested statistically due to lack of variance
** Significantly different from control (Dunnett's)
n.s. statistically not significant

Reduced body weight (males) and body weight change were observed at the high dose without an effect on food consumption. The increase in serum γ-glutamyltransferase in the high dose animals is in agreement with the increased liver weights, and indicates the possibility of either frank liver toxicity or of an adaptive response to handling the chemical. There were no gross pathological findings considered related to treatment.

Conclusion:

The liver was identified as target organ. The examinations performed were limited. Therefore, it is not considered appropriate to derive a general NOAEL for risk assessment purposes.

Re-evaluation by the RMS (2015):

This study was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566). The study is still considered to be supplementary.

Data point: KCA 5.3.1

Report: [redacted] 1987 (TOX1999-468)
SAN 582H: 5 weeks pilot feeding study in rats
[redacted]
unpublished, 27 October 1987, BASF RegDoc.#87/11227
(Experimental work from 19 February 1985 – 29 March 1985)

Guideline(s):	Range-finding study; method was determined by the sponsor (guideline was not specified)
Deviations:	Deviations from OECD Guideline No. 407 (adopted 27.07.1995) (1) Clotting time/potential was not measured; (2) Adrenals and epididymides were not weighed; (3) Spinal chord was not subjected to histopathological investigation. The deviations were not considered to have compromised the validity of the study results.
GLP:	Yes (laboratory certified by Eidgenössisches Departement des Innern, Berne, Switzerland)
Acceptability:	The study is considered to be acceptable as a range-finding study.

Materials and methods:

Test Material:	Racemic dimethenamid, batch no. [lab batch, March 27, 1984], purity 99 %
Test Animals:	Han Wistar rats; age 6 weeks at start of treatment, body weight (Day 1): males 146.7 ± 10.0 g, females 117.6 ± 6.9 g, Source: Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland

In a range-finding study, 8 rats/sex/dose group received racemic dimethenamid at feed concentrations of 0, 30, 100, 300, 1000 or 3000 ppm for 5 weeks. The animals were examined at least once daily for mortality and behavioural/neurological symptoms. Body weights and food consumption were recorded weekly. Blood profiles, clinical chemistry and urinalyses were determined on all animals after 4 weeks on test. Necropsy was performed on all rats under study. Organ weights were determined for kidneys, liver, testes/ovaries, heart, brain, spleen, thymus and lungs. The livers of all rats were subjected to histopathological examination. Histopathology was also performed on an extensive selection of organs and tissues from all controls and 3000 ppm-group rats.

Results:

The test substance intake is given in the following Table B.6.3-3:

Table B.6.3-3: Test substance intake

Dietary dose level	Test substance intake (mg/kg bw/d)	
(ppm)	Males	Females
30	2.92	3.32
100	9.5	10.8
300	28.8	35.7
1000	95.6	109
3000	285	328

No mortality or clinical symptoms that could be related to substance intake were observed. Body weight gain was reduced significantly at 3000 ppm in males only (-22 % vs. controls, $p = 0.01$), which was accompanied by a significantly reduced food consumption during the first study week (-16 % vs. control). Over the whole study, food intake was only slightly reduced (7 % vs. control, statistically non significant) in 3000 ppm-females, a non-significant, 11 % reduction of body weight gain was observed. Assessment of haematology did not reveal biologically relevant differences between control and dose groups. The majority of clinical chemistry parameters were not affected by treatment. The following exceptions are summarised (see Table B.6.3-4).

Table B.6.3-4: Clinical chemistry findings

Findings		Control	30 ppm	100 ppm	300 ppm	1000 ppm	3000 ppm
Total cholesterol [mM]	m	1.04 ± 0.48	0.92 ± 0.26	1.08 ± 0.37	1.21 ± 0.37	1.34 ± 0.30	1.59 ± 0.40*
	f	1.11 ± 0.34	0.85 ± 0.46	0.94 ± 0.35	1.09 ± 0.31	1.09 ± 0.27	1.44 ± 0.34
Triglycerides [mM]	m	0.99 ± 0.36	1.01 ± 0.45	0.86 ± 0.20	0.98 ± 0.18	0.98 ± 0.36	0.99 ± 0.29
	f	0.81 ± 0.22	0.67 ± 0.18	0.71 ± 0.20	0.64 ± 0.20	0.69 ± 0.28	0.67 ± 0.14
Total bilirubin [µM]	m	0.59 ± 0.34	0.59 ± 0.36	0.64 ± 0.31	0.61 ± 0.38	0.65 ± 0.45	0.67 ± 0.46
	f	1.29 ± 0.88	1.22 ± 0.42	1.11 ± 0.39	1.07 ± 0.52	1.00 ± 0.30	0.97 ± 0.28
GGT [U/L]	m	0.64 ± 1.24	0.70 ± 0.98	1.33 ± 1.93	0.86 ± 1.19	1.01 ± 1.51	2.25 ± 2.55
	f	0.38 ± 0.73	0.56 ± 1.12	1.11 ± 1.94	0.62 ± 1.16	0.93 ± 1.23	2.19 ± 1.98

* Statistically different from control value: 2p < 0.05 (Dunnett Test)

The only clinical chemistry parameter to reach statistical significance compared to controls was total cholesterol, which was increased by 53 % in high-dose group males, although a dose-related increase was indicated already at 300 ppm and above. In females, cholesterol was increased only in the high-dose group (+30 %, non-significant). Triglycerides remained unchanged in males but tended to be decreased by 12 to 20 % vs. controls in females. Total bilirubin tended to be slightly increased in males, while a slight dose-dependent reduction was observed in females. GGT activity values, which showed comparably large standard deviations, were increased at 3000 ppm in both male and female groups.
No obvious effects were observed upon urinalysis. Absolute liver weights were increased among both 3000 ppm males (+14 %, ns) and females (+27 %, p<0.01). Relative liver weights of this group were significantly increased for both sexes. At 1000 ppm, a statistically non-significant, approx. 10 % increase in relative liver weight was established in both males and females. The organ weight findings are summarised in Table B.6.3-5.

Table B.6.3-5: Organ weight findings

Findings		Control	30 ppm	100 ppm	300 ppm	1000 ppm	3000 ppm
Final body wt. [g]	m	298	279	277	300	280	261**
	f	180	179	178	177	174	173
Abs. liver wt. [g]	m	9.8	9.4	8.8	10.3	10.2	11.2
	f	6.2	6.1	5.8	6.5	6.6	7.9**
Rel. liver wt. [% bw]	m	3.28	3.39	3.17	3.43	3.65	4.27$$
	f	3.44	3.41	3.27	3.67	3.79	4.56$$

** Significantly different from control (p < 0.01, Dunnett Test)
$$ Significantly different from control (p < 0.01, Kruskal-Wallis and Dunn-Bonferroni Test)

Histopathological findings that were regarded to be related to treatment were confined to the liver and consisted of cytoplasmic swelling of predominantly centrolobular hepatocytes in conjunction with lighter cytoplasmic staining, which was observed at 3000 ppm in both sexes (see Table B.6.3-6). Other liver findings observed in both control and treatment groups were mild "mononuclear hepatitis"; hepatocytes with mild vacuolation were predominantly located centrolobular at 100 and 1000 ppm, and peripherolobular at 0, 30 and 3000 ppm (no specific pattern of distribution observed at 300 ppm). An alteration of hepatocytes termed as "individualisation" (not further specified) was reported for several males of the 30, 100, 300, and 1000 ppm groups and in 1 female at 1000 ppm. No such observations were reported for controls and for animals exposed to 3000 ppm.

Table B.6.3-6: Histopathological findings

Findings		Control	30 ppm	100 ppm	300 ppm	1000 ppm	3000 ppm
Hepatocellular vacuolation (predom. peripherolobular)	m	2/8	4/8	3/8	2/8	3/8	4/8
	f	5/8	5/8	3/8	1/8	1/8	3/8
Hepatitis mononuclear, mild	m	4/8	8/8	8/8	8/8	4/8	4/8
	f	4/8	6/8	7/8	6/8	7/8	3/8
Lymphoreticular proliferation and infiltration	m	0/8	0/8	0/8	0/8	0/8	1/8
	f	0/8	0/8	0/8	0/8	0/8	0/8
Cytoplasmic swelling centrolobular	m	0/8	0/8	0/8	0/8	0/8	4/8
	f	0/8	0/8	0/8	0/8	0/8	4/8
Individualisation of hepatocytes	m	0/8	6/8 (I)	6/8 (I–II)	4/8 (I)	7/8 (I)	0/8
	f	0/8	0/8	0/8	0/8	1/8 (I)	0/8

I: mild; II: moderate

Conclusion:

Following administration of racemic dimethenamid via the feed daily for 5 weeks, a LOAEL was established at 1000 ppm, based on slight liver findings (increased liver wt, and increased total cholesterol at 1000 ppm and above). At the highest dose level of 3000 ppm, reduction of body weight gain and decreased food consumption was accompanied by increased GGT serum activity levels and hepatocellular cytoplasmic swelling. At 300 ppm, the only change observed was a slight increase in cholesterol levels, which was found in males only and not considered to represent an adverse effect; therefore 300 ppm, equivalent to 29 mg/kg bw/d, is considered to be the NOAEL.

Re-evaluation by the RMS (2015):

The study was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566). The study is still considered to be acceptable.

B.6.3.2 Oral 90-day study

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.3.2
Report:	[redacted] 1996 (TOX1999-421) A subchronic (3-month) toxicity study of SAN 1289 H in the rat via dietary administration [redacted] unpublished, 15 November 1996, BASF RegDoc.#96/5420 (Experimental work from January 1996 –April 1996)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 82-1 (November 1984)
Deviations:	Deviations from OECD Guideline No. 408 (adopted 12 May 1981): (1) Temperature and humidity ranges were exceeded at some time points (18–26 °C, rel. humidity 29–76 %); (2) No microscopic investigation of the spinal chord was performed
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability:	The study is considered to be acceptable.
Data point:	KCA 5.3.2
Report:	[redacted] 1999 (TOX1999-467) Statement: Review of substance-related findings in the liver after administration of dimethenamid (racemice and s-form) over 3 months to rats [redacted] unpublished, 8 March 1999, BASF RegDoc.#99/10270
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 82-1 (November 1984)
Deviations:	Deviations from OECD Guideline No. 408 (adopted 12 May 1981): (1) Temperature and humidity ranges were exceeded at some time points (18–26 °C, rel. humidity 29–76 %); (2) No microscopic investigation of the spinal chord was performed
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	S-dimethenamid Technical (SAN 1289 H Technical); batch No. 6663-50-1; purity: 96.3 % Total dimethenamid (91.1 % S-dimethenamid)
Test Animals:	[Crl: CD®(SD)BR] rats; weights (Day 0): males 203 g (188 - 224 g), females 168 g (152 - 185 g), Source: Charles River Laboratories, King ston, New York

The test article was administered to 10 male and 10 female Sprague-Dawley CD rats at dietary concentrations of 0, 500, 1500 and 3000 ppm over a period of 90 d. Analyses for stability and homogeneity of the test substance in the diet as well as for correct concentration were performed.
Body weight and food consumption were determined weekly. The animals were examined twice daily for mortality and gross signs of toxicologic or pharmacologic effects. Moreover, comprehensive clinical examinations of the animals were performed once a week. Clinical chemistry and haematological examinations as well as urinalyses were carried out at the end of the administration period. Ophthalmological examinations were carried out in all animals before the start and at the end of the administration period. At the end of the treatment period all animals were sacrificed, subjected to complete gross examinations and selected organs were weighed. Tissues were examined histologically for all animals in the high dose and control, and in addition kidneys, liver and lungs were examined for all animals. The test substance intake is given in (see Table B.6.3-7).

Table B.6.3-7: Test substance intake

Dietary dose level	Test substance intake (mg/kg bw/d)	
(ppm)	Males	Females
500	37	40
1500	110	125
3000	222	256

Results:

Stability analyses demonstrated that dimethenamid-P was stable in the diet for the period of time presented to the animals and that the test substance was homogeneously distributed in the feed.

Concentration verification analyses confirmed the theoretical dietary concentrations.
No mortalities occurred during the study, and there were no clinical signs or ophthalmoscopy findings noted which were considered related to treatment. The clinical and clinicochemical findings, as well as organ weight changes are summarised in Table B.6.3-8).

Table B.6.3-8: Clinical, clinicochemical, and organ weight findings

Sex	Male				Female			
Dose (ppm)	0	500	1500	3000	0	500	1500	3000
Clinical findings								
Body wt. change (g)	373	377	344	330	141	135	128	127
Haematology								
APTT (sec)	12.4	13.0	11.6	12.3	21.3	21.6	23.4	25.1**
Clinical chemistry								
Cholesterol (mg/dL)	65	73	80	96**	91	98	108	116
GGT (IU/L)	1	0	3*	5**	0	0	0	2
Organ weight								
Terminal body wt.	521	528	497	486	283	275	268	266
Abs. liver wt. (g)	15.24	17.12	17.65	19.30**	8.83	8.74	9.42	10.40**
Rel liver wt. (g/kg)	2.92	3.25**	3.53**	8.54**	3.13	3.18	3.51**	3.91**
Abs. kidney wt. (g)	4.37	4.48	4.40	4.73	2.50	2.48	2.46	2.52
Rel. kidney wt. (g/kg)	8.41	8.52	8.90	9.78**	8.84	9.02	9.17	9.48

Significantly different from control: (* = p <0.05; ** = p <0.01, Dunnett test)

Table B.6.3-9: Amendment of clinicochemical findings, cholesterol values at termination

Sex	Male				Female			
Dose (ppm)	0	500	1500	3000	0	500	1500	3000
Cholesterol (mg/dL)								
Mean value	65	73	80	96**	91	98	108	116
Standard deviation	19	8	14	17	20	20	22	21
Number of animals	10	10	10	10	10	10	10	10

Significantly different from control: (* = p <0.05; ** = p <0.01, Dunnett test)

Statistically non-significant decreases of terminal body weights were observed in high dose males and females (7 and 5 %, respectively) and at 1500 ppm (by 5 and 4 % in males and females, respectively). The mean body weight and body weight change in animals receiving 500 ppm were considered to be comparable to the control values. Food consumption was not affected by treatment.
The only change noted in haematology was an increase in clotting time as measured by activated partial thromboplastin time (APTT). There were no effects on the urinanalysis parameters investigated.
Serum γ-glutamyl transpeptidase (GGT) was increased in both sexes at the high dose and in males at 1500 ppm. There was a trend toward increased cholesterol values in the treated animals (statistically increased for high-dose group males only).
Liver weights were increased in males of all treatment groups and in females at dose levels of 1500 ppm and above; statistical significance was reached for absolute liver weights for both sexes at the high dose (36 and 25 % in males and females, respectively) and in males at the mid dose. At

500 ppm, relative liver weights were slightly increased (11 %) in males only; the increased absolute liver weight (12 %) was statistically non-significant. A slight increase in absolute and relative kidney weights for males receiving 3000 ppm was not associated with morphological or clinicochemical changes and was not considered treatment-related.
Histological examination revealed hepatocellular hypertrophy in both sexes at 3000 and 1500 ppm and in females only at 500 ppm. There were no corresponding histological liver changes in males of the 500 ppm group. The localisation of hypertrophy appeared to be sex-dependent.
The liver slides of this study were re-assessed blindly by BASF AG in 1999 to allow for a better comparison with histopathological liver findings from the corresponding 90-day study conducted with racemic dimethenamid. Only slight differences compared to the original evaluation were found. Therefore, only the results of the re-assessment are reproduced in Table B.6.3-10 below.

Table B.6.3-10: Histopathological liver findings (re-assessment)

Sex	Male				Female			
Dose (ppm)	0	500	1500	3000	0	500	1500	3000
Centrilobular hypertrophy	0/10	0/10	0/10	0/10	0/10	3/10	9/10	8/10
Minimal	0/10	0/10	0/10	0/10	0/10	2/10	6/10	4/10
Slight	0/10	0/10	0/10	0/10	0/10	1/10	3/10	4/10
Periportal hypertrophy	0/10	0/10	7/10	10/10	0/10	0/10	0/10	0/10
Minimal	0/10	0/10	4/10	5/10	0/10	0/10	0/10	0/10
Slight	0/10	0/10	3/10	5/10	0/10	0/10	0/10	0/10
Periportal eosinophilic inclusions	0/10	0/10	2/10	6/10	0/10	0/10	0/10	0/10
Minimal	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10
Slight	0/10	0/10	2/10	4/10	0/10	0/10	0/10	0/10

In summary, definite signs of toxicity were observed at the high dose of 3000 ppm including decreased body weight gains, increased serum GGT and increased cholesterol. Lesser effects on body weight gain and GGT were also observed at the mid dose of 1500 ppm. Noteworthy increases in absolute and/or relative liver weights were found in all treatment-group males and in high-dose females. In males at 500 ppm, a slight increase in relative liver weight males was not accompanied by corresponding histopathological or clinicochemical changes. The observation of minimal to slight hypertrophy was confined to periportal hepatocytes of the mid- and high-dose groups. In females, minimal to slight centrilobular, hepatocellular hypertrophy was observed in all treatment groups. However, at 500 ppm, these changes were not accompanied by corresponding liver weight increases or clinicochemical alterations.

Conclusion:

The slight changes at 500 ppm are not considered to represent adverse effects and, therefore, 500 ppm (37 mg/kg bw/d) is considered a no observed adverse effect level (NOAEL).

Re-evaluation by the RMS (2015):

The study was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566).
As a result of the evaluation of dimethenamid-P, for the inclusion of this substance in Annex I to the Directive 91/414/EEC, no NOAEL was derived because of elevated blood cholesterol and centrilobular hypertrophy in females observed at the lowest dose of 500 ppm. Furthermore the study was considered as not appropriate for bridging, since a NOAEL was not derived. However, the RMS brought forward the argument that “e.g. a minimal or slight hypertrophy of hepatocytes in 1 or 2 of 10 animals should be considered to be a non adverse effect. The finding was only observed in few

animals and is a typical adaptive phenomenon. The expression of the finding was not exactly quantified and can be influenced by a subjective factor of the pathologist." It was additionally proposed "to compare also the absolute liver weights of the female animals in dose group 500 ppm and the control group. The liver weight of females in dose group 500 ppm was not increased. Overall the study is considered to be acceptable." (Reporting table, Dimethenamid-P (Hb) section 4, 11587/ECCO/BBA/01, rev. 3 (13.06.2002) 57/93; ASB2015-560).
Thus, the following can still be concluded. In males at 500 ppm, a slight increase in relative liver weight males was not accompanied by corresponding histopathological or clinicochemical changes. The observation of minimal to slight hypertrophy was confined to periportal hepatocytes of the mid- and high-dose groups. In females, minimal to slight centrilobular hepatocellular hypertrophy was observed in all treatment groups. However, at 500 ppm, these changes were not accompanied by corresponding liver weight increases or clinicochemical alterations. It should be noted that an additional table demonstrating cholesterol values at termination was included in the results and discussions section. In conclusion, the slight changes at 500 ppm are not considered to represent adverse effects. Therefore 500 ppm (37 mg/kg bw/d) is considered a no observed adverse effect level (NOAEL). The study is still considered to be acceptable.

Data point:	KCA 5.3.2
Report:	[redacted] 1987 (TOX1999-457) SAN 582 H toxicity to rats by repeated dietary administration for 13 weeks followed by a 4-week withdrawal period [redacted]. unpublished, 16 October 1986, BASF RegDoc.#86/11183 (Experimental work from 18 June 1986 – 16 October 1986)
Guideline(s):	OECD Guideline No. 408 (adopted 14.05.1981) U.S. EPA FIFRA, Subdivision F, Para. 82-1 (November 1984)
Deviations:	Deviations from OECD Guideline No. 408 (adopted 14.05.1981): none
GLP:	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.3.2
Report:	[redacted] 1999 (TOX1999-467) Statement: Review of substance-related findings in the liver after administration of dimethenamid (racemice and s-form) over 3 months to rats [redacted] unpublished, 8 March 1999, BASF RegDoc.#99/10270
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 82-1 (November 1984)
Deviations:	Deviations from OECD Guideline No. 408 (adopted 12 May 1981): (1) Temperature and humidity ranges were exceeded at some time points (18–26 °C, rel. humidity 29–76 %); (2) No microscopic investigation of the spinal chord was performed
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; SAN 582 H, batch no. 8605, purity: approx.

	91.5 %
Test Animals:	CD rats; age (weeks 0): approx. 7 weeks, body weights (weeks 0): males 242 g (217 - 284 g), females 165 g (142 - 195 g), Source: Charles River, U.S.A. (no further details reported)

The subchronic toxicity of racemic dimethenamid was tested in male and female Sprague-Dawley rats. Ten rats per sex and dose level were fed 0 (control); 50, 150, 500, 1500 or 3000 ppm test substance over a period of 90 d. An additional ten rats per sex were used in the control and high dose groups to determine effects after 4-wk recovery from treatment. Homogeneity and stability of the test material in the diet were determined prior to study start, and dietary level confirmation was performed for all levels during the first and last weeks of treatment. The animals were examined twice daily for mortality and gross signs of toxicologic effects. Detailed clinical observations were performed daily during the pre-test period and during the first four treatment weeks; thereafter, detailed examinations were recorded weekly. Body weight and food consumption was recorded on a weekly basis. Ophthalmoscopy was performed at the beginning and at the end of the administration period in all animals. After 90 d of treatment, the main study animals were sacrificed, selected organs weighed and gross pathology performed. Additional high dose and control group animals were maintained on control diet for 4 further weeks and then sacrificed. Clinical chemistry, haematological examinations and urinalysis were carried out for all animals at the end of the study. Standard tissues were evaluated histopathologically for all animals in all dose groups.
The group mean achieved intakes of test substance is given in Table B.6.3-11.

Table B.6.3-11: Test substance intake

	Test substance intake [mg/kg bw/d]				
	50 ppm	150 ppm	500 ppm	1500 ppm	3000 ppm
Males	3.5	10	34	98	204
Females	3.9	12	40	119	238

Results:

No deaths occurred during the study, and there were no clinical observations or ophthalmoscopic effects considered related to treatment. The mean body weights and body weight gains of the animals treated with 1500 and 3000 ppm were lower than control (see Table B.6.3-12). For the 90-day treatment period, the mean body weight gains of mid and high dose males were approx. 17 and 23 % below controls, respectively, and the females were 13 and 24 % below controls at the mid and high doses, respectively. The body weight change at 500 ppm males was only marginally reduced (by 6 %). For males and females at 50 and 150 ppm, body weight gain was comparable to controls. During the recovery period, males and females previously given 3000 ppm showed a higher body weight gain than controls. Food consumption was marginally lower in 1500 and 3000 ppm animals. No treatment-related effects were observed on food consumption at concentration of 500 ppm and less or in high-dose animals after a 4-week recovery period.

Table B.6.3-12: Effects on body weight gain and food consumption

Dosage (ppm)	Body weight gain				Mean total food consumption			
	Males		Females		Males		Females	
	(g)	% Control	(g)	% Control	(g/rat)	% Control	(g/rat)	% Control
0	259	–	100	–	2388	–	1704	–
50	268	103	94	94	2585	108	1654	97
150	252	97	96	96	2485	104	1666	98
500	244	94	64	94	2429	102	1705	100
1500	215**	83	87*	87	2265	95	1626	95
3000	200**	77	76**	76	2271	95	1574*	92
0[R]	30	–	9	–	706	–	508	–
3000[R]	52[$$$]	173	16	178	727	103	488	96

[R] = Effects observed during 4-wk recovery period
Statistically significant from control: * = $p < 0.05$ ** = $p < 0.01$ (Williams Test); [$$$] = $p < 0.001$ (t-Test)

Results of haematological examinations and urinalysis did not indicate any treatment-related effects. At the end of 13-wk treatment, increased levels of serum GGT values were observed in males and possibly in females at 3000 ppm (see Table B.6.3-13). Cholesterol levels were increased in both sexes at 3000 ppm and in females at 1500 ppm. The statistically significant increase in females at 500 ppm is slight and not considered to be of biological significance. At 1500 ppm and above, total protein levels, in some cases in conjunction with albumin and/or globulin, were slightly increased over control levels (≤10 %). Liver enzyme, protein and cholesterol levels at 3000 ppm were generally comparable to control following the recovery period.

Table B.6.3-13: Clinical chemistry

	Main Groups				Satellite Groups							
Dose	13-wk treatment				13-wk treatment				13-wk treatment + 4-wk recovery			
	Chol (mg/dL)		GGT (U/L)		Chol (mg/dL)		GGT (U/L)		Chol (mg/dL)		GGT (U/L)	
(ppm)	M	F	M	F	M	F	M	F	M	F	M	F
0	73 (13.8)	77 (14.3)	2	1	72	76	2	1	89 (20.5)	94 (15.9)	1	1
50	68 (15.6)	88 (9.3)	1	1								
150	76 (22.0)	89 (22)	2	1								
500	77 (18)	93* (17.6)	1	1								
1500	87 (23.3	101** (19.3)	2	1								
3000	104** (23)	107** (15.3)	6**	2**	114	108	5	2	95 (18.9)	90 (21.0)	1	2

Statistically significant from control (Williams Test): * = $p < 0.05$ ** = $p < 0.01$

Treatment-related organ weight and histopathological changes were confined to the liver (see Table B.6.3-14).

Table B.6.3-14: Final body weight, liver weight and histopathology

Dosage (ppm)		0	50	150	500	1500	3000	0R	3000[R]
Final mean body wt (g)	M	489	499	492	478	449	420	530	506
	F	270	258	258	259	251	242	262	248
Mean liver wt (g)	M	23.0	20.8	22.1	20.8	20.8	21.7	24.7	23.2
	F	10.6	10.1	10.1	10.8	11.4	12.4	10.1	11.2
Adjusted mean liver wt (g)[1]	M	22.0	19.2	20.8	20.4	22.1	24.6*	23.8	24.1
	F	10.1	10.0	10.1	10.7	11.5**	12.9**	N.D.	N.D.
Rel. liver wt (10 x g/kg bw)	M	471	419	444	435	462	517	465	455
	F	390	390	393	417	454	515	387	454
Hepatocellular hypertrophy (centrilobular)	M	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Hepatocellular hypertrophy (centrilobular)	F	0/10	0/10	0/10	1/10	9/10	10/10	0/10	2/10
Minimal		0/10	0/10	0/10	1/10	9/10	9/10	0/10	2/10
Moderate		0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10

[1] = For statistical analysis, liver weight data was adjusted for final body weight as co-variate, in an attempt to correct for differences in body weight capable of influencing the liver weights.
* = $p < 0.05$; ** = $p < 0.01$ (statistically significant from control, Williams test); N.D. = not determined
[R] = results after 4 wk recovery period

A dose related increase of liver weights compared to control values was found for female rats given 1500 or 3000 ppm, both for absolute and relative liver weights. Slight increases of relative liver weights were found for high-dose group males. These findings were confirmed by statistical analysis of liver weights after adjustment for final body weight as co-variate. Following the 4-week recovery period, livers of animals that received prior treatment with 3000 ppm were comparable to controls in males and only slightly increased in females. There were no treatment-related effects on the other weighed organs.
Microscopically, minimal to moderate enlargement of centrilobular hepatocytes was observed in females at 1500 and 3000 ppm. The centrilobular enlargement was still present in 2/10 females at 3000 ppm following the four week recovery period. There were no other microscopic findings considered related to treatment.
The liver slides of this study were re-assessed blindly by BASF AG in 1999 to allow for a better comparison with histopathological liver findings from the corresponding 90-day study conducted with dimethenamid-P (see Table B.6.3-15). Substance-related findings were comparable between the original data and after review for females only: Minimal to slight centrilobular hepatocellular hypertrophy was noted in few female treated with 500 ppm and in most females treated with 1500 ppm. There were no substance-related findings described in male animals in the original report. However, in the review, in males treated with 3000 ppm, a substance-related effect was evident: A minimal or slight periportal hepatocellular hypertrophy was observed in five males. Also in six males, eosinophilic inclusions were visible in the cytoplasm of few periportal hepatocytes. These findings were comparable to those seen after treatment with dimethenamid-P.

Table B.6.3-15: Histopathological liver findings (re-assessment)

Sex	Male				Female			
Dose (ppm)	0	500	1500	3000	0	500	1500	3000
Centrilobular hypertrophy					0/10	3/10	9/10	9/10
Minimal					0/10	3/10	8/10	3/10
Slight					0/10	0/10	1/10	6/10
Moderate					0/10	0/10	0/10	0/10
Periportal hypertrophy	0/10	0/10	0/10	5/10				
Minimal	0/10	0/10	0/10	3/10				
Slight	0/10	0/10	0/10	2/10				
Periportal eosinophilic inclusions	0/10	0/10	0/10	6/10				
Minimal	0/10	0/10	0/10	4/10				
Slight	0/10	0/10	0/10	2/10				

Conclusion:

In summary, the only effect observed at 500 ppm was a marginal decrease in body weight gain. Therefore, the NOAEL is considered to be 500 ppm (equivalent to 34 mg/kg bw/d).

Re-evaluation by the RMS (2015):

The study was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566).
As a result of the evaluation of dimethenamid-P, for the inclusion of this substance in Annex I to the Directive 91/414/EEC, a NOAEL of 150 ppm (10 mg/kg bw/d) was derived based on elevated blood cholesterol and hepatocellular hypertrophy in females at 500 ppm. However, the RMS brought forward the argument that e.g. a minimal or slight hypertrophy of hepatocytes in 1 or 2 of 10 animals should be considered to be a non adverse effect. The finding was only observed in few animals and is a typical adaptive phenomenon. The expression of the finding was not exactly quantified and can be influenced by a subjective factor of the pathologist" (Reporting table, Dimethenamid-P (Hb) section 4, 11587/ECCO/BBA/01, rev. 3 (13.06.2002) 57/93; ASB2015-560).
Thus, the following can still be concluded. The liver is identified as target organ at 3000 and 1500 ppm. Liver changes showed evidence of recovery. Minimal to slight centrilobular hepatocellular hypertrophy was noted in few female treated with 500 ppm. In accordance with the authors of the study this was considered to be of spontaneous origin and of no toxicological importance. It should be noted that standard deviations of cholesterol values were partially amended in the Table B.6.3-17 of the section "Results and discussions". The only effect observed at 500 ppm was a marginal decrease in body weight gain. Therefore, the NOAEL is still considered to be 500 ppm (equivalent to 34 mg/kg bw/d), on the basis of reduced body weights and body-weight gains in males and females and increased liver weights and centrilobular hepatocyte hypertrophy and increased serum cholesterol in females at 1500 ppm, equal to 98 mg/kg bw per day. The study is still considered to be acceptable.

Data point: KCA 5.3.2

Report: [redacted]., 1988 (TOX1999-422)
SAN 582 H: 13-week dose-range finding study in CD-1 mice
[redacted]
unpublished, March 1, 1988, BASF RegDoc.#88/11360
(Experimental work from September 1986 – December 1986)

Guideline(s):	Range-finding study for oncogenicity study, no guideline specified
Deviations:	Deviations from OECD Guideline No. 408 (adopted 14.05.1981): (1) No examinations performed regarding opthalmology, haematology and clinical chemistry; (2) adrenals were not weighed; (3) histopathology performed only on two tissues (liver and kidney) for control and for 2 of three treatment groups (low and high dose)
GLP:	Yes (laboratory certified by Eidgenössisches Departement des Innern, Berne, Switzerland)
Acceptability:	Due to the limited examinations performed, the study is not considered appropriate to derive a NOAEL for risk assessment purposes. The study is considered to be supplementary.

Materials and methods:

Test Material:	Racemic dimethenamid; SAN 582 H, Lot No. 8605; purity: 91.5 ± 1 %
Test Animals:	CD-1 mice, aged approx. 5 weeks at start of treatment (week 0), mean bw (week 0): males 26 g (23 - 30 g), females 22 g (19 - 25 g), Source: Charles River Breeding Lab., Wiga, Sulzfeld, Germany

Racemic dimethenamid was administered to 12 male and 12 female CD-1 mice at dietary concentrations of 0; 300; 700; 2000 and 5000 ppm over a period of 13 weeks. Analyses for correct concentration were performed prior to study start and at monthly intervals thereafter.
Food consumption and body weight were determined once a week. The state of health was checked twice daily. All animals were subjected to complete gross examinations, and weights of brain, heart, liver, kidney, spleen and testes were determined. Histopathological examinations were confined to the liver and kidney of control, low and high dose animals. The calculated mean test substance intakes are given in Table B.6.3-16.

Table B.6.3-16: Test substance intake

Dietary dose level	Test substance intake (mg/kg bw/d)	
(ppm)	Males	Females
300	46	60
700	105	137
2000	301	383
5000	805	972

Results:

The findings regarding clinical effects and organ weight are summarised in Table B.6.3-17. There was no mortality observed at any dose level. Clinical signs of "subdued behaviour" (no further details given in the report) were observed in 10 male and 10 female mice at 5000 ppm and in one male animal at 2000 ppm. Significant body weight gain depression (43 %) accompanied by only a slight decrease (8 %) in mean weekly food consumption was observed in high dose males; a reduced efficiency of food utilisation was found for these animals. There was no statistically significant effect on body weight at other dose levels in males or at any dose level in females. Absolute and relative liver weights and relative kidney weights were increased in both male and female animals at 2000 and 5000 ppm. Absolute and relative liver weights were also slightly increased in males at 700 ppm.

Table B.6.3-17: Clinical and organ weight findings

	Sex	Dose Level (ppm)				
		0	300	700	2000	5000
Clinical Findings:						
Body wt gain (wk 0-13) [g/mouse/wk; mean±SD]	m	1.1 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	0.9 ± 0.2	0.6 ± 0.1**
	f	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
Food consumption (wk 0–13) [g/mouse/wk; mean±SD; %ctrl]	m	36 ± 1	100 %	97 %	100 %	92 %**
	f	34 ± 2	103 %	100 %	100 %	100 %
Terminal bw [g] (mean±SD; % control)	m	39.8 ± 3.9	97.5 %	96 %	95 %	84 %
	f	28.5 ± 2.4	98 %	98 %	98 %	98 %
Organ Weights:						
Abs. liver wt [g] (mean±SD; % control)	m	1.80 ± 0.24	100 %	113 %	120 %*	125 %**
	f	1.39 ± 0.15	94 %	102 %	115 %**	136 %**
Rel. liver wt [% bw] (mean±SD; % control)	m	4.52 ± 0.44	103 %	117 %$	126 %$$	148 %$$
	f	4.90 ± 0.41	95 %	104 %	118 %$	139 %$$
Rel. kidney wt [% bw] (mean±SD; % control)	m	1.98 ± 0.27	102 %	101 %	111 %$	121 %$$
	f	1.65 ± 0.16	108 %	108 %	114 %$	112 %$

m = male, f = female;
Statistically significant: * = $p < 0.05$; ** = $p < 0.01$ (Dunnett's test)
$ = $p < 0.05$; $$ = $p < 0.01$ (Kruskal-Wallis test)

No treatment-related histopathological changes of the liver or kidney were recorded for any dose group (see Table B.6.3-18). Despite the observation of clearly increased liver weights in high-dose group animals, incidences of hepatocellular hypertrophy or hyperplasia were not reported.

Table B.6.3-18: Histopathological findings

Sex	Male			Female		
Dose Level (ppm)	0	700	5000	0	700	5000
No. Animals examined	12	12	12	12	12	12
Liver						
chronic inflammation, focal	10	5	4	9	8	7
Kidney						
Round cell infiltration	3	1	1	0	2	1
Pelvic lymphoid aggregation	7	8	6	2	8	8
Tubular casts	12	12	12	11	12	12
Cortical cysts	0	0	0	1	0	0

Conclusion:

In a 13-week oral toxicity study with mice that was limited with regard to the extent of examinations performed, a NOAEL of 300 ppm (equivalent to 46 mg/kg bw/d) was found.

Re-evaluation by the RMS (2015):

The study was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566) and was considered to be acceptable as a range-finding study. However, due to the limited examinations performed, it was not considered appropriate to derive a NOAEL for risk assessment purposes from this study. The study is still not considered appropriate to derive a NOAEL for risk assessment purposes. The study is now considered to be supplementary.

Data point:	KCA 5.3.3
Report:	[redacted] 1986 (TOX1999-423) SAN 582 H: 13 week oral toxicity study in dogs [redacted] unpublished, 5 December 1986, BASF RegDoc.#86/11159 (Experimental work from September 1986 –December 1986) Amendment: [redacted] 1986 (TOX1999-424), SAN 582 H: 13 week oral toxicity study in dogs [redacted] unpublished, 15 February 1986, BASF RegDoc.#86/11178 (Additional details on clinical observations, gross necropsy and histological findings)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 82-1 (November 1984)
Deviations:	Deviations from OECD Guideline No. 409 (adopted 12.05.1981): Ornithin decarboxylase and GGT were not determined (LDH and AP were measured instead) The deviations are not considered to have compromised the validity of the study.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; SAN 582 H, Lot No. 8605; purity: 91.4 %
Test Animals:	Purpose bred Beagle dogs; age at start of study: 5 - 7 mo; body weights at start of study: males 5.8 - 7.2 kg, females: 5.3 - 7.2 kg, Source: Marshall Farms, North Rose, New York, U.S.A.

Racemic dimethenamid was administered to groups of 4 male and 4 female purebred Beagle dogs in the diet at concentrations of 0, 100, 750 and 2000 ppm over a period of 13 weeks. Analyses for correct concentrations in the diet were performed periodically during the study.
Food consumption of the animals was determined daily and their body weight once a week. The state of health was checked each day, and detailed physical observations were made during treatment weeks 6 and 13.
Clinical chemistry and haematological examinations as well as urinalyses were carried out once before treatment began and during weeks 6 and 12 of the treatment period.
Ophthalmological examinations were carried out before the beginning of the administration period and at treatment weeks 6 and 13. All animals were subjected to complete gross and histopathological examinations, and selected organs were weighed.

Results:

The correct concentrations of test material were confirmed by analysis for dose groups at 750 and 2000 ppm. At 100 ppm, analytical values were consistently below target and the actual dose was approx. 91.5 ppm. The test substance intake is given in Table B.6.3-19.

Table B.6.3-19: 90-day oral dog study: test substance intake

Dietary dose level	Test substance intake (mg/kg bw/d)	
(ppm)	Males	Females
91.5	4.3	4.6
750	34	40
2000	90	87

The clinical and clinicochemical findings are summarised in Table B.6.3-20). No mortality or treatment-related clinical observations (including opthalmoscopy) occurred at any dose level, with the possible exception of thin appearance noted in 1 to 2 high-dose group dogs. Body weight gains were reduced in both sexes at 2000 ppm and in females at 750 ppm.
Haematology and urinalysis examinations did not reveal any changes that could be associated with treatment with the test substance. In clinicochemical examinations of animals of the highest dose group serum alkaline phosphatase was increased in 7 out of 8 dogs. Elevated plasma ALAT levels were recorded for 2 of 4 high-dose dogs at the last time-point of investigation.

Table B.6.3-20: 90-day oral dog study: clinical and clinicochemical findings

	Sex	Dose level (ppm)			
		0	91.5	750	2000
Clinical findings (Weeks 0–13)					
Body weight gain [kg]	m	2.0	1.9	1.7 ns (-15 %)	0.6** (-70 %)
	f	1.9	2.0	1.3** (-32 %)	0.8*** (-58 %)
Clinical chemistry (Week 12):					
Alkaline phosphatase[IU/L]	m	283 ± 83	275 ± 75 (-3 %)	288 ± 42 ns (+2 %)	482 ± 179 ns (+70 %)
	f	285 ± 130	422 ± 220 ns (+48 %)	302 ± 97 ns (+6 %)	672 ± 197** (+136 %)
ALAT [IU/L]	m	22 ± 6	27 ± 6 ns (+23 %)	27 ± 5 ns (+23 %)	43 ± 37 ns (+95 %)
	f	37 ± 27	26 ± 5 (-30 %)	22 ± 4 ns (-41 %)	49 ± 36 ns (+32 %)
Cholesterol [mM]	m	3.3 ± 0.5	3.8 ± 0.2 ns (+15 %)	3.8 ± 1.1 ns (+15 %)	4.2 ± 0.5 ns (+27 %)
	f	3.3 ± 0.5	3.9 ± 0.4 (+18 %)	4.1 ± 1.3 ns (+24 %)	5.4 ± 0.9** (+64 %)

Statistically significant: * p< 0.05 ** p< 0.01 *** p< 0.001 (Student's t-test)

Pronounced organ weight increases occurred in the livers of high-, and to a lesser degree in mid-dose animals of both sexes (see Table B.6.3-21). Relative thyroid weights were statistically increased in high-dose males; a similar increase in high-dose females was statistically non-significant. Slight effects on organ weight were also noted for the adrenals, heart, kidney, pituitary and thymus, which were regarded to be of doubtful biological significance.

Table B.6.3-21: 90-day oral dog study: organ weight findings

Sex	Males				Females			
Dose level (ppm)	0	91.5	750	2000	0	91.5	750	2000
Terminal bw (kg)	8.4	8.4	8.2	7.1	7.9	7.6	6.9	6.5
Abs. liver wt (g)	261	280	294	326**	237	238	253	313**
Rel. liver wt (% bw x 10)	31.15	33.56	35.9*	46.2***	29.9	31.5	36.4*	48.2***
Abs. thyroid wt (mg)	665	734	723	844	619	803	620	783
Rel. thyroid wt (% bw x 1000)	7.83	8.74	8.68	12.01**	7.78	10.63	8.94	12.12
Abs. thymus wt (g)	8.77	10.28	7.78	4.20*	7.46	10.46	6.47	6.93
Rel. thymus wt (% bw x 100)	10.28	12.39	9.45	5.78*	7.57	13.47	9.52	10.57
Abs. pituitary wt (mg)	60	66	65	65	74	58*	64	55**
Rel. adrenal wt (% bw x 100)	1.11	1.38	1.28	1.65**	1.57	1.45	1.49	1.56
Rel. heart wt (% bw x 10)	8.16	8.19	8.27	7.98	8.03	7.42	8.14	9.08*
Rel. kidney wt(% bw x 10)	6.01	5.90	6.43	6.50	5.12	5.01	5.71	5.88*
Rel. lung wt (% bw x 10)	8.03	8.19	8.05	9.23*	7.41	9.62	8.25	9.29

Statistically significant: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ (Student's t-test)

The histopathological assessment (see Table B.6.3-22) revealed treatment-related changes were confined to the liver. Periportal hepatocellular vacuolation was found in livers of all high-dose dogs, and in 1 male and 1 female dog of the mid-dose group. Inusoidal dilatation was observed at increased incidences in the high-dose group only.

Table B.6.3-22: 90-day oral dog study: histopathological liver findings

Sex	Males				Females			
Dose level (ppm)	0	91.5	750	2000	0	91.5	750	2000
Periportal hepatocellular vacuolation	0/4	0/4	1/4	4/4*	0/4	0/4	1/4	4/4*
Sinusoidal dilatation	0/4	0/4	0/4	3/4	0/4	0/4	1/4	3/4

Statistically significant * $p < 0.05$ (Fisher's Test)

Conclusion:

The liver proved to be the target organ following 13-wk dietary exposure of dogs to the test substance. Based on reduced body weight gains, increased relative liver weights and histopathological liver changes at 750 ppm, the NOAEL was found to be 91.5 ppm (equivalent to 4.3 mg/kg bw/d.

Re-evaluation by the RMS (2015):

The study was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566). The study is still considered to be acceptable.

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point: KCA 5.3.4

Report: [redacted] 1988

	(TOX1999-433; TOX1999-434) SAN 582 H: 52 Week Oral Toxicity Study in Dogs [redacted] unpublished, 24 March 1988, BASF RegDoc.#88/11361 (Experimental work from 24 March 1987 – 24 March 1988)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 83-1, generally followed OECD 452
Deviations:	Deviations from OECD-Guideline 452 (adopted 12.05.1981): Spinal chord was neither preserved nor examined; Historical control data were not included in the report. The deviations were not considered to have compromised the validity of the study.
GLP:	Yes (laboratory certified by The Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; batch No: 8605; purity: 91.3 %
Test Animals:	Beagle dogs, age: 5 - 6 months at start of treatment, bw: 6.5 - 9.6 kg (males) and 5.4 - 7.5 kg (females), Source: Marshall Farms, North Rose, New York, U.S.A.

Racemic dimethenamid was administered to groups of 4 male and 4 female purebred Beagle dogs in the diet at concentrations of 0, 50, 250 and 1500 ppm over a period of 12 month. Analyses to determine stability and homogeneity of the test substance in the diet as well as for correct concentrations were performed.
Food consumption of the animals was determined daily and their body weight once a week. The dogs' state of health was checked once each day.
Clinical chemistry and haematological examinations as well as urinalyses were carried out once before treatment began, and on study weeks 13, 26 and 51.
Ophthalmological examinations were carried out before the beginning of treatment and at study weeks 26 and 51.
All animals were subjected to complete gross and histopathological examinations, and selected organs were weighed.
The mean test substance intake is shown in Table B.6.3-23.

Table B.6.3-23: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)
50	2
250	10
1500	49

Results:

The stability and homogeneity of the test substance in the diet and the correct concentrations were confirmed by analysis.
No mortality occurred during the study and there were no treatment-related effects on clinical signs or food consumption. A significant body weight gain decrease was observed at the high dose indicating that sufficient high dose toxicity was achieved (see Table B.6.3-24). At the mid dose of 250 ppm, the mean body weight gain was decreased. However, this was due to only one animal. The mean body weight gains of the other three animals is very similar to control. In fact, one animal exceeded the

body weight gain of all the control animals. Therefore, the mean body weight gain decrease at 250 ppm is believed to be spurious.

Table B.6.3-24: Body weight gain

Sex	wk	Males				Females			
Diet concentration [ppm]		0	50	250	1500	0	50	250	1500
Mean bw gain [kg] (% controls)	52	2.6 –	2.2 -15	1.7 -35	0.9 -65	2.5 –	2.8 +12	2.3 -8	1.7 -32

Table B.6.3-25: 52 week oral toxicity study in dogs; individual body weight at weekly intervals (kg); male animals in dose group 250 ppm

Group/ Dose Level	Pre treatment		Weeks dosed					Body weight gain (Weeks 0- 52)
3 (250 ppm)	Animal	-2	0	11	25	44	52	
	17	7.2	7.8	9.4	10	10.2	10.4	2.6
	18	6.9	7.3	8.0	8.0	6.9	6.9	- 0.4
	19	7.8	8.5	11.0	11.9	12.1	11.9	3.4
	20	7.5	7.9	9.4	9.4	9.8	9.3	1.4

Table B.6.3-26: 52 week oral toxicity study in dogs; individual daily food consumption at weekly intervals (kg); male animals in dose group 250 ppm

Group/ Dose Level	Pre treatment		Week dosed				
3 (250 ppm)	Animal	-2	1	11	25	44	52
	17	388	375	400	400	400	400
	18	400	400	400	400	400	400
	19	380	400	400	372	400	400
	20	396	384	291	269	400	242

Daily food ration = 400 g per dog per day
* Mean calculated over 6/7 days to accommodate terminal studies

The liver was the target of a mild hepatotoxic effect with several alterations at the high dose (see Table B.6.3-27). These consisted of increased serum alkaline phosphatase and cholesterol observed at some time-points of investigation, increased liver weights and liver histopathology. In addition, liver weights were also increased in females at 250 and 50 ppm.
Histopathological findings that were related to the treatment were confined to the liver of high dose animals. Periportal hepatocyte vacuolation was present in 2/4 males and in all females of the high-dose group. The severity of this change was predominantly minimal/mild (moderate in one animal). In the absence of any evidence of lipid or glycogen accumulation, this finding is most likely to indicate a degenerative change of a hydropic type. This is supported by the corresponding observation of increased AP serum activity levels.

Table B.6.3-27: Clinicochemical, organ weight and histopathological findings

Sex	wk	Males				Females			
Diet concentration [ppm]		0	50	250	1500	0	50	250	1500
AP [U/L]	13	190	157	180	196	162	168	186	421***
	26	141	150	167	254**	134	164	206	426**
	51	133	139	162	196	145	140	140	303**
ALAT [U/L]	26	34	34	42	37	29	24	28	37*
	51	31	34	41	71	23	25	28	41
Cholesterol [mM]	13	3.1	3.6	3.7	4.1	3.9	3.3	4.4	5.5
	26	3.0	3.5	3.6	4.7**	5.2	4.7	5.2	4.9
	51	2.7	3.1	3.4	3.0	4.8	3.8	4.5	5.1
Absolute liver wt [g] (% controls)	52	316	286	296	343	260 –	314 (+21)	291 (+12)	310 (+19)
Relative liver wt [% bw] (% controls)	52	3.07 –	2.87 (-7)	3.24 (+6)	4.00** (+30)	2.91 –	3.47** (+19)	3.34* (+15)	3.85*** (+32)
Periportal hepatocyte vacuolation	52	0/4	0/4	0/4	2/4	0/4	0/4	0/4	4/4
Hepatocell. hypertrophy (predominantly midzonal)	52	0/4	0/4	0/4	2/4	0/4	0/4	0/4	1/4

Statistically significant: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table B.6.3-28: Comparison of relative liver weights of male animals in dose group 250 ppm

Animal	Body weight [kg]	Liver weight [g]	Relative liver weight [%]
17	10.1	332.14	3.29
18	6.8	286.47	4.21
19	11.7	320.89	2.74
20	9.0	243.13	2.70

Table B.6.3-29: Comparison of relative liver weights of male animals in dose group 1500 ppm

Animal	Body weight [kg]	Liver weight [g]	Relative liver weight [%]
25	9.5	411.66	4.33
26	9.1	342.98	3.76
27	7.0	285.51	4.08
28	8.7	333.74	3.83

Table B.6.3-30: 52 week oral toxicity study in dogs; clinical chemistry: during week 51; individual values: males

Group / Dose level	Animal	AST	ALT	AP	Chol
1 (0 ppm)	1	38	43	185	2.7
	2	27	31	94	2.8
	3	30	24	121	2.5
	4	29	26	131	2.7
2 (50 ppm)	9	18	27	108	3.2
	10	22	41	170	3.1
	11	27	41	93	2.8
	12	30	27	184	3.3
3 (250 ppm)	17	32	30	143	2.3
	18	34	55	253	4.5
	19	29	34	135	3.6
	20	26	44	116	3.3
4 (1250 ppm)	25	24	43	264	2.9
	26	23	167	110	3.9
	27	45	42	244	2.4
	28	24	30	167	2.9

Mean values of decreased body weights, cholesterol and AP for male animals in the dose group 250 ppm were decisively influenced by the male dog No 18. Dog No 18, started with a lower body weight than the other 3 male animals in the dose group 250 ppm. The body weight of male dog No 18 increased till week 11 and did not change till week 26. Afterwards there was a loss of the body weight from week 27 to week 52. At the end of the study the male dog No 18 was the only animal in this group that had a loss body weight (0.4 kg) (see Table B.6.3-25). No difference of food consumption was observed in this dose group (see Table B.6.3-26). Dog No 18 showed also higher values of cholesterol and AP in comparison to the three other male animals in the mid dose group 250 ppm (see Table B.6.3-30).
The relative liver weight of dog No 18 was the highest in the mid dose group und was in the range of the male animals in the highest dose group (see Table B.6.3-28 and Table B.6.3-29). Necropsy findings showed many roundworms present in the jejunum of dog No 18. Only single, few or several roundworms were present in single male and female animals of the low and mid dose groups (see Table B.6.3-31 and Table B.6.3-32).
There is evidence that dog No 18 was compromised by a low starting body weight. In addition it can be assumed that the parasite infestation influenced the body weight change from week 27 to week 52. Moreover it cannot be excluded that other effects observed with dog No 18 could also be influenced by parasite infestation.

Table B.6.3-31: Necropsy findings of worms present in male animals

	Animal No.				
Dose group [ppm]					
0		1	2	3	4
		-	-	Several roundworms in the jejunum	-
50		9	10	11	12
		-	-	Few worms in the jejunum	Several roundworms in the jejunum
250		17	18	19	20
		-	Many roundworms in the jejunum	-	-
1500		25	26	27	28
		-	-	-	

Table B.6.3-32: Necropsy findings of worms present in female animals

	Animal No.				
Dose group [ppm]					
0		5	6	7	8
		-	-	-	-
50		13	14	15	16
		-	-	One roundworm in the duodenum	Several roundworms in the duodenum
250		21	22	23	24
		-	Several roundworms in the jejunum	-	-
1500		29	30	31	32
		-	-	-	-

Conclusion:

Following a 52-week dietary administration of racemic dimethenamid to Beagle dogs, adverse effects were observed at feed concentrations of 1500 ppm (reduced body weight gain, increased serum levels of liver specific serum enzymes and transient increase of cholesterol, increased liver weights and histopathological liver findings). Increased liver weights were also observed in females at feed concentrations of 250 and 50 ppm. However, in the absence of a dose response relationship and of corresponding clinicochemical or histopathological findings, these liver weight increases were considered spurious and not toxicologically significant. Therefore, the NOAEL was established at 250 ppm, equivalent to 10 mg/kg bw/d.

Re-evaluation by the RMS (2015):

The study was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566). The study is still considered to be acceptable. It should be noted that the "Result and discussion" section was revised.
As a result of the evaluation of dimethenamid-P, for the inclusion of this substance in Annex I to the Directive 91/414/EEC, a NOAEL of 50 ppm (2 mg/kg bw/d) was derived based on a NOAEL for racemic dimethenamid of 50 ppm (2 mg/kg bw/d). This NOAEL was based on AP, cholesterol increase and body weight decrease. Further details are included in the “Result and discussions” section above.

B.6.3.3 Other routes

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.3.7
Report:	[redacted]., 1990 (TOX1999-420) SAN 582 H: 3-week repeated dermal limit test in rabbits [redacted] unpublished, 3 May 1990, BASF RegDoc.#90/11142 (Experimental work in July 1989
Guideline(s):	OECD Guideline No. 410 (adopted 12 May 1981)
Deviations:	Deviations from OECD Guideline No. 410 (adopted 12 May 1981): None
GLP:	Yes (laboratory certified by Eidgenössisches Departement des Innern, Bern, Switzerland)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; SAN 582 H, Lot No. 8605; purity: 91.6 %; density 1.19 g/cm³, pH 4.23
Test Animals:	Albino New Zealand White rabbits; body weights (Day 0): males 2.391 ± 0.107 kg, females 2.403 ± 0.111 kg, Source: Interfauna, Hun tingdon (U.K.)

Dimethenamid was administered to groups of 5 male and 5 female New Zealand White rabbits dermally (6 h/d; 5 d/week, semi-occlusive dressing) for 3 weeks at doses of 0 (demineralised water) and 1000 µL/kg bw/d (equivalent to 1190 mg/kg bw/d). The test material was applied undiluted. After 6 h the test substance was washed from the skin of animals using polyethylene glycol and water. The cleaning procedure was also performed on the control animals.
Skin reactions were recorded daily. Food consumption and body weight were determined twice weekly. The state of health was checked at least daily. Clinicochemical and hematological examinations were carried out on Days 9 and 19 of treatment. At necropsy, all animals were assessed by gross pathology, and selected organs were weighed. Histopathological examinations were also performed on all animals.

Results:

Skin oedema and erythema were observed in treated animals. This effect reached a maximum during the first week of treatment followed by a gradual recovery which was nearly complete by the end of the study.
Body weights were decreased in the treated group during the first week of treatment. However, this change was transitory and overall body weight gains for the treatment period was similar between controls and treated animals for both sexes. The slight and transient change in body weight is not considered toxicologically significant. Food consumption was not affected by treatment, nor were any alterations of haematology or clinical chemistry parameters observed. There were no treatment-related effects on organ weight and macroscopic findings. Histologically, the only change observed was a minimal to slight hyperkeratosis and acanthosis of the skin with inflammatory cell infiltration.

Conclusion:

Toxicologically significant signs of systemic toxicity were not observed in treated animals under the study conditions. The systemic NOAEL was found to be 1190 mg/kg bw/d.

Re-evaluation by the RMS (2015):

This study with was evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566). The study is still considered to be acceptable.
No significant signs of systemic toxicity were observed. Microscopic findings revealed a minimal to slight hyperkeratosis and acanthosis of the skin with inflammatory cell infiltration followed by complete recovery at study termination. The systemic NOAEL was found to be 1190 mg/kg bw/d, day, the only dose tested. No dermal NOAEL can be derived (<1190 mg/kg bw/d).

B.6.4 Genotoxicity

B.6.4.1 *In vitro* studies

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.4.1
Report:	Wagner V.O. and Coffman N., 1996 (TOX1999-425) Salmonella/Escherichia coli Plate Incorporation Mutagenicity Assay: SAN 1289 H Technical Microbiological Associates, Rockville, Maryland, USA unpublished, 14 March 1996, BASF RegDoc.#96/5403 (Experimental work from January – February 1996)
Guideline(s):	The study was designed to fulfill Japanese MHW guidelines (Notification No. 24). The protocol has been written to comply with EU Legislation No. L 383 A/148; OECD-Guideline 471 and EPA Health Effects Testing Guidelines, Subpart 798.5625.
Deviations:	No deviations from OECD-Guideline 471 (adopted 21.07.1997).
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160, Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid-P; SAN 1289 H Technical; batch No. 6663-50-1; purity: 93.3 % (total dimethenamid), 91.1 % (S-isomer)
Test System:	*Salmonella typhimurium*, strains TA98, TA100, TA1535, TA1537, received from Dr. Bruce Ames, University of California; *Escherischia coli*, strain WP2 uvrA from National Collection of Industrial and Marine Bacteria, Scotland.

Dimethenamid-P (purity 91.1 %) was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 and *Escherichia coli* strain WP2 uvrA were exposed to the test substance dissolved in DMSO at doses ranging from 100 to 5000 µg/plate. The study consisted of a standard plate test both with and without metabolic activation (Aroclor-induced rat liver S-9 mix). Three plates were used per dose for each strain.
For control purposes and to demonstrate the sensitivity of the test system, a negative control and positive controls both without S-9 mix and with metabolic activation were tested.

Results:

The mean number of revertant colonies was not increased in *Salmonella* strains TA 98, TA 1535, TA

1537 and *E coli* strain WP2 uvr A, either with or without S-9 activation and with strain TA100 with S-9 activation. However, an increase in revertants was observed with strain TA 100 without S-9 activation.
Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:

According to the results of the study, dimethenamid-P is not mutagenic in the Ames reverse mutation assay with strains TA 98, TA 1535, TA 1537 and *E. coli* strain WP2 uvr A with or without metabolic activation and with strain TA100 with activation. A dose-related positive response (4.1-fold, maximum increase) was observed with tester strain TA 100 without activation, which was confirmed in a repeat experiment (2.3-fold, maximum increase).
The response pattern noted with TA 100 was possibly indicative of a contaminant-induced effect. To test this hypothesis two additional Ames assays were conducted which compared results using: (a) the previously tested technical grade material (Engelhardt G., Hoffmann H., 1997; TOX1999-426) and (b) a highly purified batch of dimethenamid-P (Engelhardt G. and Hoffman H., 1997; TOX1999-427).

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.4.1
Report:	Engelhardt G., Hoffmann H., 1997 (TOX1999-426) Salmonella Typhimurium/Escherichia coli Reverse Mutation Assay with S-dimethenamid Technical BASF AG, Ludwigshafen, Germany unpublished, 5 June 1997, BASF RegDoc.#97/10622 (Experimental work: March/April 1997)
Guideline(s):	OECD Guidelines No. 471 and Draft of OECD-Guideline 472; EEC Directive 92/69, B14 and B13
Deviations:	Deviations from OECD Guideline No. 471 (adopted 21.07.1997): Stability of test substance in DMSO and in water was not determined analytically. Stability of test substance throughout study period was not determined analytically. The deviations are not considered to have had an impact on the validity of the study results.
GLP:	Yes (laboratory certified by Ministerium für Arbeit, Soziales und Gesundheit, Postfach 31 80, 55021 Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid-P; batch No. 6663-50-1; purity: 91.1 %
Test System:	*Salmonella typhimurium*, strains TA98, TA100, TA1535, TA1537, received from Knoll AG; *Escherischia coli*, strain WP2 uvrA from Merck

Dimethenamid-P (purity 91.1 %) was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay (standard plate test and preincubation test). The *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 and *Escherichia coli* strain WP2 uvrA were exposed to the test substance dissolved in DMSO at doses ranging from 20 to 5000 µg/plate. The study consisted of a standard plate test and preincubation test both with and without metabolic activation (Aroclor induced rat liver

S-9 mix). Three plates were used per dose for each strain.
For control purposes and to demonstrate the sensitivity of the test system, a negative control and positive controls both without S-9 mix and with metabolic activation were tested.

Results:

Depending on the strain and test conditions, a bacteriotoxic effect was observed at doses from about 500 µg/plate (strain TA 98) - 2500 µg/plate (other strains) onward. The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:

According to the results of the study, dimethenamid-P is not mutagenic in the Ames bacterial reverse mutation assay.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.4.1
Report:	Engelhardt G. and Hoffman H., 1997 (TOX1999-427) Salmonella typhimurium/Escherichia coli Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with s-dimethenamid BASF AG, Ludwigshafen, Germany unpublished, 5 June 1997, BASF Reg.Doc.#97/10621 (Experimental work: March/April 1997)
Guideline(s):	OECD Guidelines 471 and Draft of OECD-Guideline 472; EEC Directive 92/69, B14 and B13
Deviations:	Deviations from OECD Guideline No. 471 (adopted 21.07.1997): Stability of test substance in DMSO and in water was not determined analytically Stability of test substance throughout study period was not determined analytically The deviations are not considered to have had an impact on the validity of the study results.
GLP:	Yes (Laboratory certified by Ministerium für Arbeit, Soziales und Gesundheit, Postfach 31 80, 55021 Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid-P; analytical grade reference standard; batch No. RS 1289-111596; purity: 99.4 %
Test System:	*Salmonella typhimurium*, strains TA98, TA100, TA1535, TA1537, received from KNOLL AG; *Escherischia coli*, strain WP2 uvrA from Merck

Dimethenamid-P (99.4 % purity, analytical grade reference standard) was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 and *Escherichia coli* strain WP2 uvrA were exposed to the test substance dissolved in DMSO at doses ranging from 20 to 5000 µg/plate (standard plate test) and from 4 to 2500 µg/plate (preincubation test). The study consisted of a standard plate test and preincubation test both with and

without metabolic activation (Aroclor induced rat liver S-9 mix). Three plates were used per dose for each strain.
For control purposes and to demonstrate the sensitivity of the test system, a negative control and positive controls both without S-9 mix and with metabolic activation were tested.

Results:

Depending on the strain and test conditions a bacteriotoxic effect was observed at doses ≥2500 µg/plate in the standard plate test and at doses ≥500 µg/plate in the preincubation test. The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:

According to the results of the study, analytically grade pure dimethenamid-P is not mutagenic in the Ames bacterial reverse mutation assay.
Thus, conflicting results had been obtained between the two studies with dimethenamid-P technical. To further evaluate the findings, a third Ames assay was conducted at the original laboratory in which the positive finding was noted.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.4.1
Report:	Wagner V.O. and Klug M.L., 1997 (TOX1999-428) Bacterial reverse mutation assay: SAN 1289 H technical MA BioServices, Inc., Rockville, Maryland, USA unpublished, 24 June 1997, BASF RegDoc.#97/5271 (Experimental work: June 1997)
Guideline(s):	OECD Guidelines 471 and Draft of OECD-Guideline 472
Deviations:	Deviations from OECD Guideline No. 471 (adopted 21.07.1997): Only 1 strain of *Salmonella typh.* was used. The test article was only tested in the absence of S9 activation. The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility. Analyses of the test or control mixtures were not performed by the testing facility. The stability of the test or control article under the test conditions has not been determined by the testing facility.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be supplementary.

Materials and methods:

Test Material:	Dimethenamid-P (SAN 1289); batch no. 6663-50-1, purity: 91.1 %
Test System:	*Salmonella typhimurium*, strain TA100, received from Dr. Bruce Ames, Uni versity of California.

Dimethenamid-P was tested for its mutagenic potential based on the ability to induce back mutations in the Ames reverse mutation assay. The *Salmonella typhimurium* strain TA 100, was exposed to the

test substance dissolved in DMSO at doses ranging from 100 to 5000 µg/plate. The study consisted of a standard plate test without metabolic activation (Aroclor induced rat liver S-9 mix). Three plates were used per dose.
For control purposes and to demonstrate the sensitivity of the test system, a negative control and positive controls were tested.

Results:

At 3333 µg/plate and above, bacterial toxicity was indicated by a slight to moderate reduction of the background microcolony lawn. A positive response was not observed for revertant colonies.
Expected increases in revertant colonies were obtained with the positive control.

Conclusion:

According to the results of the study, dimethenamid-P is not mutagenic in the Ames reverse mutation assay with strain TA100 without metabolic activation.

Re-evaluation by the RMS (2015):

Due to the deviations from OECD Guideline the study is still considered to be supplementary.

Data point:	KCA 5.4.1
Report:	Haworth L. and Lawlor T.E., 1989 (TOX1999-459) Mutagenicity test on SAN 582 H in the ames salmonella/ microsome reverse mutation assay Hazleton Laboratories America, Inc., Kensington, Maryland, U.S.A. unpublished, 8 June 1989, BASF RegDoc.# 89/11032 (Experimental work from 16 March 1989 – 17 May 1989)
Guideline(s):	Not specified, but generally followed OECD 471
Deviations:	Deviations from OECD Guideline No. 471 (adopted 21.07.1997): Analyses of the test or control mixtures were not performed by the testing facility. The stability of the test or control article under the test conditions has not been determined by the testing facility.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid (582 H), batch no. 8605, purity 91.4 %
Test System:	*Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, received from Dr. Bruce Ames, University of California.

The potential of racemic dimethenamid (lot no. 8605; 91.4 % pure) to induce point or gene mutations in bacteria was tested using the Ames reverse mutation assay racemic dimethenamid diluted in DMSO was tested using *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 either with or without metabolic activation. The doses applied ranged from 50 to 6500 µg/plate in the non-activation experiments and from 100 to 10000 µg/plate in the activation tests.

Results:

Slight test article precipitate was observed at concentrations of 2500 and 5000 µg/plate in the

activation experiments and at 2500 µg/plate in the nonactivation test. Moderate precipitation was observed at 10000 and 6500 µg/plate in the activation and nonactivation tests, respectively. No evidence of mutagenic activity was observed with any of the tester strains used either with or without metabolic activation. The positive control treatments in both the non-activation and S9 activation assays induced expected increases in the revertant numbers with all the indicator strains under study, demonstrating the effectiveness of the S9 activation system and the ability of the test system to detect known mutagens.

Conclusion:

Racemic dimethenamid was not mutagenic in the Ames reverse mutation assay.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.4.3
Report:	[redacted], 1996 (TOX1999-429) CHO/HGPRT Mutation Assay: SAN 1289 H Technical [redacted] unpublished, 5 April 1996, BASF RegDoc.#96/5404 (Experimental work: January – February 1996)
Guideline(s):	OECD Guideline No. 476 (adopted April 1984)
Deviations:	Deviations from OECD Guideline No. 476 (adopted 21.07.1997): The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility. Analyses of the test or control mixtures were not performed by the testing facility. The stability of the test or control article under the test conditions has not been determined by the testing facility. Exposure to the maximum dose level tested (400 µg/mL) without activation was not high enough to result in cytotoxicity in the range of 10–20 % relative cloning efficiency as required by Guidelines. The negative test result was not confirmed by a second trial, a justification was not given.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be supplementary.

Materials and methods:

Test Material:	Dimethenamid-P; batch No. 6663-50-1; purity: 96.3 % (total dimethenamid technical); 91.1 % (S-dimethenamid)
Test System:	CHO-K1-BH4 cells from Dr. A. Hsie, Oak Ridge National Laboratories, Oak Ridge, Tennessee, USA.

Dimethenamid-P was tested for its ability to induce gene mutations at the HGPRT locus in Chinese hamster ovary (CHO) cells *in vitro*. Tests were conducted both with and without exogenous metabolic activation (Aroclor induced rat liver S-9 mix). Dose levels were selected based on results from a preliminary toxicity assay. The cell line (CHO-K1-BH4) was treated for 5 h. The concentrations of test substance dissolved in DMSO were from 100 - 400 µg/mL without S-9 mix, and from 100 - 450 µg/mL with S-9 mix. A vehicle negative control was used as well as appropriate positive controls

to demonstrate the sensitivity of the test system both with and without metabolic activation.

Results:

The treatment medium was cloudy, but without visible precipitate at concentrations ≥400 µg/mL. Concentrations of ≤350 µg/mL were soluble in treatment medium.
Toxicity (i.e. reduction of the relative cloning efficiency) occurred at doses of 350 µg/mL without activation (41 %) and at 400 and 450 µg/mL with activation (33 % and 22 %, respectively).
Positive controls gave the expected results. With the test material, no biologically significant increases in the mutant frequency were observed either with or without metabolic activation.

Conclusion:

It is concluded that dimethenamid-P is not mutagenic in the CHO/HGPRT test.

Re-evaluation by the RMS (2015):

Due to the many deviations from OECD Guideline the study is now considered to be supplementary.

Data point:	KCA 5.4.3
Report:	[redacted]., 1986 (TOX1999-460) Evaluation of the mutagenic activity of SAN 582H in an *in vitro* mammalian cell gene mutation test with V79 Chinese hamster cells [redacted] unpublished, 6 June 1986, BASF RegDoc.# 86/11167 (Experimental work from 24 October 1985 – 1 May 1986)
Guideline(s):	OECD Guideline No. 476 (adopted 4 April 1984)
Deviations:	Deviations from OECD Guideline No. 476 (adopted 21.07.1997): Cells were exposed to the test substance for 2 h only (OECD Guideline recommends an effective exposure period of 3 - 6 h). The authors justified the short exposure duration by claiming that S9-mix toxicity would pose a problem in case of longer exposure duration.
GLP:	Yes (laboratory certified by Staatstoezicht op de Volksgezondheid, NL, Section GLP of the Veterinary Public Health Inspectorate)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid (SAN 582 H), batch no. 8502, purity 92 %
Test System:	V79 Chinese hamster cells, provided by Department of Toxicology, Agricultural University of Wageningen, Netherlands.

The potential of racemic dimethenamid (lot no. 8502; 92 % pure) to induce point mutations in mammalian cells was investigated at the hypoxanthine guanine-phosphoribosyl transferase (HGPRT) locus of V79 Chinese hamster ovary (CHO) cells. The CHO cells were exposed for 2 h to the test substance (dissolved in ethanol) at dose levels ranging from 33 - 333 µg/mL both with and without metabolic activation (Arochlor 1254-induced rat liver S-9 mix). An independent repeat of the study was conducted.

Results:

Cytotoxicity was expressed by a reduced cloning efficiency compared to control. In the preliminary toxicity test, cytotoxicity was observed at concentrations of 167 µg/mL and higher, and complete cell

death occurred at concentrations of 500 µg/mL and higher. In the first mutagenicity test, cytotoxicity was observed at a concentration of 333 µg/mL with and without S-9. Cytotoxicity was not observed in the repeat mutagenicity experiment.
The frequency of mutant colonies was not increased with the application of the test substance in either mutagenicity experiment. Under the same conditions positive control chemicals, ethyl methanesulphonate and dimethylnitrosamine, produced a significant increase in the incidence of mutant colonies.

Conclusion:

Racemic dimethenamid was found to be non-mutagenic in the V79/HGPRT mammalian cell mutation assay under the experimental conditions described in this report.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.4.3
Report:	[redacted] 2013b (ASB2014-8389) BAS 656-PH - *In vitro* gene mutation test in L5178Y mouse lymphoma cells (TK +/- locus assay, microwell version) [redacted] BASF RegDoc 2013/1003738 ! 52M0442/08M015 (Experimental work from 24-Jul-2012 to 16-May-2013)
Guideline(s):	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, OECD 476 (1997), EPA 870.5300
Deviations:	Deviations from OECD Guideline No. 476 (adopted 21.07.1997): no deviations which compromise the conclusions of the study
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	BAS 656-PH
Description:	Liquid; brown, clear
Lot/Batch #:	COD-001509
Purity:	95.9 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in DMSO. The stability of the test substance under storage conditions over the test period was guaranteed until 01 Oct 2013 by the sponsor, and the sponsor holds this response bility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control -S9:	Methyl methanesulfonate (MMS): 15 µg/mL (4-hour exposure period) and 5 µg/mL (24-hour exposure period)

Positive control +S9: Cyclophosphamide (CPA) 2.5 µg/mL
Activation:
S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3) parts followed by centrifugation at 9000 g.
An appropriate quantity of S9 supernatant was mixed with an equal volume of S9 cofactor solution.
Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
Phosphate buffer (pH 7.4)	15 mM

Test organism:
The L5178Y cell line, which is characterised by a high proliferation rate (doubling time 9-10 h in stock cultures) and a high cloning efficiency of about 90 %. The cells have a stable karyotype with a near diploid number of 40 ± 1 chromosomes. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in “THMG" medium (pretreatment medium A), and for the following 3 days in “THG" medium (pretreatment medium B).
Culture media:
Complete culture medium:
RPMI 1640 medium supplemented with 1 % (v/v) penicillin/streptomycin (10,000 IU/10,000 µg/mL) 1 % (v/v) sodium pyruvate (10 mM) (RPMI-0); foetal calf serum was added with respect to the respective treatment conditions: 5 % for the 4-hour exposure interval (RPMI-5), 10 % for the 24-hour exposure interval (RPMI-10), 20 % for cloning efficiency and selection (RPMI-20).
Selection medium: RPMI-20 (complete culture medium) by addition of 4 µg/mL TFT
Pretreatment medium A
("THMG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), methotrexate (0.1 µg/mL), glycine (7.5 µg/mL).
Pretreatment medium B
("THG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), glycine (7.5 µg/mL).
Locus examined: Thymidine Kinase Locus (TK+/-)
Test concentrations:
a) Preliminary toxicity assay: Nine concentrations ranging from 11.3 to 2900 µg/mL
b) Mutation assay:
1st experiment: 6.25, 12.5, 25.0, 50.0, 100. 0, 200.0 and 400.0 µg/mL without metabolic activation
3.13, 6.25, 12.5, 25.0, 50.0, 100.0 and 200.0 µg/mL with metabolic activation
2nd experiment: 3.13, 6.25, 12.5, 25.0, 50.0, 100.0 and 200.0 µg/mL without metabolic activation
4.69, 9.38, 18.75, 37.50, 75.0, 150.0 and 200.0 µg/mL with metabolic activation
A slight increase in the mutation frequency was observed in the 2nd Experiment in the presence of metabolic activation. To corroborate this finding a further experiment was performed designated 3rd Experiment.
3rd experiment: 6.25, 12.5, 25.0, 50.0, 100. 0, 150.0 and 200.0 µg/mL with metabolic activation
Preliminary cytotoxicity assay:
In the pretest for toxicity based on the purity and the molecular weight of the test substance 2900 µg/mL (approx. 10 mM) BAS 656-PH was used as top concentration both with and without S9 mix at 4 hour exposure time and without S9 mix at 24 hour exposure time. The pretest was performed

following the method described for the main experiment. The relative suspension growth (RSG) was determined as toxicity indicator for dose selection and as well as precipitation, pH value and osmolarity.
Mutation Assay:
Cell treatment and expression:
For each test group, about 1 x 10^7 cells per flask were seeded into 75 cm^2 flasks. Two cultures were treated in parallel for each test group. Subsequently the treatment medium was added. The cultures were incubated for the respective exposure period. In case of experiments without metabolic activation the treatment medium consisted of 19.8 mL RPMI-5/10 plus 0.2 mL positive control, test substance preparation or vehicle, respectively. In case of metabolic activation the treatment medium consisted of 19 mL RPMI-5/10 medium, 0.2 mL positive control, test substance preparation or vehicle and 0.8 mL S9-mix, respectively.
Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5 % CO_2, 37 °C and ≥90 % humidity. At the end of the exposure period, the cells were transferred in tubes, centrifuged for 5 minutes at 1000 rpm and resuspended in RPMI-5 medium. The washing of the cells was repeated at least once. Then the cells were centrifuged and resuspended in RPMI-10 medium. From each test group a sample of treated cells (2 x 10^5 cells/mL or 6 x 10^6 cells/flask) were pipetted in 75 cm^2 flasks and were incubated for a 2-day expression period. To maintain exponential growth during this phase, each culture was counted daily and the cell numbers were adjusted at each day to 2 x 10^5 cells/mL in 30 mL RPMI-10 medium.
For the selection of the mutants, 5 x 10^5 cells from each test group were resuspended in 50 mL selection medium ("TFT" medium; 1 x 10^4 cells/mL). Per test group 200 µL were dispensed in each well of two 96-well plates (2000 cells/well). After incubation for at least 9 days, both the number of negative wells and the number of wells containing small or large colonies were scored for calculation of the mutant frequency (MF). The viability (cloning efficiency) was determined after the expression period, 2 days after end of exposure. The cells were centrifuged and 400 cells from each test group were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per test group 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well) and evaluated after at least 9 days of incubation. For calculation of the suspension growth (SG) and the relative total growth (RTG) the cell counts determined within the expression period at 2^{nd} and 3^{rd} passage after exposure in the case of 4-hour exposure and 1^{st}, 2^{nd} and 3^{rd} passage after exposure in the case of 24-hour exposure were used.
Size distribution of the colonies:
The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Small colonies are defined as less than 1/4 of the diameter of the well. Size is the key factor and morphology (the optical density of the small colonies is considerably higher) should be secondary.

Calculations:
Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{-\ln(total\ number\ of\ empty\ wells / total\ number\ of\ seeded\ wells(96))}{number\ of\ seeded\ cells(2000)}\ x\,10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_2} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_x = \frac{-\ln(total\ number\ of\ empty\ wells / total\ number\ of\ seeded\ wells(96))}{number\ of\ seeded\ cells\ perwell(1.6)}\ x100$$

relative, in comparison to control:

$$RCE_x = \frac{CE_x\ of\ the\ test\ group}{CE_x\ of\ the\ negative\ /\ vehicle\ control} \times 100$$

Relative total growth (RTG):

$$RTG = \frac{RSG \times RCE_2}{100}$$

$$RSG = \frac{Suspension\ Growth\ of\ the\ test\ group}{Suspension\ growth\ of\ the\ negativ\ /\ vhiclecontrol} \times 100$$

Statistics:
An appropriate statistical trend test (SAS procedure PROC REG; 9) was performed to assess a possible dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective negative/vehicle control groups. A trend was judged as statistically significant whenever the p-value was below 0.10 and the slope was greater than 0. However, both, biological and statistical significance has been considered together.
Evaluation criteria:
The test item is considered mutagenic if all of the following criteria are met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 106 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures of one experiment.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 106 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.

However, in the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls and the mutation rates of all negative and vehicle controls of this study were taken into consideration.
Results of test groups have been rejected if the relative total growth (RTG) and/or the cloning efficiency 1 (CE1) were less than 10 % of the respective negative/vehicle control.
Whenever a test substance is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose-related shift in the ratio of small versus large colonies clastogenic effects are indicated.

Results:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (BASF study code 01Y0442/08Y014).
The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 11.3 µg/mL and 2900 µg/mL (equal to a molar concentration of approximately 10 mM) were used. After 4 hours treatment in the absence of S9 mix cytotoxicity indicated by reduced relative suspension growth of about or below 20 % was observed at 362.5 µg/mL and above. In addition, in the presence of S9 mix, clearly reduced relative suspension growth was observed after treatment with 90.6 µg/mL and above. After 24 hours treatment in the absence of S9 mix reduced relative suspension growth of below 20 % was observed after treatment with 90.6 µg/mL and above. In culture medium test substance precipitation occurred at 725 µg/mL and above after 4 hours treatment in the absence and the presence of S9 mix, and from 1450 µg/mL onward after 24 hours treatment in the absence of S9 mix. There was no relevant shift of the osmolarity and pH value even at the maximum concentration of the test item. The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 400.0 and 200.0 µg/mL without and with metabolic activation.
In this study, in the absence and the presence of S9 mix no precipitation in culture medium was observed up to the highest applied test substance concentration in the main experiments.
In the absence of S9 mix in the 1st experiment there was a strong decrease in the number of colonies at 400 µg/mL after an exposure period of 4 hours. In the 2nd experiment after an exposure period of 24 hours the relative total growth was strongly reduced from 100 µg/mL onward. In both experimental parts the cell densities were distinctly reduced at the highest applied concentrations each. Thus, these test groups were discontinued within the expression period.
In addition, in the presence of metabolic activation, a clear reduction of the relative total growth was observed at 100 µg/mL in the 1st experiment and at 200 µg/mL in the 2nd experiment. In the 3rd experiment, a reduction of the relative total growth was observed at 200 µg/mL. In the 1st and 3rd experiment the cell densities were distinctly reduced at the highest applied concentration of 200 µg/mL each. Thus, these test groups were discontinued within the expression period.
No biologically relevant increase in the number of mutant colonies was observed without S9 mix. In detail, in the 1st experiment after 4 hours treatment the values for the corrected mutation frequencies were close to the respective vehicle control value and clearly within the range of the historical negative control data. In addition, in the 2nd experiment after 24 hours treatment the values for the corrected mutation frequencies were close to the respective vehicle control value and nearby the range of historical negative control data. The corrected mutant frequencies obtained for both experiments were always below the calculated threshold.
In the presence of S9 mix a statistically significant dose-related increase of mutant frequencies was observed in the 1st and 2nd experiment. However, these results were not confirmed in a 3rd experiment. In detail, in the 1st experiment after 4 hours treatment the values for the corrected mutation frequencies were close to the respective vehicle control value and nearby the range of the historical negative control data. In the 2nd experiment the corrected mutant frequencies were clearly increased at higher concentrations showing dose-related cytotoxicity as indicated by relative total growth below 50 % of control. The values of the test groups from 37.5 µg/mL onward were either clearly above the respective vehicle control value or clearly above the range of the historical negative control data. At 75 and 200 µg/mL, strong growth depression and mutation frequencies clearly exceeding the mutation frequency threshold were observed. In the confirmatory 3rd experiment in the presence of S9 mix the values for the corrected mutation frequencies were close to the respective vehicle control values and nearby the historical negative control range data. In this experimental part, the highest applied concentration of 200 µg/mL was not scorable for mutation potency due to strong cytotoxicity (Table B.6.4-1, Table B.6.4-2).
The statistical analyses of all data sets by testing for linear trend led to a negative finding for the 2nd experiment in the absence of S9 mix and for the 3rd experiment in the presence of S9 mix. In the 1st experiment in the absence and presence of S9 mix a statistically significant dose-related increase of mutant frequencies was obtained. However, in this experiment all values were clearly below the respective mutant frequency threshold and, therefore, the statistical finding was regarded as

biologically irrelevant. In addition, in the 2nd experiment in the presence of S9 mix the linear trend analyses led to a statistically significant dose-related increase of mutant frequencies. Two values were clearly above the respective mutant frequency threshold. However, this finding occurred at a clearly cytotoxic concentration and it was not corroborated in a confirmatory experiment. Therefore, this finding has to be regarded as biologically irrelevant.
The positive control substances MMS and CPP induced clearly increased mutant frequencies as expected. The values of the corrected mutant frequencies clearly exceeded the respective calculated thresholds for a mutagenic effect based on the global evaluation factor (GEF: 126 plus the mutant frequency of the respective negative control). In addition, the corrected mutant frequencies were clearly within the historical positive control data range.

Table B.6.4-1: Gene mutation in mammalian cells – experimental parts without S9 mix

				Cytotoxicity		**Genotoxicity (colonies per 10^6 cells)**	
	Conc. µg/mL	S9 mix	Prec.*	Relative cloning efficiency 1 (RCE_1, %)	Relative total growth (RTG, %)	Corrected mutant frequency ($MF_{corr.}$)	Mutant frequency threshold**
Experiment I/4 h treatment							
Vehicle control[1]		-	n.d.	100.0	100.0	64	190
Test item	6.25	-	-	99.2	93.7	47	190
Test item	12.50	-	-	95.6	73.3	58	190
Test item	25.00	-	-	101.5	68.2	54	190
Test item	50.00	-	-	103.1	68.0	57	190
Test item	100.00	-	-	90.7	60.0	70	190
Test item	200.00	-	-	77.4	37.9	66	190
Test item	400.00	-	-	2.2	n.c.	n.c.	190
Positive ontrol[2]		-	n.d.	62.2	34.2	1078	190
Experiment II/24 h treatment							
Vehicle control[1]		-	n.d.	100.0	100.0	36	162
Test item	3.13	-	-	131.5	77.8	51	162
Test item	6.25	-	-	125.7	73.2	42	162
Test item	12.50	-	-	100.8	75.2	39	162
Test item	25.00	-	-	117.3	54.4	49	162
Test item	50.00	-	-	101.5	42.9	50	162
Test item	100.00	-	-	40.3	7.9	69	162
Test item	200.00	-	-	3.5	n.c.	n.c.	162
Positive ontrol[3]		-	n.d.	69.5	43.9	397	162

* : Precipitation in culture medium at the end of exposure period
** : Mutant frequency threshold: number of mutant colonies per 10^6 cells of current vehicle plus 126
n.c. : Culture was not continued due to strong cytotoxicity
1 : DMSO 1 % (v/v)
2 : MMS 15.0 µg/mL
3 : MMS 5.0 µg/mL

Table B.6.4-2: Gene mutation in mammalian cells – experimental parts with S9 mix

				Cytotoxicity		Genotoxicity (colonies per 10^6 cells)	
	Conc. µg/mL	S9 mix	Prec.*	Relative cloning efficiency 1 (RCE_1, %)	Relative total growth (RTG, %)	Corrected mutant frequency ($MF_{corr.}$)	Mutant frequency threshold**
Experiment I/4 h treatment							
Vehicle control[1]		+	n.d.	100.0	100.0	49	175
Test item	3.13	+	-	124.9	81.0	51	175
Test item	6.25	+	-	99.3	70.1	46	175
Test item	12.50	+	-	103.0	55.4	59	175
Test item	25.00	+	-	96.4	34.0	53	175
Test item	50.00	+	-	92.3	21.7	99	175
Test item	100.00	+	-	85.9	15.4	97	175
Test item	200.00	+	-	59.7	n.c.	n.c.	175
Positive ontrol[2]		+	n.d.	82.9	37.1	499	175
Experiment II/4 h treatment							
Vehicle control[1]		+	n.d.	100.0	100.0	59	185
Test item	4.69	+	-	105.3	109.1	66	185
Test item	9.38	+	-	101.5	82.5	95	185
Test item	18.75	+	-	84.8	78.7	74	185
Test item	37.50	+	-	61.1	43.4	140	185
Test item	75.00	+	-	56.7	32.0	191	185
Test item	150.00	+	-	56.7	26.2	156	185
Test item	200.00	+	-	45.0	14.1	270	185
Positive ontrol[2]		+	n.d.	48.7	28.1	922	185
Experiment III/4 h treatment							
Vehicle control[1]		+	n.d.	100.0	100.0	61	187
Test item	6.25	+	-	100.8	83.8	77	187
Test item	12.50	+	-	101.6	81.9	70	187
Test item	25.00	+	-	89.8	64.0	68	187
Test item	50.00	+	-	86.5	49.3	84	187
Test item	100.00	+	-	79.3	45.1	118	187
Test item	150.00	+	-	65.4	28.1	75	187

				Cytotoxicity		Genotoxicity (colonies per 10^6 cells)	
Test item	200.00	+	-	41.6	n.c.	n.c.	187
Positive ontrol[2]		+	n.d.	61.8	48.4	685	187

* : Precipitation in culture medium at the end of exposure period
** : Mutant frequency threshold: number of mutant colonies per 10^6 cells of current vehicle plus 126
n.c. : Culture was not continued due to strong cytotoxicity
[1] : DMSO 1 % (v/v)
[2] : CPP 2.5 µg/mL

Conclusion:

This study was submitted with the dossier for the Renewal Assessment Report. The study is considered to be acceptable.
Based on the results of the study it is concluded that under the conditions of the test BAS 656-PH does not induce forward mutations in the TK+/- locus in L5178Y cells *in vitro*.

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point: KCA 5.4.2

Report: [redacted]., 1996 (TOX1999-430)
Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells: SAN 1289 H Technical
[redacted]
unpublished, 23 February 1996, BASF RegDoc.#96/5400
(Experimental work: December 1995-February 1996)

Guideline(s): Not specified, generally followed OECD No. Guideline 473

Deviations: Deviations from OECD Guideline No. 473 (adopted 21.07.1997):
The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility.
Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.
The stability of the test or control article under the test conditions has not been determined by the testing facility.
The equivocal test result was not clarified by further testing.

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is now considered to be not acceptable.

Materials and methods:

Test Material: Dimethenamid-P; batch No. 6663-50-1; purity: 96.3 % (total dimethenamid technical); 91.1 % (S-dimethenamid)
Test System: CHO-K1 cells (repository number CCL 61) from American Type Culture Col lection, Rockville, MD

Dimethenamid-P was assessed for its potential to induce structural chromosome aberrations in Chinese hamster ovary cells *in vitro* both with and without metabolic activation (Aroclor-induced S-9). The test concentrations were selected according to results from a preliminary toxicity assay. The test

concentrations used in the mutagenicity assay ranged from 2 - 120 µg/mL for the non-activated portion and from 8 - 500 µg/mL in the activated portion of the study. The test substance was dissolved in DMSO and the treatment period was approx. 20 h for the non-activated portion and 4 h for the activated portion of the study. Chromosomes were prepared approx. 20 h after the initiation of treatment for both the activated and non-activated portions of the study. In each experiment, duplicate cultures were used. About 2 h prior to harvesting the cells, colcemid was added to arrest the cells in metaphase. 200 metaphases per concentration tested were scored for structural chromosome aberrations. Appropriate negative and positive controls were included in the testing.

Results:

Cytotoxicity was evidenced at the highest doses tested in both the non-activated and activated portions of the study as evidenced by cell growth inhibition. A statistically significant increase in chromosomally damaged cells was observed without S-9 mix at 60 µg/mL, but not at 120 µg/mL, the highest concentration tested (Table B.6.4-3). In the presence of exogenous metabolic activation, a small, dose related increase of chromosomal aberrations was established, which however was statistically non-significant. The positive control substances showed distinct increases in cells with structural chromosome aberrations, indicating the sensitivity of the assay.

Table B.6.4-3: Results of *in vitro* cytogenetic test

Treatment		**Cells scored**	**Number and type of aberration**							**No. of aberrations per cell[1] (Mean)**	**% Cells with aberrations**
			Chromatid			Chromosome			SDC		
µg/mL	S9		G	TB	EX	SB	D	R			
0	–	200	5	0	0	0	0	0	0	0.000	0.0
15	–	200	6	0	0	0	0	0	0	0.000	0.0
30	–	200	5	0	0	0	0	0	0	0.000	0.0
60	–	200	6	5	0	0	0	0	0	0.025*	2.5*
120	–	200	8	3	0	0	0	0	0	0.015	1.5
MMC 0.08	–	200	10	28	5	3	0	0	0	0.185**	15.5**
0	+	200	1	1	0	0	0	0	0	0.005	0.5
63	+	200	5	0	1	0	0	0	0	0.005	0.5
125	+	182	3	0	1	1	0	0	0	0.011	1.1
250	+	160	5	1	3	0	0	0	0	0.025	2.5
500	+	95	3	3	0	0	0	0	0	0.032	3.2
CP 10	+	200	4	24	8	5	0	0	1	0.235**	16.5**

MMC = Mitomycin C (pos. control); CP = Cyclophosphamide (pos. control); G = Gaps; SB = Chromosome break; TB = Chromatid break; D = Dicentric; EX = Exchange; R = Ring; SDC = Severely Damaged Cells

[1] Severely damaged cells (SDC) were counted as 10 aberrations.

Significantly different from control (Fisher's Exact Test): * $p < 0.05$, ** $p < 0.01$

Note: The historical control data supplied within the original study report were not appropriate for comparison with the DMSO control values obtained in this study, because the historical control data included treatments with other solvents (ethanol, acetone) and with a variety of substances that were not used in this case. Therefore, the percentage range of aberrant cells reported for historical controls is not comparable with the solvent control data of this study.

Conclusion:

In the absence of metabolic activation, the increase of aberrant cells was statistically increased at one dose level; however, a dose-response relationship could not be established. In the presence of S9-mix, a small, albeit dose-related increase in the percentage of aberrant cells was observed, which however was statistically non-significant. It is concluded that under the study conditions employed in the cytogenetic test, dimethenamid-P gave an equivocal test result.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable due to many deviations from the Guideline.

Data point:	KCA 5.4.2
Report:	[redacted] 1985 (TOX1999-461) SAN 582 H: *In vitro* Chromosome Aberration Assay Using Chinese Hamster Ovary (CHO) Cells, [redacted] unpublished, 19 December 1985, BASF RegDoc.# 85/10795 (Experimental work from 2 October 1985 – 28 November 1985)
Guideline(s):	Not specified
Deviations:	The test was performed in 1985 prior to the establishment of several criteria of the current OECD Guideline No. 473 Deviations from OECD Guideline No. 473 (adopted 21 July 1997): The concentration of S9-mix in the test medium was not reported. Cells were exposed only for max. 2.5 h in the range-finding tests, and for only 2 h in the main test with metabolic activation (S9 mix). Guidelines recommend an exposure period of 3 to 6 h. Cells exposed in the presence of S9 mix were harvested 10 h after start of treatment. Guidelines recommend a total culture duration of about 1.5 normal cell cycle lengths (approx. 1.5×14 h = 21 h) For tests performed in the absence of S9 mix, exposure of cells was increased from 2.5 to 17 h. Cells were harvested 3 h later, resulting in a total culture period of 20 h. Despite severe substance-mediated mitotic suppression had been established in a range-finding test, the treatment /sampling times did not exceed 1.5 cycle lengths as recommended in the Guidelines for such a case. Absence of mycoplasma was not demonstrated. Karyotype features of the cell type used were not reported. Historical control data was not included in the report. Cytotoxity of test concentrations not reported for cells scored. The deviations may have compromised the reliability and validity of the study results.
GLP:	Yes (laboratory certified by Staatstoezicht op de Volksgezondheid, NL, Section GLP of the Veterinary Public Health Inspectorate)
Acceptability:	The study is considered to be not acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid (SAN 582 H), batch no. 8502, purity 92 % according to the applicant
Test System:	Chinese hamster ovary (CHO-WBI) cells, mean cycle time: 12 - 14 h; Source: Dr. S. Wolff's lab., University of California, San Francisco, cloned in Dr. A.

Bloom's lab., Columbia University, New York.

To assess the potential of racemic dimethenamid to induce chromosome aberrations *in vitro*, a study using Chinese hamster ovary (CHO) cells was conducted. The cells were exposed to the test compound dissolved in DMSO in the presence and absence of metabolic activation (S9 mix of male Sprague-Dawley rats treated with Arochlor). In a dose range-finding test, inhibition of cell cycling was assessed via BrdUrd-staining. Since the results showed that severe mitotic suppression and delay in cellular proliferation occurred in the absence of exogenous metabolic activation at and above dose levels of 50 µg/mL, the duration of exposure to the compound was increased from 2.5 to 17 h for the aberration test. In the presence of S9 mix, however, the duration of exposure was maintained at 2 h, and the cells harvested after 8 h. The experimental design is summarised in Table B.6.4-4.

Table B.6.4-4: Exposure scheme and dose levels for racemic dimethenamid *in vitro* chromosome aberration study

Harvest Time	Exposure period	
	17 h without S-9 mix	2 h with S-9 mix
3 h	10-150 µg/mL	-
8 h	–	150-500 mg/mL

Each exposure was run in duplicate.
Statistical analysis employed the Chi-square test to compare the percentage of aberrant cells in treated cultures with pooled results from solvent and untreated control. The difference was considered significant where $p<0.05$.

Results:

Severe cytotoxicity was observed at doses of 125 and 150 µg/mL without activation and at a dose of 500 mg/mL with activation. Therefore results were analysed for doses from 10-100 µg/mL without activation and from 150-400 µg/mL with activation. There were no statistically significant increases in aberrant cells at any dose tested either with or without metabolic activation (Table B.6.4-5 and Table B.6.4-6). However, a small, dose-dependent increased incidence of aberrant cells (statistically insignificant) was observed at the highest concentration (% cells with alterations), which was primarily due to an increased number of chromatid breaks. The relevance of this finding is unclear, since historical control data was not submitted.

Table B.6.4-5: Chromosome aberration in the absence of metabolic activation

Treatment	**Cells scored**	**Number and type of aberration**								**No. of aberrations per cell**	**% Cells with aberrations**	**% Cells with >1 aberration**
		Chromatid			Chromosome				Other			
		TB	TR	QR	SB	AF	D	R				
Neg. Ctrl.	200	2	0	0	0	0	0	0	0	0.010	1.0	0.0
Pos. Ctrl.	25	21	5	1	1	4	0	0	0	1.280	80.0*	28.0
10 µg/mL	200	1	0	0	0	0	0	0	0	0.005	0.5	0.0
25 µg/mL	200	0	0	0	0	1	0	0	0	0.005	0.5	0.0
50 µg/mL	200	3	0	0	0	0	1	0	DM6	0.005	2.0	1.0
75 µg/mL	200	2	0	0	0	0	2	2	0	0.030	2.5	0.5
100 µg/mL	200	14	0	0	1	0	0	0	0	0.090	4.1	1.2

TB = Chromatid break; AF = Acentric Fragment; TR = Triradial chromatid interchange; D = Dicentric; QR = Quadriradial chromatid interchange; R = Ring; SB = Chromosome break; DM = Double minute fragment

Table B.6.4-6: Chromosome aberration in the presence of metabolic activation

Treatment	Cells scored	Number and type of aberration								No. of aberrations per cell	% Cells with aberrations	% Cells with >1 aberration
		Chromatid			Chromosome				Other			
		TB	TR	QR	SB	AF	D	R				
Neg. Ctrl.	200	3	0	0	0	0	0	0	0	0.015	1.5	0.0
Pos. Ctrl.	25	7	2	3	0	0	0	0	ID1	0.520	36.0*	12.0
150 µg/mL	200	4	0	0	0	0	0	0	0	0.020	2.0	0.0
200 µg/mL	200	8	0	0	0	0	0	0	0	0.040	4.0	0.0
300 µg/mL	200	6	0	0	0	0	0	0	0	0.030	2.5	0.5
400 µg/mL	200	11	0	0	0	0	0	0	0	0.055	4.5	1.0

TB = Chromatid break; ID = Interstitial deletion; TR = Triradial chromatid interchange; QR = Quadriradial chromatid interchange

Conclusion:

Under the study conditions, exposure of CHO cells to the test substance resulted in an increased incidence of aberrant cells (statistically insignificant) at cytotoxic dose levels only and thus did not fulfil the criteria for a positive test result. However, due to limitations with regard to the study design, no clear conclusions can be drawn. Result: Inconclusive.

Re-evaluation by the RMS (2015):

The study is still considered to be not acceptable.

Data point: KCA 5.4.1

Report: [redacted], 1996 (TOX1999-431)
Unscheduled DNA synthesis assay in rat primary hepatocytes
[redacted]
unpublished, 11 April 1996, BASF RegDoc.#96/5399
(Experimental work: January–February 1996)

Guideline(s): OECD Guideline 482

Deviations: Deviations from OECD Guideline No. 482 (adopted 21.07.1997):
The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility.
Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.
The stability of the test or control article under the test conditions has not been determined by the testing facility.

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is now considered to be supplementary.

Materials and methods:

Test Material: Dimethenamid-P; batch No. 6663-50-1; purity: 96.3 % (total dimethenamid technical); 91.1 % (S-dimethenamid)

Test System: Primary rat hepatocytes, from liver of an adult male Spraque-Dawley rat from Harlan Spraque-Dawley, Inc. (Frederick, MD)

Dimethenamid-P was tested for its potential to induce DNA-damage and -repair in the *in vitro* unscheduled DNA synthesis (UDS) test using rat hepatocytes. Hepatocytes were isolated from Sprague-Dawley rats and exposed to the test substance for 18 - 20 h. The concentration range was 7.8 -1000 µg/mL. The selection of these concentrations was based on the results of a preliminary cytotoxicity study. DMSO was selected as the vehicle. Appropriate negative and positive controls were also tested.
The quantification of UDS was performed microscopically by determining net nuclear grain counts, using 3 slides per test group. A total of 50 cells in good morphological condition were randomly selected per slide and examined to achieve a total number of 150 cells/dose group scored.

Results:

Precipitate was observed at doses of 250 µg/mL and higher. Cytotoxicity as evidenced by released LDH was observed at doses of 62.5 µg/mL and higher. Based on the microscopical assessment of cell morphology, it was determined that cells treated with ≥250 µg/mL could not be evaluated for UDS due to excessive toxicity. Therefore, the five dose levels evaluated for UDS were 7.8, 15.6, 31.3, 62.5 and 125 µg/mL.
In none of the experiments, at any dose level was an increase in the mean number of net nuclear grain counts observed.
The negative and positive controls demonstrated the sensitivity of the test system.

Conclusion:

Under the experimental conditions of this assay, dimethenamid-P is considered to be negative in the *in vitro* UDS assay using primary rat hepatocytes.

Re-evaluation by the RMS (2015):

The study is now considered to supplementary due to the deviations from the Guideline.

Data point:	KCA 5.4.1
Report:	[redacted] 1986 (TOX1999-462) SAN 582H: UDS in rats hepatocytes *in vitro*, [redacted] unpublished, 21 February 1986, BASF RegDoc.# 86/11169 (Experimental work from 20 January 1986 – 22 January 1986)
Guideline(s):	Not specified
Deviations:	Deviations from OECD Guideline No. 482 (adopted 23.10.1986): (1) The results of only five of the six cell culture dishes per test concentration were presented. (2) The result was not confirmed in an independent experiment. (3) Primary hepatocytes were exposed to the test substance for 3 h only. This duration may have been not sufficient, although the OECD Guideline No.482 does not give a specific recommendation. (4) Data on cytotoxicity resulting from exposure to the test concentrations were not reported
GLP:	Yes (laboratory certified by Hessisches Umweltministerium für Arbeit, Umwelt und Soziales, Wiesbaden, Germany)
Acceptability:	The study is considered to be not acceptable.

Materials and methods:

Test Material: Racemic dimethenamid (300-069 = SAN 582 H), batch no. and purity not specified.
Test System: Primary hepatocytes prepared from liver of 8 - 12 week old male Wistar CF HB rats (bw 150 - 200 g).
Source: not specified

In the UDS assay, six replicate dishes each containing the rat hepatocytes and ^{3}H-thymidine were exposed to the test material dissolved in DMSO or DMSO alone for 3 h. Dose levels were 1, 3, 10, 30 and 100 nL/mL. The nuclei were lysed and the DNA isolated. A liquid scintillation counter was used to determine radioactivity and a colorimetric method was used to determine DNA content. The ^{3}H-thymidine incorporation was reported as dpm/µg DNA.
Note: On the basis of results from cell survival assessment, it appears that concentrations of at least 400 nL/mL could have been tested. The survival data shown in Table B.6.4-7 indicate that survival is significantly reduced at test concentrations above 400 nL/mL.

Table B.6.4-7: Survival Data (Trypan Blue Test)

Concentration		% Survival after 3-h treatment	
µg/mL	nL/mL*	absolute	relative
0	0	76	100
0.05	40	55	72
0.15	120	70	92
0.5	400	58	76
1.5	1200	4	5
5.0	4000	0	0
15.0	12000	0	0

* Calculated from reported µg/mL data by assumption of 1.2 g/cm³ density of the test compound

Results:

Since the experimental design did not allow to discriminate between DNA replication and DNA repair, an increase of ^{3}H-thymidine incorporation into DNA would not necessarily have indicated a DNA-damaging potential of the test substance. However, in this case, no increase in ^{3}H-thymidine incorporation into DNA was found at any of the treatment levels. The positive control substance DMBA induced a 3.2 fold increase of the dpm/µg DNA.

Conclusion:

Racemic dimethenamid did not appear to induce unscheduled DNA synthesis in rat hepatocytes *in vitro* under the conditions of the study. However, an incubation period of only 3 h may not have been sufficient to enable optimal detection of unscheduled DNA synthesis. This is suggested by the comparably low fold increase induced by the positive control, DMBA. In addition, recent UDS assays with primary hepatocytes typically involved incubation durations of approx. 20 h. Also, as indicated above, it would have been desirable to include higher dimethenamid concentrations that elicit some cytotoxic effects as recommended in OECD Guideline No. 482. Result: Inconclusive

Re-evaluation by the RMS (2015):

Results of the *in vitro* UDS assay were inconclusive due to the limitations with regard to the study design. Therefore the study is now considered to be not acceptable.

Data point:	KCA 5.4.1
Report:	[redacted] 1989 (TOX1999-463) Mutagenicity Test on SAN 582H in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay [redacted] unpublished, 7 November 1989, BASF RegDoc.# 89/11033 (Experimental work from 6 April 1989 – 27 July 1989)
Guideline(s):	Not specified
Deviations:	Deviations from OECD Guideline No. 482 (adopted 23.10.1986): None
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid (SAN 582 H Technical), batch no. 8605, purity 91.4 %
Test System:	Primary hepatocytes prepared from liver of adult male Fischer 344 rats (F344/NhsdBR) bw 226.6 - 260.5 g. Source: Harlan Sprague Dawley Lab., Inc.

In the UDS assay, rat primary hepatocytes were exposed to the test material dissolved in DMSO or DMSO alone for 18 - 19 h in the presence of ^{3}H-thymidine. In each of 2 separate trials, 15 dimethenamid concentrations ranging from 0.1 - 500 µg/mL and 0.01 - 50 µg/mL, respectively, were tested. Each treatment was performed on five cultures (three with coverslips and 2 without). The 2 cultures without coverslips were used to monitor the cytoxicity (Trypan Blue exclusion) of each treatment) while the remaining three cultures were used for quantitation of UDS. Based on the high toxicity encountered at concentrations of 15 µg/mL and above, the concentration levels selected for UDS analysis in the first trial were 0.025, 0.05, 0.1, 0.5, 1, 2.5, 5 and 10 µg/mL, and in the second trial were 0.1, 0.25, 1, 2.5, 5 and 10 µg/mL. Following the exposure period, the cells were washed, and the cover slips were coated with Kodak NTB-2 emulsion and stored in darkness for 7 to 10 d. The slides were then developed, fixed and stained with hematoxylin and eosin. Nuclear grains were counted in fifty cells in random areas on each of three coverslips for a total of 150 cells per treatment. The net nuclear grain counts were determined.

Results:

In the first trial, the percentage of nuclei with ≥6 grains was significantly increased at the lowest dose of 0.025 µg/mL, and slightly at 0.050 µg/mL. These increases were accompanied by non-significantly increased mean net grain counts. The response appeared to be inversely related to the concentration level; survival was not reduced with increasing concentration, therefore this finding could not explain the inverse concentration-effect relationship. In summary, the results indicated a borderline effect.
In the second trial, nuclear grains were significantly increased at levels of 0.25 and 1 µg/mL, and a dose-related trend was observed. The percentage of nuclei with ≥6 grains was significantly increased at concentration range of 0.1 - 2.5 µg/mL. For both parameters, non-significant, inversely concentration-related increases were observed at higher concentration levels. Cytotoxicity was indicated by a slight reduction in survival the highest test concentration of 10 µg/mL only. In summary, a positive test result was established. However, it was regarded unusual that the decrease of UDS response observed at and above 2.5 µg/mL was not accompanied by a corresponding decrease of cell survival.

Conclusion:

Positive UDS responses were observed over 2 or more concentrations in 2 separate trials. The fact that the response observed in Trial 2 was larger than found in Trial 1 and occurred at higher concentrations may be related to different sensitivities of the animals. The decrease of the UDS response at high concentration levels could not be sufficiently explained by increased cytotoxicity. However, it cannot be ruled out that DNA repair may be significantly compromised at concentrations that have no immediate impact on cell survival. Therefore, it is concluded that racemic dimethenamid was active in the rat primary hepatocyte UDS assay under the conditions of the study. Result: positive.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.4.1
Report:	[redacted] 1990 (TOX1999-464) Study to Evaluate the Potential of SAN 582 H to Induce Unscheduled DNA Synthesis in Isolated Rat Hepatocytes *In vitro* [redacted] unpublished, 1 August 1990, BASF RegDoc.# 90/11185 (Experimental work from 4 April 1990 – 4 June 1990)
Guideline(s):	Not specified
Deviations:	Deviations from OECD Guideline No. 482 (adopted 23.10.1986): The duration of the overnight exposure was not specified. Other Deviations: The determination of the net grain count did not follow the procedure usually employed in current UDS assays and may have resulted in a decreased test sensitivity. The concentration of the positive control (2-AAF) used was considered excessive. The deviation may have compromised the validity of the test result.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be not acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid (SAN 582 H), batch no. 8605, purity 92.1 %
Test System:	Primary hepatocytes prepared from liver of male Wistar rats, aged 56 - 58 d, bw 203 - 206 g, Source: Charles River U.K. Ltd., Margate, Kent, U.K.

In the UDS assay, triplicate cultures each containing the rat hepatocytes and ^{3}H-thymidine were exposed overnight to the test material dissolved in DMSO or DMSO alone. Two separate trials were performed. Concentration levels that were scored in the first trial were 0.32, 1.6, 8, 40 and 200 µg/mL. In the second trial, the dose levels were 7.813, 15.63, 31.25, 62.5 and 125 µg/mL. Following the exposure period, the cells were washed and mounted on cover slips, coated with Kodak NTB-2 emulsion and stored in darkness for 14 d. The slides were then developed, fixed and stained with hematoxylin and eosin. The net nuclear grain counts were determined.

Results:

In experiment 1, cell viability was below 50 % at and above dose levels of 40 µg/mL (63.5 % of DMSO control). A marginal increase in the net grain count was observed only at cytotoxic dose levels. In experiment 2, viability was below 50 % of control values at and above 62.5 µg/mL. A dose-related

increase in NG count indicating the presence of UDS was seen from 0 - 31.25 µg/mL, which further increased at cytotoxic concentration levels. However the criteria for a positive test result as set up by the authors were not met.

Table B.6.4-8: Results of UDS test *in-vitro*

Exp.-No	Dose [µg/mL]	Mean NG	Mean % cells in repair (NG ≥ 5)	% Viability	% Viability of control
1	0	-12.68	2.4	64.1	100
	0.3200	-8.27	0	Not scored	Not scored
	1.6000	-9.87	1.3	Not scored	Not scored
	8.0000	-9.67	0	64.7	101
	40.0000	-8.03	1.3	40.7	63.5
	200.0000	-4.20	2.7	11.6	18.1
	2.5 µg/mL 2-AAF	+18.7	94.7	No data	No data
2	0	-26.7	0	59.1	100.0
	7.8130	-29.0	0	52.3	88.5
	15.6300	-22.2	0	39.0	66.0
	31.2500	-16.1	0	29.6	50.1
	62.5000	-13.1	0	11.0	18.6
	125.0000	- 7.9	0	Toxicity	Toxicity
	2.5 µg/mL 2-AAF	+47.5	100	No data	No data

Note: The mean NG counts established in negative controls were exceptionally low, (-12.7 in Exp. 1, and -26.7 in Exp. 2); also mean NG counts of treatment groups were considerably lower than zero, indicating that grain counting may not have been performed correctly. Positive values were established only for the positive control 2-AAF, which however was used at a 25 fold higher concentration (2.5 µg/mL) than usually applied in UDS assays (0.1 µg/mL). It seems that only potent DNA-damaging agents would have been detected under these study conditions.

Conclusion:

A dose-dependent increase in the net grain count suggesting UDS was found in 1 of 2 independent trials. Other criteria for a positive test result that were defined by the study authors were difficult to meet, due to the method used for grain count determination. On the basis of the reservations discussed above, the reliability of the study must be questioned. Result: Inconclusive

Re-evaluation by the RMS (2015):

The study is still considered to be not acceptable.

B.6.4.2 *In vivo* studies in somatic cells

Studies evaluated in the addendum of the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point: KCA 5.4.2

Report: [redacted] 1993 (TOX2001-472)
Study to evaluate the potential of SAN 582 H to induce unscheduled DNA synthesis in rat liver using an *in vivo/in vitro* procedure
[redacted]
unpublished, 1993, BASF RegDoc.# 93/11757
(Experimental work from 10 June – 19 July 1993)

Guideline(s): Mirsalis J.C. and Butterworth B.E. (1980): Carcinogenesis 1, 621 - 625; modified by Ashby J. et al. (1985): Mutation Res. 156, 1-18.;
Generally compliant to OECD Test Guideline No. 486 (21 July 1997)

Deviations: None that compromised the validity of the study results.

GLP: Yes

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test material: Racemic dimethenamid technical (SAN 582 H), batch no. 5835-15; purity: 97.6 %.

Test animals: Male Fischer rats, age 57 - 66 days, bw 176 - 203 g, Source: Charles River Margate, Kent, UK.

Racemic dimethenamid was tested for its ability to induce unscheduled DNA synthesis (UDS) in the livers of orally dosed male rats using an *in vivo/in vitro* procedure.
Groups of 6 male rats were treated once with dimethenamid at 158 or 500 mg/kg bw by oral gavage at a dose volume of 10 mL/kg. The selection of doses was based on results from a preliminary toxicity study, in which mortality occurred after single oral gavage of dimethenamid at dose levels of 1000 and 2000 mg/kg bw, while no signs of toxicity were observed at 250 and 500 mg/kg bw throughout the 3-day observation period. For the negative control, 6 male rats received the vehicle, corn oil, orally at the same dose volume. Two groups consisting of six male rats each were used as positive controls: For the 2 - 4 h experiment, dimethylnitrosamine (DMN) at 10 mg/kg bw was orally administered, while for the 12 - 14 h experiment, rats were treated with 2-acetamidofluroene (2-AAF) at 75 mg/kg bw. Hepatocytes from 5 animals of each group were isolated at two expression times, 2 - 4 and 12 - 14 hours, in order to allow for variations in the rate of absorption, metabolism and accumulation of DNA damage. Gross and net nuclear grain (NG) counts from treated compared to control rats were assessed and the incidence of S-phase cells was determined.
The UDS assay was considered to give a positive test result if the chemical yielded at least 0 NG and ≥20 % of cells responding ("cells in repair", i.e. cells with NG of ≥5). A dose related increase on both NG and the percentage of cells in repair would also be required.

Results:

One animal dosed at 500 mg/kg bw was hunched and lethargic by day 1 post dosing. All other animals in the main study showed no toxic effects. The results of the net grain count quantitation are summarised in Table B.6.4-9. Negative (vehicle) control animals gave a mean NG value of less than 0 with only 0.2 - 3.6 % cells in repair. NG values were increased by 2-AAF and DMN treatment to more than 5, and more than 50 % cells were found to be in repair. In this study, the vehicle control NG was consistent with published data, and the system was shown to be sensitive to two known DNA damaging agents requiring metabolism for their action. The assay was therefore accepted as valid. Dimethenamid treatment at 158 or 500 mg/kg bw did not produce a group mean NG value greater than -0.3 nor were any more than 5.8 % cells found in repair at either dose.

Table B.6.4-9: *In vivo* UDS assay: group mean net grain count values

12-14 h experiment	Net nuclear grain count (NG)		Net grain count of cells in repair		Pecent of cells in repair (NG ≥5)	
Dose (mg/kg bw)	mean	SD	mean	SD	mean	SD
0	-0.5	0.2	5.3	0.0	0.2	0.4
158	-0.5	0.3	6.8	1.8	1.8	1.5
500	-0.3	0.6	7.8	1.4	2.2	2.9
75 2-AAF	14.2	3.1	14.8	2.9	94.4	2.3
2-4 h experiment						
0	-0.5	0.5	5.8	0.4	3.6	2.3
158	-0.6	0.5	6.7	0.6	4.8	5.4
500	-0.8	0.5	6.6	0.7	5.8	1.9
10 DMN	21.9	1.5	24.4	1.4	90.0	1.4

Conclusion:

It was concluded that racemic dimethenamid has no genotoxic activity in this test system under the experimental conditions employed.

Re-evaluation by the RMS (2015):

This study was evaluated in the addendum to the monograph of rapporteur member state Germany of Sep. 12, 2000 (ASB2010-10566). The study is still considered to be acceptable.

Data point:	KCA 5.4.2
Report:	[redacted] 1996 (TOX1999-432) Micronucleus Cytogenetic Assay in Mice: SAN 1289 H Technical [redacted] unpublished, 28 February 1996, BASF RegDoc.#96/5401 (Experimental work: January-February 1996)
Guideline(s):	OECD-Guideline 474; EEC 79/831, B12
Deviations:	Deviations from OECD Guideline No. 474 (adopted 21.07.1997): The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility. Analyses to determine the concentration of the test or control mixtures were not performed by the testing facility. The stability of the test or control article under the test conditions has not been determined by the testing facility. Only 1000 polychromatic erythrocytes (PCE) per animal were analysed for the presence of micronuclei (Guideline requirement: 2000 PCE/animal).
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is now considered to be supplementary.

Materials and methods:

Test Material:	Dimethenamid-P; batch No. 6663-50-1; purity: 96.3 % (total dimethenamid technical); 91.1 % (S-dimethenamid).
Test Animals:	ICR mice, age: 6 - 8 weeks, bw: 31 - 37 g (males) and 26 - 31 g (females), Source: Harlan Sprague Dawley, Inc., Frederick, MD.

Dimethenamid-P (SAN 1289 H) was tested for clastogenicity and the ability to have a spindle poison effect in ICR mice using a micronucleus test.
The test substance dissolved in corn oil was administered once intraperitoneally to 15 male and 15 female animals per group at dose levels of 103, 205 and 410 mg/kg bw in a volume of 20 mL/kg bw. These dose levels were selected based on a preliminary toxicity study where mortality was observed at doses of 600 mg/kg bw and above. The negative control group received the vehicle only. Cyclophosphamide was used as a positive control.
24, 48 and 72 h after the administration of the test substance, 5 animals/sex/group were sacrificed and the bone marrow of 2 femora was prepared. The positive controls were sacrificed 24 h after administration. After staining of the preparations, 1000 polychromatic erythrocytes were evaluated per animal.
Test substance concentrations were determined analytically.

Results:

Target concentrations were verified analytically.
No mortality occurred at any dose level. The administration of the test substance led to clinical symptoms in animals at 205 and 410 mg/kg bw. These symptoms included lethargy, hyperactivity and aggressiveness. There were no clinical signs of toxicity in the vehicle control, the low dose or the positive control groups. No inhibition of erythropoiesis as determined from the ratio of polychromatic to normochromatic erythrocytes was detected.
The administration of dimethenamid-P did not lead to an increase in the number of polychromatic erythrocytes containing micronuclei in any of the dose groups at any time period measured after treatment. The administration of the positive control resulted in the expected increase in micronuclei, demonstrating the sensitivity of the assay.

Conclusion:

Under the conditions of this test, dimethenamid-P does not have a chromosome damaging (clastogenic) effect *in vivo*.

Re-evaluation by the RMS (2015):

The study is now considered to be supplementary due to the deviations from the guideline.

Data point:	KCA 5.4.1
Report:	[redacted] 1986 (TOX1999-465) SAN 582 H: Micronucleus Test in Bone Marrow Cells of the Mouse [redacted] unpublished, 15 January 1986, BASF RegDoc.# 86/11168 (Experimental work from 2 December 1986 – 8 January 1986)
Guideline(s):	Not specified
Deviations:	Deviations from OECD Guideline No. 474 (adopted 21.07.1997): No information on acclimatisation period of test animals and of randomisation procedures was provided in the report. Only one dose level was used (1000 mg/kg bw) which caused high toxicity (Guideline requirement: at least three dose levels or, provided

	cytotoxicity is absent, one limit test dose dose level at 2000 mg/kg bw) Only 1000 polychromatic erythrocytes (PCE) per animal were analysed for the presence of micronuclei (Guideline requirement: 2000 PCE/animal).
GLP:	Yes (laboratory certified by Hessisches Umweltministerium für Arbeit, Umwelt und Soziales, Wiesbaden, Germany)
Acceptability:	The study is considered to be supplementary.

Materials and methods:

Test Material:	Racemic dimethenamid (300-069 = SAN 582 H), batch no. 85002, purity and stability not specified.
Test Animals:	NMRI mice, age: 2.5 - 4 months, bw not specified, Source: Sueddeutsche Versuchstierfarm, Tuttlingen, Germany.

Racemic dimethenamid dissolved in DMSO was administered to mice orally via gavage at a single dose of 1000 mg/kg bw. Blood cells from the femoral bone marrow were prepared 24, 48 and 72 h after treatment and 1000 polychromatic erythrocytes per animal were scored for the presence of micronuclei.

Results:

The numbers of micronuclei were not increased by treatment with racemic dimethenamid in relation to control values.

Table B.6.4-10: Results of Micronucleus Test

Sampling time point (h)	**24**			**48**		**72**	
Treatment group	Dimethenamid		CPA	Dimethenamid		Dimethenamid	
Dose (mg/kg bw)	0	1000	30	0	1000	0	1000
% cells with micronuclei	0.06	0.04	0.45	0.11	0.15	0.10	0.03
range	0-2	0-1	0-8	0-3	0-4	0-4	0-1
Ratio PCE/NCE	1.73	1.43	1.49	2.31	1.83	2.42	1.78

PCE = polychromatic erythrocytes; CPA = Cyclophosphamid (positive control); NCE = normochromatic erythrocytes

Conclusion:

Dimethenamid did not induce micronuclei in polychromatic erythrocyte stem cells after single oral treatment in mice under the study conditions employed. However, due to limitations in experimental design and reporting, the study can only be considered as supplementary information.

Re-evaluation by the RMS (2015):

The study is still considered to be supplementary due to the limitations in experimental design and reporting.

Data point:	KCA 5.4.2
Report:	[redacted] 1993 (TOX1999-466) Study to Evaluate the Potential of SAN 582 H Technical to Induce Micronuclei in the Polychromatic Erythrocytes of CD-1 Mice [redacted]

	unpublished, 26 August 1993, BASF RegDoc.# 93/11758 (Experimental work from 6 May 1993 – 1 June 1993)
Guideline(s):	Not specified
Deviations:	Deviations from OECD Guideline No. 474 (adopted 21.07.1997): Only 1 dose level was used (710 mg/kg bw ≈ 50 % LD_{50}) which caused general toxicity (Guideline requirement: at least three dose levels or, provided cytotoxicity is absent one limit test dose at 2000 mg/kg bw) Slides from dose and vehicle control groups were sorted by sampling time and sex prior to blind analysis; positive control slides were not analysed blind
GLP:	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be not acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid (SAN 582 H Technical), batch no. 5835-15, purity 97.6 % (w/w).
Test Animals:	Outbred CD-1 mice, age: 35 - 42 days, bw 23 - 29 g (males), 22 - 25 g (females), Source: Charles River U.K. Ltd., Margate, Kent, U.K.

Racemic dimethenamid in corn oil was orally administered to 5 male and 5 female mice via gavage once a day for 2 d at a dose of 710 mg/kg bw/d. This dose is approx. 50 % of the oral LD_{50} in mice that was determined in preliminary range-finding test. Negative controls received the vehicle, while positive control animals received cyclophosphamid (CPA) at a single dose of 80 mg/kg bw 24 h before sacrifice. Blood cells from the femoral bone marrow were prepared on glass slides and 2000 polychromatic erythrocytes per animal were scored for the presence of micronuclei.

Results:

Signs of toxicity were apparent in several treated animals; one of which had to be sacrificed in extremis, indicating a higher treatment dose is not possible. In both male and female treatment groups, the number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes was not increased compared to corresponding vehicle control values.

Table B.6.4-11: Summary of results

Sampling time	**24 h**			**48 h**	
Treatment group	Racemic dimethenamid		CPA	Racemic dimethenamid	
Dose (mg/kg bw)	0	2 x 710	1 x 80	0	2 x 710
% cells with micronuclei	0.040	0.055	2.144***	0.065	0.035
range per 1000 cells	0-1	0-1	11.5-28.9	0-1.5	0-1.5
Ratio PCE/NCE	1.02	1.16	0.56	1.02	1.12

PCE = polychromatic erythrocytes; NCE = normochromatic erythrocytes
*** Statistically different from vehicle control value ($p < 0.001$)

Conclusion:

Dimethenamid did not induce micronuclei in polychromatic erythrocyte stem cells after single oral treatment in mice under the study conditions employed. However, the study can only be considered as supplementary information because only one dose level was tested.

Re-evaluation by the RMS (2015):

The study is considered to be non-acceptable due to the limitations in experimental design and reporting.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.4.2
Report:	[redacted] 2014e (ASB2014-8390) BAS 656-PH - Micronucleus assay in bone marrow cells of the mouse [redacted] unpublished, BASF RegDoc2014/1038343 (Experimental work from 06-Feb-2014 to 05-Mar-2014) Grauert E.,Kamp H., 2014b (ASB2014-8391) Analytical report - BAS 656-PH Concentration control analyses in corn oil BASF SE Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany unpublished, BASF RegDoc 2014/1104188
Guideline(s):	OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395, EPA 712-C-98-226
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden and by Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material	BAS 656 PH (Dimethenamid-P)
Description:	Liquid, brown
Lot/Batch #:	0258B01BH
Purity:	97.6 %
Stability of test compound:	stable
Solvent used:	Corn oil
Control Materials:	
Negative:	No negative control was employed in this study.
Solvent control:	Corn oil
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw
Test animals:	
Species:	Albino mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 12 weeks
Weight at dosing:	mean value 35.4 g (SD +/- 1.6)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males/dose/test group, 5 males per vehicle and control group, respectively

Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	The animals were housed in groups in Makrolon Type II/III, with wire mesh top.
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	45 - 65 %
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00-18:00, 18:00-06:00)
Test compound concentration:	
Range finding test:	500, 1000 mg/kg bw
Micronucleus assay:	125, 250 and 500 mg/kg bw (doses were corrected for purity with a correction factor of 1.02. Test item dose levels as is were 127.5, 255 and 510 mg/kg bw). The test substance was administered once orally using an application volume of 10 mL/kg.

Preliminary cytotoxicity assay:
Male and female NMRI mice were administered the test substance once by oral administration at a dose of 500 and 1000 mg/kg bw.
Micronucleus test:
Treatment and sampling:
Groups of male mice were treated once with either the vehicle, positive control substance or 125, 250 and 500 mg test substance/kg bw by oral administration. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The animals were surveyed for evident clinical signs of toxicity throughout the study.
Twenty-four or 48 hours after the administration the mice were killed and the two femora were prepared free of all soft tissue. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with foetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet resuspended.
Slide preparation:
A small drop of the resuspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide evaluation:
In general, 2000 (6000 in one animal of the 125 mg/kg bw dose group (24 h treatment)) polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.
Statistics:
Statistical significance at the five per cent level ($p<0.05$) was evaluated by means of the non-parametric Mann-Whitney test.
Evaluation criteria:
A test item is considered as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods (nonparametric Mann-Whitney test) were used as an aid in evaluating the results, if necessary. However, the primary point of consideration is the biological relevance of the results.
A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-mutagenic in this system.

Results:

The stability of the test substance in the vehicle was verified in a separate study under the

responsibility of the sponsor and the results are reported in a separate report (BASF study code 04Y0442/08Y018).
Preliminary range finding test:
In the 1000 mg/kg bw dose group reduction of spontaneous activity, abdominal position, eyelid closure, tremor, shortness of breath, hunchback, salivation, uncoordinated movements and whitely lacrimation were observed within one hour after application. Due to the severe clinical signs, all animals were euthanised one hour after application. In the 500 mg/kg bw dose group, reduction of spontaneous activity, eyelid closure, excitement, rapid breathing, hunchback, whitely lacrimation, salivation, incontinence and hyperemia were observed in animals of both sexes starting after application of the test substance until hour 6 post-application. No animals died. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.
Micronucleus assay:
Clinical signs were noted only on the application day and comprised ruffled fur, reduction of spontaneous activity, abdominal position, eyelid closure, hunchback, whitely lacrimation, salivation, excitement and hyperaemia at ≥125 mg/kg bw as well as rapid breathing at 500 mg/kg bw.
After treatment with the test item at 24 h and 48 h preparation interval the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that the test substance did not induce cytotoxic effects in the bone marrow.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant increase in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were below the value of the vehicle control group and all values in all dose groups were very well within the historical vehicle control data range (Table B.6.4-12).
The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (2.25 %), thereby demonstrating the sensitivity of the test system.

Table B.6.4-12: Micronucleus test in mice administered BAS 656 PH

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Corn oil	24	0.190	2-6	1250
BAS 656 PH				
125 mg/kg bw	24	0.171	1-6	1231
250 mg/kg bw	24	0.186	1-6	1277
500 mg/kg bw	24	0.171	1-7	1219
Positive control				
Cyclophosphamide	24	2.250	23-61	1186
48 h sampling				
Corn oil	48	0.110	1-5	1194
BAS 656 PH				
500 mg/kg bw	48	0.093	0-4	1187

Conclusion:

The study is considered to be acceptable.
Based on the results of this study, BAS 656 PH does not induce the formation of micronuclei in mouse polychromatic erythrocytes under *in vivo* conditions.

B.6.4.3 *In vivo* studies in germ cells

The results of the *in vitro* as well as the *in vivo* studies demonstrated, that dimethenamid-P has no mutagenic or clastogenic potential. Therefore, there was no necessity to evaluate the test substance in an *in vivo* study using germ cells.

B.6.5 Long-term toxicity and carcinogenicity

Data point:	KCA 5.5.2
Report:	[redacted]., 1990 (TOX1999-435) SAN 582 H: Potential Tumorigenic and Toxic Effects in Prolonged Dietary Administration to Rats [redacted] unpublished, 1 March 1990, BASF RegDoc.#90/11138 (Experimental work from 16 December 1986 – 3 January 1989) Amendments: [redacted] 1990 (TOX1999-436), BASF RegDoc. #90/11179 [redacted] 1993 (TOX1999-437) Review of Ovarian Neoplasia in Sandoz Study SDZ335 (Compound SAN 582H), 22March 1993; unpublished BASF RegDoc. #93/11798
Guideline(s):	OECD-Guideline 453
Deviations:	Deviations from OECD-Guideline 453 (adopted 12.05.1981): After 5 wk on study clinical examinations were performed only at weekly intervals; Haematology was performed only on 10 rats/sex/group The number of surviving animals in some dose groups (control and low dose males and low dose females) was lower than the guideline recommends.
GLP:	Yes (laboratory certified by The Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid (SAN 582 H); batch No. 8605; purity: 91.3 %.
Test Animals:	Crl:CD (SD) BR rats, age at start of treatment: bw: Source: Charles River Breeding Lab., Portage, Michigan, U.S.

Racemic dimethenamid was administered to groups of 70 male and 70 female Sprague-Dawley rats at dietary concentrations of 0, 100, 700 and 1500 ppm. 50 animals/sex/group were treated for 24 months. Satellite animals of 20/sex/group were used in the chronic toxicity evaluations and sacrificed after 12 months of treatment. Analyses for stability and homogeneity of the test substance in the diet were performed prior to study start. Analyses to verify correct concentrations in the diet were conducted throughout the treatment period.
Food consumption and body weight were determined once a week. Water consumption was determined daily during weeks 12, 25 and 51 for the satellite animals. The animals were examined for mortality once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Ophthalmological examinations were carried out prior to study start and towards the end of dosing on all animals, and also at week 53 for control and high dose animals. Urinalysis, clinicochemical and hematological examinations were carried out during weeks 13, 26, 52, 78 and 104 of the administration period using 10 animals/sex/group. Satellite animals were used

through week 52, and main group animals were used for weeks 78 and 104. All animals were subjected to gross pathological assessment and selected organs were weighed. Histopathological examinations were performed on all control and high dose animals, animals that died during the study, lungs liver, kidney and any macroscopically abnormal tissue from low and intermediate dose animals, and on tissues from low and intermediate dose animals for which a treatment-related change was noted in the high dose group.

Results:

The stability and homogeneity of the test substance in the diet was demonstrated. Verification of correct concentrations were also confirmed by analysis. The test substance intake is given in Table B.6.5-1.

Table B.6.5-1: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	males	Females
100	5	7
700	36	49
1500	80	109

There was no treatment-related impairment of survival (see Table B.6.5-2).

Table B.6.5-2: Survival in chronic rat study (week 104)

	Dose Group (ppm)			
	0	100	700	1500
No. of male survivors	18/50	20/50	25/50	31/50
% male survival	36	40	50	62
No. of female survivors	25/50	22/50	30/50	31/50
% female survival	50	44	60	62

Note:
Survival rates were below 50 % for male controls and for low-dose group males and females. According to OECD Guidelines, the study cannot be accepted to prove the absence of a tumorigenic effect when groups have survival rates below 50 %. However, since survival was still at 50 % for both the low dose and control at week 96 (very near end of the study) it can be assumed that carcinogenic effects would have been detected in these groups. Also, survival was sufficient in the two highest dose groups where one would expect to see tumour development if it occurs. It is unlikely that the tumour profile in the control and low dose groups would have changed significantly in the last few weeks of the study. The clinical, ophthalmoscopical and clinicochemical are summarised in Table B.6.5-3. Organ weight changes and histopathological findings are summarised in Table B.6.5-4.

Table B.6.5-3: Clinical, ophthalmoscopical and clinicochemical findings

	Sex	Dose level (ppm)			
		0	100	700	1500
Clinical findings:					
Food consumption, week 1-10 (% of control)	m	100	98	95**	92*
	f	100	99	96*	91**
Body weight change week 0-104 (% of control)	m	100	94	93	84*
	f	100	88	90	69**
Body weight change week 0-10 (% of control)	m	100	97	94**	86**
	f	100	99	91**	81**
Ophthalmoscopy:					
Posterior capsular lenticular opacities (incidence)	m	4/20	5/21	6/27	13/32
	f	4/26	4/23	5/33	11/34
Clinicochemical findings:					
Cholesterol, week 104; % of control; females	f	100	98.7	125.6	147.4*
Gamma-GT, week 104, % of control, males	m	100	123.3	210.0	263.3**

*= p<0.05 (Williams test), **= p<0.01 (Williams test), m = male; f = female

Table B.6.5-4: Organ weight and histopathological findings

	Sex	Dose level (ppm)			
		0	100	700	1500
Organ weights (% of control):					
Relative liver weight, week 53	f	100	95.5	114.9**	115.7**
Relative liver weight, week 105	f	100	100.6	104.6	111.0*
Histopathology (incidence):					
Stomach: epithelial hyperplasia at limiting ridge	m	1/18	7/20	8/25	18/31
Liver: bile duct hyperplasia	f	0/25	4/22	9/29	13/31
Liver: cystically dilatated bile ducts	f	1/25	1/22	3/29	6/31
Parathyroid: focal or diffuse hyperplasia	m	4/18	7/20	8/25	13/31

**= p<0.01 (Williams test), m = male; f = female

The maximum tolerated dose (MTD) was reached at the high dose in both sexes as indicated by a body weight gain depression of 16 % in males and 31 % in females (week 104). A body weight gain decrease (13 %) was also observed in females at 700 ppm (week 0-10 and 10-80). The incidence of posterior capsular lenticular opacities was increased in both sexes at 1500 ppm (41 % vs. 20 %. and 32 % vs. 16 %, respectively). Although the increased incidence was still within historical control ranges (males: 22 – 44 %, females: 12 – 50 %), the finding was regarded to be a treatment-related exacerbation of a normal age-related change. In addition, in high-dose males, hyperplasia in the stomach and parathyroid were observed.
The liver was a target organ for dimethenamid in rats. Alterations at the high dose included increased cholesterol and liver weight in females and increased serum GGT in males.
Urinary ketone bodies were observed in males of the 1500 ppm group in weeks 26, 52 and 78.
The original report indicated a slight increase in ovarian tubular adenomas. Regarding the ovarian findings and taking into account recent advances in diagnostic criteria for rodent ovarian neoplasia, a pathology peer review was conducted following the issue of the final report. Table B.6.5-5 shows the

original and peer review analyses for ovarian tumours and hyperplasia. The peer review found one additional tumour in the control, two additional in the low and mid dose groups and one less at the high dose.
Between the original review and the peer review, pathology terminology had changed. Lesions originally diagnosed as ovarian tubular adenomas or hyperplasia were re-diagnosed as sertoliform tubular adenoma or hyperplasia. This change in terminology reflects a change from the original classification of these neoplasms as epithelial in nature to their current grouping with the other sex cord-stromal neoplasms. Neoplasms diagnosed by the original pathologist as "tubular adenomas" have been reclassified by the reviewers as "Sertoliform tubular adenomas". They consist of tubular structures lined by Sertoli-like cells. They differ from true Sertoli cell tumours in that the tubular cells lack basal nuclei and vertically oriented cytoplasm.
In general, the differentiation between Sertoliform tubular hyperplasia and adenoma is difficult and subjective because of the diffuse nature of the lesion. There is a biological continuum from hyperplasia to adenoma. In the original report pathologists diagnosed adenoma when at least 50 % of the ovary was involved. Lesions below this threshold size were diagnosed as hyperplasia. The reviewers used similar criteria, but also considered compression of surrounding ovarian stroma to be indicative of neoplasia rather than hyperplasia.
The review consisted of 2 steps. The first step was a "blind" review by [redacted] of all ovaries from the study. In a second step [redacted] made his own evaluation of the slides in the light of the findings of the original report and [redacted]. All discrepancies were examined and discussed and a consensus was reached between the two reviewers.
The final analysis demonstrates that there is no statistical or biologically significant evidence to indicate that dimethenamid causes ovarian tumours. The incidence at the high dose is within historical control range, and the difference in incidence from control is not statistically significant.
When adenoma and hyperplasia were combined for analysis, there was only a minimal difference between the control group and the high dose group. The organ weights of the ovaries of the high dose group were not increased in comparison with the controls.
Sertoliform tubular hyperplasia and adenoma are mainly found in Sprague-Dawley rat. These lesions are rarely found in other strains of rat, and are not found in man or domestic animals. They have therefore only very limited relevance for man.

Table B.6.5-5: Incidence of ovarian tumors and hyperplasia

Dose level (ppm)	0	100	700	1500
Animals investigated	50	50	50	50
Ovary - Original Analysis				
Granulosa cell tumor	0	1	1	0
Tubular adenoma	2	1	2	6
Tubular hyperplasia	12	7	14	22
Ovary - Peer Review Analysis				
Granulosa cell tumor	0	0	1	0
Sertoliform tubular adenoma	3	3	4	5
Sertoliform tubular hyperplasia	18	12	12	23
Sertoliform tubular hyperplasia + adenoma	21	12	14	24

Table B.6.5-6: Historical control group incidence of tubular adenoma of the ovaries in Sprague-Dawley rats at the performing laboratory

Study code	86A	86B	85A	85B	84A	84B	84C	84D	84E	83A	83B	83C
Tubular adenoma incidence	0/50	1/50	0/49	0/50	4/100	0/50	0/50	0/50	0/50	0/50	1/50	5/55

A marginal increase was observed for liver tumours in male rats. The incidence numbers are given in Table B.6.5-7.

Table B.6.5-7: Incidence of liver tumours in male rats

Dose level (ppm)	0	100	700	1500	Historical control range
Animals investigated	50	50	50	50	
Hepatocellular adenomas	0	0	1 (2 %)	3 (6 %)	HRCa: 0–1.8 % (same diet) HRC 0–4.0 % (other diet) CRLb: 0–15.4 % RITAc: 0–12.0 %
Hepatocellular carcinomas	0	0	0 (0 %)	2 (4 %)	HRC: 0–3.6 %(same diet) 0–6.0 % (other diet) CRLa: 0–7.7 % RITAc: 0–8.0 %

a Nine SD rat studies started at HRC during 04/1985–07/1986 with same stock diet (SDS).
b Nineteen studies with Charles River rats started during 04/1984–09/1986 (Charles River Historical Database, Patricia Lang, 1991, personal communication).
c Sixteen SD rat studies, different breeders, studies started between 1986–1990.

The incidence of hepatocellular carcinomas was not statistically different from control and within historical control ranges. The incidence of adenomas was only slightly outside of historical range (6 % at 1500 ppm compared to 4 % in historical control), but was not statistically significant.
The statistically non-significant increased incidence of benign liver tumours in male rats is most likely due to a large increase in survival at this dose as indicated in Table B.6.5-2.
Survival in high dose males was 72 % greater than control males. This increased survival allowed considerably more animals to reach an older age and develop the liver adenomas which are spontaneously occurring tumours that increase in incidence with age. In support of this position, high dose females had a much more modest increase in survival and there was no increase in liver tumours.
In addition, the incidence of adenomas at the high dose is well within historical control range for Sprague-Dawley rats as given in the Registry of Industrial Toxicology Animal (RITA) database. This database was started in 1988 and collects historical control data for rats and mice. For long-term studies conducted between 1986 and 1990 with Sprague-Dawley rats, the historical (spontaneous) incidence for hepatocellular adenomas has a range of 0 to 12 % and a mean of 3.6 %. In 4 of 16 studies the incidence of hepatocellular adenomas was 6 % (equal to the incidence in the dimethenamid study) or greater.
Overall, the slight increase in the benign liver tumour in high-dose males does not indicate that dimethenamid is carcinogenic. The increase was not statistically significant, was within historical control range for Sprague-Dawley rats and was most likely due to the considerable increase in survival at that dose.

Conclusion:

The maximum tolerated dose (MTD) was clearly met at the high dose of 1500 ppm as evidenced by significant body weight gain depression and liver alterations in both sexes. Histopathological changes were noted at the high dose in the liver, stomach and parathyroid. The mid dose of 700 ppm produced body weight gain decreases and liver alterations in females. Dimethenamid did not produce a carcinogenic response. The NOAEL was found to be 100 ppm (approx. 5 mg/kg bw/d).

Re-evaluation by the RMS (2015):

Despite that the survival rate was below 50 % in some dose groups (control and low dose males and low dose females), the study is considered acceptable since the survival rate in the mid and high dose groups was above 50 %.
The study shows increases in the incidence of liver and ovarian tumours, which were not considered sufficient for classification by a recent RAC decision (see Committee for Risk Assessment RAC Opinion of Dimethenamid-P, adopted 4 June 2013; CLH-O-0000003037-80-03/F; ECHA 2013, ASB2015-2797).
The NOAEL is considered to be at 100 ppm (5 mg/kg bw/d). The LOAEL is based on body weight gain decreases in both sexes and liver alterations (body weight increased, bile duct hyperplasia) in females at the next higher dose level of 700 ppm (36 mg/kg bw/d).

Data point:	KCA 5.5.3
Report:	[redacted] 1990 SAN 582 H: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice [redacted] unpublished, 24 August 1990, BASF RegDoc.#90/11139 (Experimental work: November 1987- September 1989), TOX1999-438
Guideline(s):	OECD Guideline 451
Deviations:	The number of surviving male animals in all groups (33, 48, 48, 37 and 46 % in control, low, lower mid, upper mid and high dose) was lower than the guideline recommends.
GLP:	Yes (laboratory certified by The Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; batch No. 8605; purity: 91.4 %.
Test Animals:	Charles River Crl:CD-1 (ICR)BR mice

Racemic dimethenamid was administered to groups of 52 male and female CD-1 mice at dietary concentrations of 0, 30, 300, 1500 and 3000 ppm for 94 weeks. Satellite groups of 16 animals/sex received 0 or 3000 ppm dimethenamid for 65 weeks. Analyses for stability and homogeneity of the test substance in the diet were performed prior to study start, and analyses to confirm target concentrations were performed periodically during the treatment period.
Food consumption and body weights were determined once a week. At least once a day the animals were examined for evident signs of toxicity and mortality, and once a week were subjected to an additional comprehensive clinical examination (including palpation). Blood smears were prepared from all mice killed during the study, and from all surviving mice at weeks 52, 78 and at terminal sacrifice (week 66 for satellite animals and week 95 for main study animals). At the end of the of treatment period, all surviving animals were sacrificed, subjected to gross pathological assessment, and selected organs were weighed. A histopathological examination was performed on all organs from the control and high dose satellite groups, and on all organs from all animals in the main study.
The test substance intake is given in the following table:

Table B.6.5-8: Test substance intake

Dietary dose level (ppm)	**Test substance intake (mg/kg bw/d)**	
	males	Females
30	3.8	4.1
300	41	40
1500	205	200
3000	431	411

Results:

The stability and homogeneity of the test substance in the diet, and the correct concentrations were confirmed by analysis.
There were no adverse treatment-related effects on survival or clinical observations. The clinical and clinicochemical findings, as well as organ weight changes and histopathological findings are summarised in Table B.6.5-9.
Body weight changes were reduced in males and females at 1500 and 3000 ppm. Reduced body weight gains at lower doses (30 and 300 ppm) are considered to be without toxicological relevance because they were without dose relation or were observed only in short periods and were compensated in other periods. The body weight change impairment at the high dose demonstrated that a Maximum Tolerated Dose (MTD) was attained.
Body weight adjusted liver weights were increased in females by 25 % at 1500 ppm and by 18 % at 3000 ppm. Kidney weights were also increased in females at 1500 and 3000 ppm, but considering the lack of any histopathological findings in the kidney, the toxicological significance of this finding is equivocal. There were no other organ weight changes considered related to treatment.
Effects on the liver were also noted with histopathology. The incidence of enlarged hepatocytes was increased in a dose-related manner at doses of 300 to 3000 ppm. However, at 300 ppm, enlarged hepatocytes were observed in only 1 male and 2 females and the severity was only minimal. The minimal enlargement of hepatocytes in the absence of any other toxicity at this dose is not considered an adverse effect.

Table B.6.5-9: Clinical, organ weight and histopathological findings

	Sex	Dose level (ppm)				
		0	30	300	1500	3000
Body weight gain, week 0-52, [% of control]	m	100	110.0	101.9	86.9*	85.0**
	f	100	87.7	93.6	84.2*	71.1**
Rel. liver weight, week 95 [% of control]	f	100	101.2	102.9	120.9**	117.5**
Rel. kidney weight, week 95 [% of control]	f	100	97.2	102.3	115.9**	116.8**
Liver: enlarged hepatocytes [incidence]	m	3/15	1/24	8/25	6/19	19/22
	f	0/33	2/41	5/41	14/34	29/37

*= $p<0.05$ (Williams test), **= $p<0.01$ (Williams test), m = male; f = female

Also, the incidence of hyperkeratosis of the limiting ridge of the stomach was increased at the high dose, but only minimally at the interim sacrifice. By the terminal sacrifice, this effect was not noted indicating a recovery had occurred. This effect may have been due to an irritating effect of the chemical.
There were no test substance-related findings at 30 ppm. In addition, there was no evidence that dimethenamid caused a treatment-related increase in tumours at any dose level.

Conclusion:

Evidence of toxicity was observed at the highest doses tested of 1500 and 3000 ppm in the form of reduced body weight gains, increased liver weights and enlarged liver cells. The NOAEL was found to be 300 ppm (approx. 40 mg/kg bw/d). There was no evidence that dimethenamid produced a carcinogenic effect in mice.

Re-evaluation by the RMS (2015):

Despite that the survival rate was below 50 % in all male groups (33, 48, 48, 37 and 46 % in control, low, lower mid, upper mid and high dose), the study is considered acceptable since the survival rate in the low, lower mid and high dose group was nearly 50 %.
No increased incidences of tumours were reported in this study.
The NOAEL is considered to be at 300 ppm (40 mg/kg bw/d). The LOAEL is based on body weight gain decreases in both sexes and liver and kidney alterations (body weight increased, enlarged livers) in females at the next higher dose level of 1500 ppm (200 mg/kg bw/d).

B.6.6 Reproductive toxicity

B.6.6.1 Two generation reproductive toxicity study in the rat

Data point:	KCA 5.6.1
Report:	[redacted]., 1990 TOX1999-439 SAN 582 H: Two-Generation Reproduction Study in the Rat [redacted] unpublished, 22 June 1989, BASF RegDoc.#90/11140 (Experimental work from July 1988 –June 1989)
Guideline(s):	OECD-Guideline 416
Deviations:	No deviations from OECD-Guideline 416
GLP:	Yes (laboratory certified by Eidgenössisches Departement des Innern, Berne, Switzerland)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; batch No. 8710; purity: 92.6 %.
Test Animals:	Wistar/HAN Rat (Kfm:WIST, outbred, SPF Quality).

Dimethenamid was administered to groups of 25 male and 25 female sexually immature Wistar rats (F0 parental generation) in the diet at concentrations of 0; 100; 500 or 2000 ppm. At 70 d after the beginning of treatment, F0 animals were mated to produce a litter (F1). Groups of 25 males and 25 females selected from F1 pups as the F1 parental generation were offered diets containing 0; 100; 500 and 2000 ppm of the test substance post weaning for 101 d, and the breeding program was repeated to produce an F2 litter. The study was terminated with the sacrifice of the F2 weanlings and F1 adult animals. Test diets containing dimethenamid were offered continuously throughout the study.
The stability and homogeneous distribution of the test substance in the diet were evaluated prior to study start. Analyses to confirm correct concentrations were performed periodically during the study.
Food consumption of the F0 and F1 parents was determined weekly up to the mating period. After mating, female food consumption was determined on Days 0, 7, 14 and 21 of gestation, and on Days 1, 4, 7 and 14 *post partum*.
In general, body weights of F0 and F1 parents were determined once weekly until the mating period.

During gestation and lactation F0/F1 females were weighed on Days 0, 7, 14 and 21 of gestation, and on Days 1, 4, 7, 14 and 21 after birth. The F1 and F2 pups were weighed on the day of or day after birth, and on Days 4, 7, 14 and 21 *post partum*.
The state of health of parents and pups was checked at least once each day, and parental animals were examined for their mating and reproductive performances. Pups were sexed and evaluated as to health, and pup viability was recorded. All pups were examined macroscopically at necropsy.
All F0 and F1 parental animals were assessed by gross pathology (including liver weight determination) and subjected to a histopathological examination, special attention being paid to the organs of the reproductive system.
The mean test substance intake during premating (F0 and F1 parental animals) is shown in the following table (see Table B.6.6-1):

Table B.6.6-1: Average test substance intake

Study Period	**100 ppm**	**500 ppm**	**2000 ppm**
	[mg/kg bw/d]		
P males premating	7.3	35.9	144.8
F1 males premating	7.3	36.6	154.5
P females premating	8.3	39.4	155.4
F1 females premating	8	41	167
P females gestation	7	36	147
P females lactation	15	74.7	300.3
F1 females gestation	7	36	140
F1 females lactation	15	75	289
Average of all values	9.4	46.8	187
Average without lactation values:	7.5	37.5	151

Results:

The stability and homogeneity of the test substance in the diet and target dietary concentrations were confirmed by analysis.
F0 and F1 Parents:
There were no effects due to treatment on any reproductive parameter. Substance-related findings in the parental generations (F0 and F1) are summarised in the following table (see

Table B.6.6-2): Clear signs of general, systemic toxicity occurred in both parental generations at 2000 ppm. Toxicity was characterised by decreased food consumption and increased liver weight in both sexes and impaired body weight gain in males. At 500 ppm the increase of liver weight was very slight (F0 males 4 %, females 10 %; F1 males 3 %, females 4 %), and therefore considered not to represent an adverse effect.

Table B.6.6-2: Results of clinical examinations and organ weight changes (F0 and F1 parental generations)

	P-gen.	Sex	Dietary dose level (ppm)			
			0	100	500	2000
Body wt gain, day 1-70, [% of control]	F0	m	100	98.4	98.4	87.6*
Body wt gain, day 1-101, [% of control]	F1	m	100	94.8	91.2	92.3$
Relative liver wt, [% of control]	F0	m	100	96.0	104.2	117.4**
		f	100	103.0	110.1*	123.9**
	F1	m	100	96.4	103.0	119.8**
		f	100	97.6	104.2	120.7**

*= statistically reduced body wt compared to control (Dunnett-test, p<0.05) from days 8-22 and days 36-70.
$= statistically reduced body wt compared to control (Dunnett-test, p<0.05) from days 29-101.
**= statistically significant (p<0.01 Dunnett-test))

F1 and F2 pups:
There were no effects on pup survival. In F1 pups (combined sexes), significantly increased mean body weights were noted at 100 ppm throughout the lactation period and at 500 ppm between days 1 and 7 postnatal (values for each sex were also decreased, but without significance). At 2000 ppm, pup body weight (gain) was reduced during the lactation period for both the F1 and F2 generations, at mid dose level of 500 ppm significantly only at one time point in the F2 Generation (see Table B.6.6-3).

Table B.6.6-3: Body weights in F1 and F2 pups

	Body weight (gram)	Dose level (ppm)			
		0	100	500	2000
Day 0	**F1: M & F combined mean (S.D.)**	5.7 (0.5)	5.7 (0.4)	5.8 (0.5)	5.6 (0.4)
Day 1 p.n.		6.1 (0.7)	6.5 (0.7)*	6.4 (0.8)*	6.2 (0.7)
Day 4 p.n		8.8 (1.1)	9.5 (1.3)*	9.4 (1.4)*	8.8 (1.2)
Day 7 p.n.		14.2 (1.7)	14.9 (1.8)*	14.8 (2.0)*	13.8 (1.8)
Day 14 p.n.		29.2 (3.4)	30.5 (3.7)*	28.7 (3.5)	27.2 (3.3)*
Day 21 p.n.		45.4 (6.2)	47.9 (6.2)*	44.4 (6.5)	41.1 (5.4)*
Day 0	**F2: M & F combined mean (S.D.)**	5.7 (0.4)	5.8 (0.5)	5.7 (0.4)	5.7 (0.5)
Day 1 p.n.		6.0 (0.6)	5.9 (0.7)	6.0 (0.6)	6.0 (0.7)
Day 4 p.n		8.8 (0.9)	8.6 (1.4)	8.7 (1.0)	8.8 (1.2)
Day 7 p.n.		14.5 (1.6)	14.1 (2.0)	14.0 (1.6)*	13.9 (1.6)*
Day 14 p.n.		30.2 (2.8)	30.4 (3.2)	30.1 (2.9)	28.6 (3.2)*
Day 21 p.n.		49.0 (4.6)	49.5 (5.6)	48.5 (4.6)	45.4 (5.0)*

*Dunnett-Test based on pooled variance significant at 5 % level

Conclusion:

Racemic dimethenamid was administered to Wistar rats over 2 parental generations with 1 litter produced in each of the first and second parental generations. There were no adverse effects on reproductive parameters of the parental animals at any dose level. Clear signs of general, systemic toxicity occurred in both parental generations at 2000 ppm. The only substance-related effect on pups was a decreased pup weight gain during lactation at 2000 ppm. Therefore, the NOAEL for reproductive function is 2000 ppm (151 mg/kg bw/d). The NOAEL for parental systemic toxicity and developmental toxicity is 500 ppm (37.5 mg/kg bw/d). Because no reproductive effects were noted up to parentally toxic doses in a 2-generation rat study, no further reproductive testing is needed.

Re-evaluation by the RMS (2015):

The study is considered acceptable.
In contrast to the previous evaluation, the NOAEL for parental toxicity is now considered to be 100 ppm (7.5 mg/kg bw/d) based on significantly increased liver weight of 10 % at 500 ppm in F0 females.
The NOAEL for reproductive toxicity of 2000 ppm (151 mg/kg bw/d), the highest dose tested, can be confirmed.
The NOAEL for offspring toxicity is still considered to be 500 ppm (75 mg/kg bw/d) based on lower body weight (gain) at 2000 ppm during lactation period. In contrast to the previous evaluation, the average test substance intake should be converted into 75 mg/kg bw/d (lactation period) instead of 37.5 mg/kg bw/d (average without lactation values).

B.6.6.2 Developmental toxicity studies

Data point: KCA 5.6.10

Report: [redacted] 1996 (TOX1999-440)
Oral (gavage) developmental toxicity study of SAN 1289 H in rats
[redacted]
unpublished, 23 October 1996, BASF RegDoc.#97/5274
(Experimental work from April 1996 – June 1996)

Guideline(s): U.S. EPA FIFRA, Subdivision F, Para. 83-3; generally followed OECD-guideline 414

Deviations: None

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: The study is considered to be acceptable.

Report: [redacted] 1999 (TOX1999-469)
Review of skeletal ossification delays in SAN 1289 developmental toxicity study
[redacted]
unpublished, 22 March 1999, BASF RegDoc.#99/504

Materials and methods:

Test Material: Dimethenamid-P (SAN 1289 H); batch No. 6663-50-1; purity: total dimethenamid: 96.3 %; S-dimethenamid: 91.1 %.
Test Animals: Crl:CD BF VAF/Plus (Sprague-Dawley) rats.

Dimethenamid-P was tested for its prenatal toxicity in Sprague-Dawley rats. The test substance was administered to 25 pregnant female rats/group by stomach tube at dosages of 25, 150 and 300 mg/kg bw on Days 6-15 *post coitum* (p.c.). The test substance was suspended in 0.5 % aqueous carboxymethylcellulose after first adhering the test substance to HiSil 233 as the carrier. A standard dose volume of 10 mL/kg bw was used. The control group, consisting of 25 dams, was dosed with the vehicle only.
Analytical verification of stability and homogeneity of dimethenamid-P in 0.5 % carboxymethylcellulose were determined prior to study start. Verification of test concentrations was performed twice during the current study.
Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked twice each day.
On Day 20 p.c. all females were sacrificed and assessed by gross pathology (including weight determination of the liver). The number of corpora lutea was determined, and the number and

distribution of implantation sites were classified. The foetuses were removed from the uterus, sexed, weighed and investigated for any external findings. Approx. 50 % of the foetuses were examined for soft tissue findings and 50 % were examined for skeletal findings.

Results:

Analytical determinations demonstrated that test substance concentrations were acceptably close to target levels.
The findings in dams listed in the following Table B.6.6-4 were obtained and assessed as substance-related:

Table B.6.6-4: Prenatal toxicity rat: maternal toxicity effects

Findings:		**Dose level (mg/kg bw/d)**			
		0	25	150	300
Food consumption (Days 6-9 p.c.)	Mean±SD [% control]	23.9 ± 2.6 100	21.7 ± 2.2** 91	18.5 ± 2.6** 77	16.5 ± 3.3** 69
Body weight gain (Days 6-16 p.c.)	Mean±SD [% control]	+59.6 ± 11.9 100	+51.3 ± 9.0** 86	+48.7 ± 8.0** 82	+44.5 ± 9.9** 75
Rel. liver weight [% body weight]	Mean±SD [% control]	4.07 ± 0.27 100	4.01 ± 0.27 98.5	4.20 ± 0.24 103	4.41 ± 0.35** 108

**= Significnatly different from the control group value (p<0.01, Dunnett's test)

No mortalities, abortions or premature deliveries occurred during the study. The 300 mg/kg bw/d group had increased incidences of excess lacrimation, piloerection, excess salivation, decreased motor activity, orange substance on fur, swollen ocular membrane, ptosis, dark pink skin, urine-stained abdominal fur and coldness to touch. There were no signs of clinical toxicity related to treatment observed in mid or low dose animals.
Body weight gains and feed consumption were reduced in all treatment groups.
Relative liver weights were increased at 300 mg/kg bw/d. There were no necropsy observations considered related to treatment.
There were no treatment-related effects on pre- or postimplantation loss, on the number of resorptions or number of viable foetuses, or on the sex distribution of foetuses. Mean foetal weight was slightly reduced at 300 mg/kg bw/d (-3 %) and 150 mg/kg bw/d (-2 %) compared to the control value (statistically not significant).
No treatment-related findings occurred in relation to external, soft tissue or skeletal malformations. Distended ureters were seen in 7 high dose foetuses in 3 litters compared to 3 control group foetuses in 2 litters. Because the litter incidence did not differ significantly from control, this increase was not considered treatment related. At 300 and 150 mg/kg bw/d there was an increase in incidence of 2 retarded ossifications, sternal centra and pelvic pubes (see Table B.6.6-5). Further evaluation of the delayed ossifications indicated that these differences were spurious, primarily due to unusually low control values, and not related to treatment.

Table B.6.6-5: Foetal skeletal alterations

Findings:		**Dose level (mg/kg bw/d)**			
		0	25	150	300
Pelvis: Pubis, incompletely ossified	Litter incidence N (%) Fetal incidence N (%)	1 (4.3) 2 (1.0)	0 (0) 0 (0)	5 (25.0)** 6 (3.6)	6 (24.0)** 12 (5.8)
Sternal centra: 2nd incompleteley ossified	Litter incidence N (%) Fetal incidence N (%)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (12.0)** 4(1.9)**

**= Significnatly different from the control group value (p<0.01)

Table B.6.6-6: Historical control range (%) for incomplete ossification

Year	Sternebrae	Pubis
1985-1998		
Litter	18.2 % - 44.4 %	18.2 % - 44.2 %
Fetus	5.5 % - 7.6 %	3.8 % - 14.8 %

Conclusion:

The administration of dimethenamid-P to pregnant Sprague-Dawley rats during organogenesis produced distinct signs of maternal toxicity at the high dose of 300 mg/kg bw/d as evidenced by initial body weight loss, subsequent reduced maternal body weight gain and food consumption, clinical observations and increased liver weight. Maternal body weight gain and food consumption were also reduced at 150 mg/kg bw/d. Slight foetal weight decreases were observed at 150 and 300 mg/kg bw/d. The only differences noted from control at 25 mg/kg bw/d were a slight and transient decrease in maternal body weight gain and reduced food consumption during the first three days of treatment. For this study, the NOAEL for maternal toxicity is <25 mg/kg bw/d. The NOAEL for developmental toxicity is 25 mg/kg bw/d.

Re-evaluation by the RMS (2015):

The study in rats is considered to be acceptable.
Based on a slight and transient decrease in maternal body weight gain and reduced food consumption at lowest dose level the NOAEL for maternal toxicity <25 mg/kg bw/d is agreed.
In contrast to the previous evaluation, the NOAEL for developmental toxicity is considered to be 25 mg/kg bw/d based on retarded ossifications at 150 and 300 mg/kg bw/d. These findings were previously regarded as spurious, primarily due to unusually low control values. However, this argument would also apply for the low dose level, where none of these findings occurred. Whereas in comparison to control values, the marginal and not significant lower foetal body weights at 150 mg/kg bw/d (2 %) and 300 mg/kg bw/d (3 %) are considered of no toxicological concern. In conclusion, the confirmed NOAEL for developmental toxicity is still 25 mg/kg bw/d, however based on retarded ossification instead of decreased foetal body weight. Teratogenic effects were not observed.

Data point: KCA 5.6.10

Report: [redacted]., 1987 (TOX1999-458)
Developmental toxicity (embryo/fetal toxicity and teratogenic potential) study of SAN 582 H administered orally via gavage to Crl:COBS^{R} CD^{R} (SD) BR presumed pregnant rats,
[redacted]
unpublished, 23 July 1987, BASF RegDoc.# 87/11225
(Experimental work from November 1986 – December 1986)

Guideline(s): Not specified, generally followed OECD Guideline No. 414

Deviations: Batch and purity not specified.
Food consumption was not recorded on Day 7 and/or 8 of presumed gestation for 13 control, 14 low, 13 middle and 12 high-dose group rats. It was nevertheless possible to determine interval values for each animal.

GLP: Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)

Acceptability: In contrast to the previous evaluation, the study is now considered supplementary due to missing information of batch and purity.

Materials and methods:

Test Material:	Racemic dimethenamid (SAN 582 H), batch and purity not specified. The test material was formulated into a powder suitable for daily suspension in aqueous 0.5 % (w/v) carboxymethyl cellulose by combining it with an equal volume of Hi-Sil® 233.
Test Animals:	Female, presumed pregnant Crl:COBS^{R} CD^{R} (SD) BR rats; age and body weight on Day 0 of gestation: approx. 12 weeks, 212 - 299 g, Source: Charles River Breeding Lab., Inc., Raleigh, NC, U.S.

The teratogenic potential and developmental toxicity of racemic dimethenamid was studied in CD rats. Groups of 25 pregnant female rats were administered the test substance as an aqueous suspension at dose levels of 0, 50, 215 and 425 mg/kg bw once daily by gavage from Days 6-15 of gestation. The dosing volume was 10 mL/kg bw. The control group received the vehicle. The animals were observed daily throughout gestation for clinical signs of toxicity, and body weights were recorded daily during the study period. Feed consumption was recorded on Day 0 and daily during Days 6-20 of gestation. On Day 20 p.c., all surviving dams were sacrificed and the foetuses were delivered by caesarean section and examined. A gross necropsy was performed on all dams, and the livers were weighed and preserved in formalin. Each foetus was weighed, sexed and examined gross external alterations. Approx. one half of the foetuses in each litter were examined for soft tissue alterations. The remaining foetuses were eviscerated, stained with Alizarin Red-S and examined for skeletal alterations.

Results:

Maternal toxicity:
Findings regarding maternal toxicity are summarised in

Table B.6.6-7. Two control dams died during the study. No other deaths or abortions occurred in the dams during the study. One dam in the 50 mg/kg bw/day group prematurely delivered.
Clinical signs which occurred included excess salivation at 425 and 215 mg/kg bw/d and urine-stained abdominal fur at 425 mg/kg bw/d.
Body weight loss was confined to dams at 425 mg/kg bw/day during the first 3 days of treatment, although reduced body weight gain also occurred in dams at 215 mg/kg bw/day during this time period. Body weight development continued to be delayed for high-dose group rats during Days 9 - 12 p.c. but was no longer observed during Days 12 - 16 p.c. or thereafter. Based on these early effects on body weight, significant decreases in body weight gain were observed for the overall treatment period (-16 % at 215 mg/kg bw/day and -35 % at 425 mg/kg bw/day). A slight decrease in body weight gain (11 %) was noted in 50 mg/kg bw/day dams during the first 3 days of treatment. This was transient in that no differences in body weight were noted on subsequent days of treatment. However, this resulted in a 9 % decrease in body weight gain during the overall treatment period. Because the effect was slight and transient, this difference is not considered to be of toxicological significance.

Table B.6.6-7: Maternal toxicity (clinical signs, body and organ weight changes)

Observations	**Dose group (mg/kg bw/d)**			
	0	50	215	425
Mortality	2	0	0	0
Excess salivation	0/25	2/25 (2 rat×days)	20/25** (31 rat×days)	20/25** (63 rat×days**)
Urine-stained fur	1/25 (1 day)	0/25	0/25	2/25 (4 rat×days**)
Thin appearance	0/25	0/25	0/25	1/25 (3 d**)
Body weight gain				
– Dosing period (days 6–16)	+57.1 g	+51.8 g	+47.8 g**	+37.2 g**
–Gestational period (days 0–20)	+160 g	+156.5 g	+152.7 g	+143.2 g**
Final body weight (day 20)	415.5 g	413.1 g	409.3 g	401.0 g
Liver weight	16.82 g	17.80 g	17.94 g*	19.31 g**
Relative liver weight	4.06 %	4.32 %*	4.39 %**	4.82 %**

* Significantly different from the control value (P<0.05)
** Significantly different from the control value (P<0.01)

Relative feed consumption was significantly reduced in the mid dose group during the first three d of treatment, and in the high dose group from Days 6 - 12. A slight (5 %) and transient (days 6 - 9 only) decrease in food consumption at 50 mg/kg bw/day was not considered to be toxicologically significant.
Liver weights were found to be significantly increased in mid- and high-dose group rats. Relative liver weight was statistically significantly increased in dams at 50, 215 and 425 mg/kg bw/day by 6, 8 and 19 %, respectively.

Caesarean-Delivery Data:
A summary of the caesarean delivery data is presented in

Table B.6.6-8.

Table B.6.6-8: Caesarean-Delivery and Litter Data

Observations		**Dose group (mg/kg bw/d)**			
		0	50	215	425
Animals tested	n	25	25	25	25
Animals pregnant	n (%)	24 (96)	25 (100)	23 (92)	23 (92)
Animals pregnant + sectioned on Day 20	n	22	24	23	23
Corpara lutea	mean	17.5	17.4	18.2	17.6
Implantations	mean	15.9	15.8	16.3	16.0
Live litter size	mean	15.2	14.8	14.9	13.9
Live foetuses	total n	335	355	342	320
Dead foetuses	total n	0	0	0	0
Early resorptions total (mean ± s.d.)		14 (0.6 ± 1.0)	21 (0.9 ± 0.8)	32 (1.4 ± 1.3)	47 (2.0 ± 2.8)
– Historical Control (810 litters, 34 groups 1985–1986)		mean (range):	0.8 (0.3-1.4)1		
Late resorptions total (mean ± s.d.)		0 (0.0)	2 (0.1 ± 0.3)	0 (0.0)	2 (0.1 ± 0.3)
% dead or resorbed conceptuses/litter ± s.d.		3.8 ± 6.1	6.2 ± 6.0	9.0 ± 9.1	10.7 ± 10.4
– Historical Control (497 litters, 36 groups, 1985–1986)		mean (range):	5.9 (2.1 – 9.4)		
Dams with any resorptions	n (%)	9 (41)	15 (63)	16 (70)	18 (78)
Dams with complete resorption	n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
Dams with viable foetuses (%)		22 (100)	24 (100)	23 (100)	22 (95.6)
No. (%) litters with altered development		6 (27.3 %)	7 (29.2 %)	4 (17.4 %)	7 (31.8 %)
No. (%) foetuses with any alteration		13 (3.9 %)	9 (2.5 %)	4 (1.2 %)	9 (2.8 %)
% foetuses with any alteration per litter		3.93 %	3.59 %	1.22 %	3.36 %

* Significantly different from the control value (P<0.05)
** Significantly different from the control value (P<0.01)
1 An early resorption incidence of 1.4 was reached in 1 of 34 historical control groups only [the next highest incidence reported was 1.3 (1x) followed by 1.2 (2x) and 1.1 (1x)].

While the resorption incidence at 50 mg/kg bw/d did not appear to be affected by treatment, a small dose-related increase in resorption was observed in groups administered 215 and 425 mg/kg bw/d, which in the high-dose group resulted in a minimal decrease in the average live litter size. Neither of these observations was significantly different from concurrent control values upon statistical data analysis. However, based on historical control data, the increased incidences of early resorptions observed at 215 and 425 mg/kg bw/d are regarded to be related to treatment. At doses of 215 and 425 mg/kg bw/d, a small dose-dependent increase in the average percentage of resorbed conceptuses per litter was observed. Although not statistically significant, the high-group value exceeded the historical control range. No other Caesarean-delivery parameter was affected.

Litter data:

Administration of the test substance to the dams, as compared with the vehicle, did not affect the incidence of foetuses with alterations or the percentage of foetuses with alterations per litter. Difference among the four groups was neither dose-dependent nor statistically significant (see

Table B.6.6-8). Foetal body weights were marginally decreased at 215 (-1 %) and at 425 (-2 %) mg/kg bw/d. However, these very slight differences from control were not considered toxicologically significant, and were not even discussed in the original report. They are mentioned here only for comparison to similar slight foetal body weight effects observed with the p isomer. Foetal sex ratio was unaffected by treatment. At the high dose, 2 foetuses in 2 litters had incompletely ossified manubria. This small incidence was not considered related to treatment. There were no other increased incidences of foetal gross, soft tissue or skeletal variations or malformations.

Conclusion:

Significant maternal toxicity at 425 mg/kg bw/day was evidenced by initial body weight loss, subsequent reduced maternal weight gain, reduced food consumption, clinical observations and increased liver weight. A reduced maternal body weight gain and reduced food consumption also occurred at 215 mg/kg bw/day. Marginal foetal body weight decreases were observed at 215 and 425 mg/kg bw/day. An increase in early resorptions occurred at the high dose and to a lesser extent at the mid dose. Slight and transient decreases in body weight gain and food consumption during the first three days of treatment at 50 mg/kg bw/day were considered to not be of toxicological significance. Therefore, the NOAEL for maternal and developmental toxicity is 50 mg/kg bw/day. There were no teratogenic effects observed which were considered related to treatment.

Re-evaluation by the RMS (2015):

In contrast to the previous evaluation, this study in rats is now considered supplementary only, because both, batch and purity of the test substance are not specified.
Under the present conditions of this study, the NOAEL for maternal toxicity remains 50 mg/kg bw/d based on significant decreased body weight gain during dosing period at 215 mg/kg bw/d and both, clinical signs and increased liver weight (>10 %) at 425 mg/kg bw/d. During treatment period the absolute food consumption (g/day) was significantly reduced at 215 and 425 mg/kg bw/d but not the relative food consumption (g/kg/day).
The NOAEL for developmental toxicity is 50 mg/kg bw/d based on early resorptions (and % dead or resorbed conceptuses/litter) at 215 mg/kg bw/d and above, which are not significant compared to the concurrent control values, but outside historical control data. Teratogenic effects were not observed.

Data point:	KCA 5.6.11
Report:	[redacted] 1988 (TOX1999-441) Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic potential) Study of SAN 582 H Administered Orally (Stomach Tube) to New Zealand White Rabbits [redacted] unpublished, 10 May 1988, BASF RegDoc.#88/11376 (Experimental work in June 1987)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 83-3; generally followed OECD-guideline 414
Deviations:	No deviations
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Racemic dimethenamid; batch No. 8605; purity: 92 %
Test Animals:	New Zealand White rabbit [Hra:(NZW)SPF)]

Dimethenamid was tested for its prenatal toxicity in New Zealand White rabbits. The test substance was combined with equal amounts of HiSil and suspended in aqueous 0.5 % carboxylmethylcellulose. 20 pregnant female rabbits/group were administered the test substance by stomach tube at doses of 37.5, 75 and 150 mg/kg bw on Days 6 - 18 post insemination (p.i.). A dose volume of 10 mL/kg bw was used. The control group, consisting of 20 does, was dosed with an amount of HiSil in carboxymethylcellulose equal to that given the high dose group.
Analytical verification of the stability and homogeneity of dimethenamid in carboxymethylcellulose were determined, and verification of test concentrations was performed three times during the current study.
Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked at least once each day.
On Day 29 post insemination, all females were sacrificed. The number of corpora lutea was determined, and the number and distribution of implantation sites were classified. The foetuses were dissected from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal findings.

Results:

Analytical determinations demonstrated that the test substance concentrations were acceptably close to target levels.
The findings given in Table B.6.6-9 were obtained and assessed as substance-related.

Table B.6.6-9: Substance-related findings

		Dose level (mg/kg bw/d)			
		0	37.5	75	150
Rabbits pregnant/tested		18/20	16/20	16/20	17/20
Abortion/premature delivery		0/18	0/16	0/16	2/17
Localised alopecia	Incidence	5/20	4/20	3/20	10/20
	Maximum incidencea	53/480	47/480	25/480	92/480**
Reduced faeces		0/20	0/20	1/20	2/20
Rel. feed consumption [g feed/kg bw/d]	(days 6-19) [% control]	100 %	95.8 %	94.7 %	76.5 %*
	(days 15-19) [% control]	100 %	89.4 %	82.7 %	60.0 %*
Body weight gain (days 15-19) [kg]		+0.04 ± 0.08	+0.00 ± 0.11	-0.04 ± 0.17	-0.07 ± 0.12
Body weight gain (days 24-29) [kg]		+0.02 ± 0.11	+0.01 ± 0.15	-0.05 ± 0.13	+0.00 ± 0.10
Body weight gain (days 6-15) [kg]		+0.14 ± 0.08	+0.12 ± 0.12	+0.18 ± 0.08	+0.03 ± 0.18
Body weight gain (days 6-19) [kg]		+0.18 ± 0.11	+0.12 ± 0.17	+0.14 ± 0.21	-0.03 ± 0.28
Body weight gain (days 6-29) [kg]		+0.23 ± 0.22	+0.19 ± 0.32	+0.13 ± 0.35	+0.20 ± 0.22

Maximum incidence: No. rabbits observed / examined multiplied by the numbers of days observed
*= Statistically significant from control ($p<0.05$) **= Statistically significant from control ($p<0.01$)

No deaths occurred during the study. At the high dose, 2 animals aborted and this is considered a treatment-related effect. Clinical signs considered related to treatment were localised alopecia at the high dose and reduced faeces at the mid and high doses.
Maternal feed consumption was reduced in the middle and high dose animals especially during the second half of the treatment period (days 15 - 19). Statistically significant reductions in feed consumption were obtained only in the high-dose group when based on body weight. During the treatment period, a body weight loss occurred in high dose animals and partly at the mid dose.
There were no gross pathological findings which were related to treatment.
There were no treatment-related effects on implantation, live litter size, foetal sex ratio or foetal body weight. Likewise, there were no effects on external, soft tissue or skeletal variations or malformations.
At lowest level of 37.5 mg/kg bw/d two does had no viable foetuses, due to early resorption (one doe

with only one and one with three implantation sites (Table B.6.6-10). This finding is considered of no toxicological concern, because it is restricted to the lowest dose level only. Late resorptions in general are increased at 150 mg/kg bw/d, however statistically not significant.

Table B.6.6-10: Litter data

	Dose level (mg/kg bw/d)			
	0	37.5	75	150
Rabbits pregnant, caesarean section d 29 p.c.	18	168	168	15
Abortion/premature delivery	0	0	0	2
Corpora lutea, Mean (S.D.)	11.4 (2.6)	11.2 (2.2)	11.7 (2.5)	12.0 (2.1)
Implantations, Mean (S.D.)	7.6 (3.9)	7.5 (3.2)	7.9 (2.6)	8.4 (3.7)
Litter size, Mean (S.D.)	7.2 (3.6)	6.8 (3.6)	7.5 (2.4)	7.4 (2.9)
Live Foetuses, N, Mean (S.D.)	129, 7.3 (3.6)	109, 6.8 (3.6)	120, 7.5 (2.4)	111, 7.4 (2.9)
Dead Foetuses, N, Mean (S.D.)	1, 0.0 (0.2)	0	0	0
Resorptions, Mean (S.D.)	0.4 (1.0)	0.7 (0.9)	0.4 (0.6)	1.0 (1.2)
Early resorptions, N, Mean (S.D.)	6, 0.3 (1.0)	8, 0.5 (0.8)	3, 0.2 (0.4)	5, 0.3 (0.6)
Late resorptions, N, Mean (S.D.)	1, 0.0 (0.2)	3, 0.2 (0.4)	4, 0.2 (0.4)	10, 0.7 (1.2)
% Dead or resorbed conceptuses/Litter, Mean (S.D.)[§]	3.9 (8.8)	6.6 (8.8)	5.5 (7.1)	9.2 (10.7)
Does with any resorptions, N (%)	4 (22.2)	8 (50.0)	6 (37.5)	7 (46.7)
Does with resorption of all conceptuses, N (%)	0	2 (12.5)	0	0
Does with viable fetuses, N (%)	18 (100.0)	14 (87.5)	16 (100.0)	15 (100.0)

[§] animals with all conceptuses resorbing excluded

Conclusion:

Racemic dimethenamid produced clear signs of maternal toxicity at 150 mg/kg bw/d as evidenced by reduced food consumption, body weight loss and clinical signs. Maternal toxicity, though less severe, was also observed at the mid dose including reduced body weight gain, reduced absolute food consumption and clinical signs. Although two abortions occurred in the high-dose group, this finding must be seen in connection with the accompanied clear maternal toxicity, especially for rabbits.
For this prenatal toxicity study in New Zealand White rabbits, the no observed adverse effect level (NOAEL) for maternal toxicity was 37.5 mg/kg bw/d, and the developmental toxicity NOAEL was 75 mg/kg bw/d.

Re-evaluation by the RMS (2015):

The developmental toxicity study in rabbits is still considered acceptable.
At highest dose level clear maternal toxicity was observed, evident as clinical signs, reduced food consumption and transient body weight loss. Two abortions were considered related to severe maternal toxicity: individual data revealed remarkable decreased food consumption and body weight loss for these two does.
The maternal toxicity NOAEL of 37.5 mg/kg bw/d is confirmed based on reduced food consumption, lower body weight gain and reduced faeces at mid dose level of 75 mg/kg bw/d.
The NOAEL for developmental toxicity of 75 mg/kg bw/d is confirmed and based on embryolethality at highest dose level of 150 mg/kg bw/d. Teratogenic effects were not observed.

B.6.7 Neurotoxicity

B.6.7.1 Neurotoxicity studies in rodents

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.7.1
Report:	[redacted]., 2013a (ASB2014-8392) BAS 656-PH: Acute oral neurotoxicity study in Wistar rats - Administration via gavage [redacted] unpublished, 31 July 2013, BASF DocID 2013/1028330 Experimental work from 16-Oct-2012 - 25-Apr-2013
Guideline(s):	OECD 424; EPA 870.6200; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.43
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	dimethenamid-P
Description:	liquid/brown, clear
Batch/purity #:	COD-001509, dimethenamid-P: 95.9 %
Stability of test compound:	The test substance was stable over the study period under storage conditions (ambient, room temperature; protect from temperatures below 0 °C; protect from temperatures above 40 °C); Expiry date 01-Oct-2013
Vehicle:	1 % aqueous carboxymethylcellulose (CMC)
Test animals:	
Species:	Rat
Strain:	Crl:WI(Han)
Sex:	Male and female
Age:	Males: 35-37 (Section A), 33-35 (Section B) Females 34-36 (Section A), 32-34 (Section B) 49 days at administration

Animal assignment and treatment:
Dimethenamid-P was administered once to groups of 10 male and 10 female Wistar rats by oral gavage at dose levels of 0, 60 (low dose), 200 (mid dose) and 600 mg/kg (high dose). The application volume was 10 mL/kg bw.
Each group per sex was subdivided into 2 subsets (Section A males and Section A females = first 5 animals of each dose group and Section B males and Section B females = second 5 animals of each dose group) in order to balance the groups for FOB and motor activity measurements. The animals were assigned to the treatment groups by means of computer generated randomisation lists based on body weights.

Test substance preparation and analysis:
The dosing suspensions were prepared by mixing weighed amounts of test substance with appropriate amounts of the vehicle (1 % aqueous CMC) with a magnetic stirrer. During administration of the test substance, preparations were kept homogeneous using a magnetic stirrer. The test-substance preparations were made once before the first administration.
Analyses performed prior to the start of the administration period revealed that the test-substance was stable in 1 % aqueous CMC for 4 days when stored at room temperature.
Homogeneity analyses of suspension preparation were performed for the lowest and the highest concentrations. For this - as laid down in the SOP - samples were taken from the top, middle and bottom of the storage containers. The homogeneity samples were also used for concentration control analysis. According to the SOP three samples were taken from the top middle and bottom of the beaker at the end of the administration period for the high and the low dose. Furthermore a single sample of the mid dose (200 mg/kg bw/day) was taken to confirm the correctness of the concentration.

Table B.6.7-1: Analysis of diet preparations for homogeneity and test-item content

Concentration [g/100 mL]	Sampling	Concentration [g/100 mL]	% of nominal concentration	Relative standard deviation
0.6	29.10.12	0.605 ± 0.006	100.8	1.1
2	29.10.12	1.867	93.4	n.a.
6	29.10.12	5.711 ± 0.157	95.2	2.8

values may not calculate exactly due to rounding of values
n.a. = not applicable

Relative standard deviations in the range of 1.1 to 2.8 % indicate the homogenous distribution of dimethenamid-P in the suspensions. The actual average test-substance concentrations were in the range of 93.4 to 100.8 % of the nominal concentrations.
Statistics:
Means, medians, and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables.

Table B.6.7-2: Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
Faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided).If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Table B.6.7-3: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

The animals were checked daily for evident signs of toxicity. Abnormalities and changes were recorded for each animal individually. A check for moribund and dead animals was made twice on each working day and once on Saturdays, Sundays, and public holidays. Animals that were in a moribund state were sacrificed and necropsied.
Body weight was determined before the first neurofunctional tests in order to randomise the animals. During the study body weights were determined on the days FOBs were conducted, i.e. Days -7, 0, 7 and 14. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change. On study day 15 the body weights of the fasted animals were used for determination of the relative organ weights.
Individual food consumption was checked daily by visual inspection. No food consumption data were recorded.
Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume. No water consumption data were recorded.
Ophthalmoscopy was not performed in this study.
Functional observation batteries (FOBs) were performed in all animals prior to administration (day -7) and on study days 0, 7 and 14. The FOBs were performed starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians being not aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation attention was paid to posture, tremors, convulsions, abnormal movements, impairment of gait and (if applicable) other findings.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behaviour when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. faeces (no. foecal pellets/appearance/consistency) within 2 minutes
8. posture	17. urine (appearance/quantity) within two minutes (Q)
9. palpebral closure	18. number of rearings within two minutes (Q)
(Q) quantitative parameter	

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response	8. behaviour during "handling"
2. touch response	9. vocalisation
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs (Q)
5. pinna reflex	12. grip strength of hind limbs (Q)
6. audition ("startle response")	13. landing foot-splay test (Q)
7. coordination of movements ("righting response")	14. other findings
(Q) quantitative parameter	

Motor activity examinations (MA) were performed in all animals prior to administration (day -7) and on study days 0, 7 and 14 with the exception of the female section B subset where MA were conducted on day 8 instead of day 7 due to a technical error. MA was performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
The first five surviving animals per sex and test group were selected for neuropathology evaluation. These animals were sacrificed by perfusion fixation under deep Isoflurane anesthesia. SOERENSEN's phosphate buffer was used as the rinsing solution and fixation was performed with a solution according to KARNOVSKY.
The remaining animals were sacrificed using CO_2 and discharged without further examination.
The sacrificed animals were necropsied and the visible organs or organ sections were assessed by gross pathology as accurately as it is possible for perfused animals. The weight of the brain (without olfactory bulb) was determined in all perfused animals after removal of the brain but before any other preparation. For determination of the relative brain weights the terminal body weights were used.
Additionally to organ/tissues listed in paragraphs below, the following organs/tissues were preserved in neutral buffered 4 % formaldehyde:

Brain (remaining material after trimming)
Spinal cord (parts of cervical and lumbar cord)
Gross lesions

Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were embedded and histologically examined. The remaining organ material and the animal bodies were stored in neutrally buffered 4 % formaldehyde solution. Details are given below:
The following organ samples were embedded in paraffin, sectioned and stained with haematoxylin-eosin (H&E) and assessed by light microscopy.

#	
	Brain (cross sections): - Frontal lobe - Parietal lobe with diencephalon and hippocampus - Midbrain with occipital and temporal lobe - Pons - Cerebellum - Medulla oblongata

#	Brain-associated organs/tissues - Eyes with retina and optical nerve
#	Spinal cord (cross and longitudinal sections): - Cervical cord (C3-C6) - Lumbar cord (L1-L4)
#	Peripheral nervous system: - Gasserian ganglia with nerve - Gastrocnemius muscle

(# only control and high dose - low and mid dose organs were stored in 4 % formaldehyde)

The following nerves were embedded in an epoxy resin, semi thin sectioned and stained with Azure II - Methylene blue basic Fuchsine (AMbf) and assessed by light microscopy.

#	Dorsal root ganglion, 3 of (C3-C6)
#	Dorsal root fiber (C3-C6)
#	Ventral root fiber (C3-C6)
#	Dorsal root ganglion, 3 of (L1-L4)
#	Dorsal root fiber (L1-L4)
#	Ventral root fiber (L1-L4)
#	Proximal sciatic nerve
#	Proximal tibial nerve (at knee)
#	Distal tibial nerve (at lower leg)

(# only control and high dose, - low and mid dose organs/tissues were stored in buffer solution)

Results:

No treatment-related clinical observations were noted during the daily standard clinical observation. No mortality was observed in this study. No ophthalmoscopy examinations were performed in this study. No treatment-related effects on body weight were observed.

Table B.6.7-4: Mean body weights and body weight gain of rats administered dimethenamid-P once and observed for 14 days

	Males				**Females**			
Dose level [mg/kg bw]	0	60	200	600	0	60	200	600
Body weight [g]								
- Day 0	205.2	204.8	199.3	202.4	149.6	152.5	154.2	155.5
- Day 14	280.3	280.4	268.9	278.8	182.7	188.5	189.2	193.4
Δ% (compared to control) #		0.0	-4.1	-0.6		3.2	3.6	5.8
Overall body weight gain [g]	75.2	75.7	69.5	76.3	33.2	36.0	35.1	37.9
Δ% (compared to control)#		0.7	-7.5	1.6		8.5	5.8	14.2

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

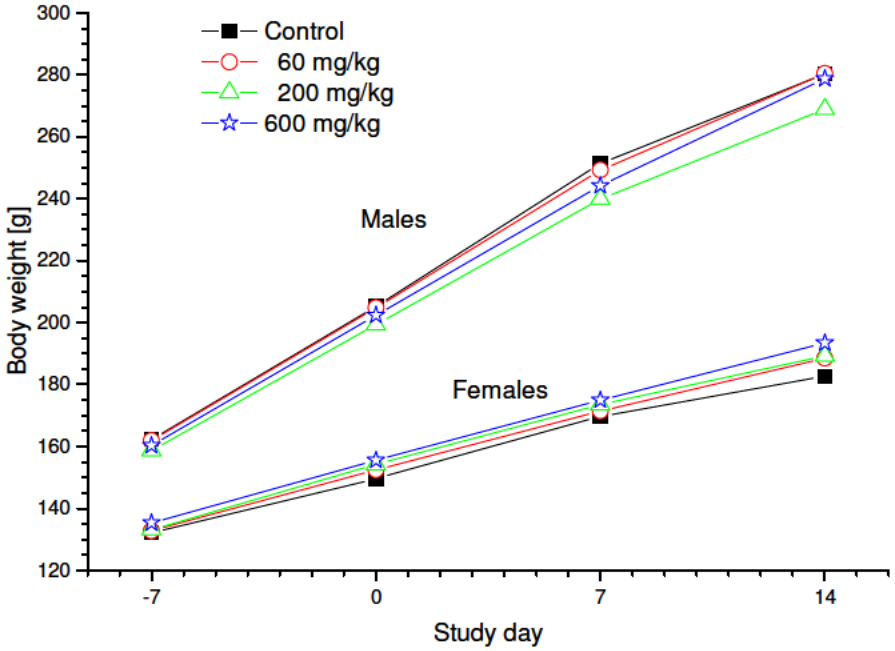


Figure B.6.7-1: Body weight development of rats administered dimethenamid-P once and observed for 14 days

Food consumption was not determined quantitatively for this study. No test-substance related effects on food consumption were observed.
Functional observation battery revealed deviations from (rank) "zero values" obtained in several animals. However, most findings were either equally distributed between treated groups and controls or displayed no dose-response relationship or occurred in single animals only, thus these observations were considered incidental.
On the day of application (study day 0), clinical findings during home cage observation were only observed in female animals of test group 3 (600 mg/kg body weight), i.e. piloerection in 6 females (Nos. 47, 52, 64, 66, 81, 87), half-closed eyelids in 3 females (Nos. 66, 81, 87) as well as permanently closed eyelids and slight salivation in 1 female animal (No. 52), and clear lacrimation in 2 females (Nos. 52, 87) (see Table B.6.7-5). These findings were assessed as being treatment-related. They were not observed on study days 7 and 14.

Table B.6.7-5: Clinical findings during home cage observation after single dose of dimethenamid-P on day of administration (d0)

	Males				Females			
Dose level [mg/kg bw]	0	60	200	600	0	60	200	600
Piloerection	0	0	0	0	1	0	0	6
Salivation, - slight	0	0	0	0	0	0	0	1
Nose discharge, - reddish	0	0	0	0	0	0	0	2
Lacrimation, - clear	0	0	0	0	0	0	0	2
Eyelids, - half-closed	0	0	0	0	0	0	0	3
- permanently closed	0	0	0	0	0	0	0	1

During home cage observation, no treatment-related findings were observed for male animals of test group 3 (600 mg/kg body weight) as well as for male and female animals of the other test groups.
Clinical findings during the open-field observations in female animals of the high dose group comprised piloerection, slight salivation, reddish discharge of the nose, slight lacrimation, half-closed eyelids, accelerated or irregular respiration, slight tremors, unsteady gate and reduced exploration of the area (see Table B.6.7-6). These findings were observed on the day of administration only and were not observed in any of the other dose groups. In contrast to the findings in females, where the slight tremors observed in 2 females were accompanied by other clinical findings and thus considered possibly treatment-related, the slight tremors observed in individual males of all dose groups are considered incidental as they were not accompanied by other clinical findings and not showing a clear dose-response relationship. The observed isolated findings of reduced exploration of the area in male and female animals of the low (60 mg/kg bw) and mid dose group (200 mg/kg bw) are occasionally observed during open field observation, were also observed in the same incidence in male control group animals and thus considered to be incidental.

Table B.6.7-6: Clinical findings during open field observation after single dose of dimethenamid-P on day of administration (d0)

	Males				**Females**			
Dose level [mg/kg bw]	0	60	200	600	0	60	200	600
Piloerection	0	0	0	0	1	0	0	7
Salivation, - slight	0	0	0	0	0	0	0	2
Nose discharge, - reddish	0	0	0	0	0	0	0	2
Lacrimation, - slight	0	0	0	0	0	0	0	2
Eyelids, - half-closed	0	0	0	0	0	0	0	4
Respiration, - accelerated	0	0	0	0	0	0	0	5
- irregular	0	0	0	0	1	0	0	1
Tremors, - slight	0	1	2	1	1	0	0	2
Impairment of gait, - slight	0	0	0	0	0	0	0	3
Exploration of area, - reduced	2	2	2	0	0	1	1	4
Contracted pupil, - strongly	0	0	0	1	0	0	0	0

The only clinical finding considered as potentially treatment-related observed in male animals during open field observation were strongly contracted pupils in one animal of the high dose group (600 mg/kg bw) (see Table B.6.7-5). This isolated finding was supported by findings in the sensorimotor testing.
A treatment-related decrease in the frequency of rearings was observed in high dose females on day 0 (see Table B.6.7-7). At the following FOBs (days 7 and 14) this value was comparable to the controls. No effect was observed in males.
All findings in females were assessed as being related to an impairment of the overall condition of the animals rather than being related to a neurotoxic mode of action.

Table B.6.7-7: Frequency of rearing in rats administered dimethenamid-P once and observed for 14 days

	Males				Females			
Dose level [mg/kg bw]	0	60	200	600	0	60	200	600
- Day -7	3 ± 4	3 ± 2	3 ± 3	4 ± 3	10 ± 5	8 ± 7	7 ± 4	9 ± 6
- Day 0	2 ± 2	3 ± 3	1 ± 1	4 ± 3	12 ± 7	8 ± 6	6 ± 7	2 ± 3**
- Day 7	3 ± 2	4 ± 4	2 ± 3	4 ± 5	13 ± 8	13 ± 8	10 ± 8	15 ± 7
- Day 14	2 ± 2	4 ± 3	4 ± 3	4 ± 4	17 ± 9	12 ± 8	15 ± 5	13 ± 7

** $p \leq 0.01$ (Kruskal-Wallis + Wilcoxon-test, two sided)

During open field observation, no treatment-related findings were observed in any dose group on day 7 and day 14 after administration.
A potentially treatment-related incidence of strong contracted pupil under incidence of light was observed in single animals of all male dose groups (see Table B.6.7-8). There was however, no clear dose response relationship and it was observed in a small subset of male animals only. Moreover, this finding was not observed in high dose group females who generally showed more pronounced toxicity effects than males. This finding was neither observed at 7, nor at 14-days after administration.
Deviations from "zero values" for non-quantitative parameters were obtained in several animals. However, as all findings were equally distributed between treated groups and controls, were without a dose-response relationship or occurred in single animals only, these observations were considered incidental.

Table B.6.7-8: Pupillary contraction determined in sensorimotor tests/ reflexes after single dose of dimethenamid-P on day of administration (d0)

	Males				Females			
Dose level [mg/kg bw]	0	60	200	600	0	60	200	600
Pupillary reflex - strong contracted pupil under incidence of light	0	1	2	2	0	0	0	0

Regarding the overall motor activity as well as single intervals, no relevant deviations were observed on study days -7, 0, 7 as well as study day 14 for male and female rats of test groups 1-3 (60, 200 and 600 mg/kg bw) when compared to the control group. The significantly lower value of interval No. 6 for male animals of test group 3 (600 mg/kg bw) on study day 14 was assessed as being incidental.
No treatment-related changes of terminal body weights or absolute and relative brain weights were noted (see Table B.6.7-9). The obvious differences in female brain weights of the low and mid dose group (60 and 200 mg/kg bw) are artificial differences due to technical reasons of brain preparation, that deviates for the control group and the high dose group (weights of trimmed brains for control and high dose group were compared to total brain weights for the low and mid dose group).

Table B.6.7-9: Mean terminal body weights and absolute and relative brain weights of rats administered dimethenamid-P once and observed for 14 days

Sex		**Males**				**Females**			
	Dose [mg/kg bw]	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%
Terminal body weight	0	263.76				176.16			
	60	262.56	(0)			177.52	(1)		
	200	243.38	(-8)			176.60	(0)		
	600	258.52	(-2)			179.96	(2)		
Brain	0	1.972		0.749		1.744		0.991	
	60	1.966	(0)	0.750	(0)	1.816**	(4)	1.025	(3)
	200	2.028	(3)	0.839	(12)	1.850*	(6)	1.051	(6)
	600	1.984	(1)	0.769	(3)	1.738	(0)	0.969	(-2)

* p≤0.05; ** p≤0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)
Values may not calculate exactly due to rounding of figures.

No treatment related gross pathology or neuro-histopathological findings were observed.
No concurrent positive control was employed in this study.
However, in several positive control studies, behavioural and neuro-pathological sequelae of substances with nervous system effects were evaluated using Functional Observational Batteries (FOB), Motor Activity Measurements and Neuropathology. Clinical signs of peripheral neuropathy (e.g. ataxia, limb weakness), central neuropathy (e.g. tremors) and autonomic signs (e.g. salivation) could be shown. Histopathologically, changes in the peripheral nervous system (e.g. Wallerian-like degeneration) and central nervous system (e.g. neuronal necrosis) were seen. The motor activity device was able to show both increased and decreased activity. The inter-observer reliability of the technicians performing FOBs was proven. Thus, the ability of the methods used to detect signs of neurotoxicity was demonstrated.
Positive control studies employed single or repeated administration of the following neurotoxicants: 3,3-iminodipropionitrile, carbaryl, nomifensin, diazepam, acrylamide and trimethyltinchloride, triethyltin bromide, methylphenidate hydrochloride, neostigmine bromide, R-(-)-apomorphine hydrochloride. Study summaries are attached to the report.

Conclusion:

This study was submitted with the dossier for the Renewal Assessment Report. The study is considered to be acceptable.
Single oral gavage of dimethenamid-P to rats at dose levels of 0, 60, 200 and 600 mg/kg bw resulted in signs of general systemic toxicity in female animals of the high group observed during home cage and open field observations on the day of administration only. All findings were assessed as being related to an impairment of the overall condition of the animals rather than being related to a neurotoxic mode of action.
The observed neuro-behavioural effect of strongly contracted pupils under incidence of light that was observed on the day of administration in a small subsets (1 or 2 out of 10 animals) of all male dose-groups might possibly be treatment-related. There was however no dose-response relationship and it was not seen in females who showed treatment related toxicity while no other toxicity effects were noticed in males. The effect was noted as a transient effect only on the day of administration. Additionally, no treatment-related neuropathological findings were noted, i.e. no brain weight changes or neurohistopathological findings were observed. Therefore, the observed pupillary reaction under light was considered to represent a transient neurotoxicological adaptive effect rather than to be indicative for adverse neurotoxicity.
Based on the afore mentioned findings the NOAEL for neurotoxicity was 600 mg/kg bw in males and

female rats. The NOAEL for systemic toxicity was 200 mg/kg bw in females and 600 mg/kg bw in males.

Data point:	KCA 5.7.4
Report:	[redacted]., 2013b (ASB2014-8393) BAS 656-PH - Repeated dose 90-day oral neurotoxicity study in Wistar rats - Administration via the diet [redacted] unpublished, 31 July 2013, BASF DocID 2013/1165818 Experimental work from 21-Jan-2013 - 24-Jul-2013
Guideline(s):	OECD 424; EPA 870.6200; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.43
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	dimethenamid-P
Description:	liquid/brown, clear
Batch/purity #:	COD-001509: 95.9 %
Stability of test compound:	Stability under storage conditions (ambient temperature +5 to +30 °C) guaranteed by the sponsor; Expiry date: 01-Oct-2013
Vehicle and/or positive control:	None
Test animals:	
Species:	Rat
Strain:	Crl:WI(Han)
Sex:	Male and female
Age:	34 - 36 days (Section A) and 33 - 35 days (Section B) at delivery, 48 - 50 days at start of treatment
Weight at dosing:	males: 196.5 ± 9.6 g; females: 150.6 ± 7.6 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld.
Acclimation period:	13 to 16 days
Diet:	Kliba maintenance diet for mouse/rat “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*.
Water:	Drinking water from water bottles, *ad libitum*.
Housing:	Group housing (5 animals per cage) polysulfonate cages from Tecniplast, Hohenpeißenberg, Germany (floor area about 2065 cm²); with dust free bedding (SSNIFF, Soest, Germany) enriched with wooden gnawing blocks (TYP NGM E-022; Abedd® Lab. and Vet. Service GmbH, Vienna, Austria). Motor activity (MA) measurements were conducted in poly carbonate cages (Tecniplast, Hohenpeißenberg, Germany; floor area about 800 cm² and small amounts of absorbent material).
Environmental conditions:	
Temperature:	20 - 24 °C

Humidity:	30 - 70 %
Air changes:	15 air changes per hour
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6.00 am to 6.00 pm

Dimethenamid-P was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 300 (low dose), 1000 (mid dose) and 4500 ppm (high dose) for at least 90 days. Each group per sex was subdivided into 2 subsets (Section A males and Section A females = first 5 animals of each dose group and Section B males and Section B females = second 5 animals of each dose group) in order to balance the groups for FOB and motor activity measurements. The animals were assigned to the treatment groups by means of computer generated randomisation lists based on body weights.
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, corresponding amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Three diet preparations were performed for this study and were mixed at least every 32 days.
Analyses performed prior to the start of the administration period revealed that the test substance was stable in the diet for at least 32 days when stored at room temperature.
According to the SOP three samples were taken from the top middle and bottom of the beaker at the end of the administration period for the high and the low dose. Furthermore a single sample of the mid dose (1000 mg/kg bw/day) was taken to confirm the correctness of the concentration.

Table B.6.7-10: Analysis of diet preparations for homogeneity and test item content

Dose level [ppm]	Sampling	Concentration# [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
300 ppm	21.01.2013	294.7 ± 3.8	98.2	1.3
1000 ppm	21.01.2013	985.3	98.5	n.a.
4500 ppm	21.01.2013	4116.5 ± 90.4	91.5	2.2

based on mean values of the three individual samples; values may not calculate exactly due to rounding of values.
n.a.: not applicable

No test article was determined in control diets. Relative standard deviations in the range of 1.3 to 2.2 % indicate the homogenous distribution of dimethenamid-P in the diet preparations. The actual average test substance concentrations were in the range of 91.5 to 98.5 % of the nominal concentrations.
Means, medians, and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables.

Table B.6.7-11: Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
Faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided).If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Table B.6.7-12: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided).If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Table B.6.7-13: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

The animals were checked daily for evident signs of toxicity. Abnormalities and changes were documented for each animal individually.
A check for moribund and dead animals was made twice on each working day and once on Saturdays, Sundays, and public holidays. Animals that were in a moribund state were sacrificed and necropsied.
Detailed clinical observations (DCO) in all animals was performed prior to the administration period and in weekly intervals thereafter. For DCO animals were transferred to a standard arena (50 x 37.5 cm with sides 25 cm high) and the following parameters were analysed:

1. abnormal behaviour during “handling”	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. faeces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

Body weight was determined before the start of the administration period, on day 0 (start of the administration period) and weekly thereafter as well as on days of FOB performance. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.
Individual food consumption was determined weekly and calculated as mean food consumption in grams per animal and day.
Food efficiency was calculated for each animal based on individual values for body weight and average food consumption for animals per cage:

$$\text{Food efficiency for day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BW_x and BW_y: body weight [g] on day x and day y (last weighing date before day x).
$FC_{y\ to\ x}$: mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days from day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values

for body weight and food consumption:

$$\text{Compound intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x: mean daily food consumption (in g/day) on day x
C: concentration in the food on day x [mg/kg] equivalent to dose in ppm on day x
BW_x: body weight on day x of the study (in g)

Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume. No water consumption data were recorded.
Ophthalmoscopy was not performed in this study.
Functional observation batteries (FOBs) were performed in all animals prior to administration (day -7) and on study days 1, 22, 50 and 85. The FOBs were performed starting at about 10.00 a.m.. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians being not aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation attention was paid to posture, tremors, convulsions, abnormal movements, impairment of gait and (if applicable) other findings.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behaviour when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. faeces (no. faecal pellets/appearance/consistency) within 2 minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response	8. behaviour during "handling"
2. touch response	9. vocalisation
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hind limbs
6. audition ("startle response")	13. landing foot-splay test

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually

for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
Clinical chemistry and urinalysis were not performed in this study.
Five animals per sex and test group were selected for neuropathology evaluation. These animals were sacrificed by perfusion fixation under deep isoflurane anesthesia. SOERENSEN's phosphate buffer was used as the rinsing solution and fixation was performed with a solution according to KARNOVSKY.
The sacrificed animals were necropsied and the visible organs or organ sections were assessed by gross pathology as accurately as it is possible for perfused animals.
Animals not selected for perfusion fixation were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.
The following weights were determined for animals sacrificed on schedule:

✓	Anesthetised animals
#	Brain (without olfactory bulb)[1]
✓	Liver
✓	Kidneys
✓: all animals/dose group sacrificed on schedule #: only animals/dose group scheduled for perfusion fixation	

[1] The weight of the brain was determined in all perfused animals after removal of the brain but before any other preparation.

Additionally to organ/ tissues listed in paragraphs below, the following organs/tissues were preserved in neutral buffered 4 % formaldehyde:

#	Brain (remaining material after trimming)
#	Spinal cord (parts of cervical and lumbar cord)
✓	Gross lesions
○	Kidneys
○	Liver
○	Remaining organ material and animal body
✓: all animals/dose group sacrificed on schedule #: only animals/dose group scheduled for perfusion fixation ○: only animals/dose group not scheduled for perfusion fixation	

Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were embedded and histologically examined. The remaining organ material and the animal bodies were stored in neutrally buffered 4 % formaldehyde solution. Details are given in Table B.6.7-14 below.

Table B.6.7-14: Histotechnical processing and histopathological evaluation

	Dose level [ppm]	0		300		1000		4500	
		M	F	M	F	M	F	M	F
#	Brain (cross sections)			FT	FT	FT	FT		
#	- Frontal lobe	P	P					P	P
#	- Parietal lobe with diencephalon and hippocampus	P	P					P	P
#	- Midbrain with occipital and temporal lobe	P	P					P	P
#	- Pons	P	P					P	P
#	- Cerebellum	P	P					P	P
#	- Medulla oblongata	P	P					P	P
#	Brain-associated organs/tissues - Eyes with retina and optical nerve	P	P	F	F	F	F	P	P
#	Spinal cord (cross and longitudinal sections): - Cervical swelling (C3-C6) - Lumbar swelling (L1-L4)	P P	P P	F F	F F	F F	F F	P P	P P
#	Peripheral nervous system: - Gasserian ganglia with nerve - Gastrocnemius muscle	P P	P P	F F	F F	F F	F F	P P	P P
°	Gross-lesions	P	P	P	P	P	P	P	P
#	Peripheral nervous system								
#	- Dorsal root ganglion, (3 of C3-C6)	PL	PL	S	S	S	S	PL	PL
#	- Dorsal root fiber (C3-C6)	PL	PL	S	S	S	S	PL	PL
#	- Ventral root fiber (C3-C6)	PL	PL	S	S	S	S	PL	PL
#	- Dorsal root ganglion, (3 of L1-L4)	PL	PL	S	S	S	S	PL	PL
#	- Dorsal root fiber (L1-L4)	PL	PL	S	S	S	S	PL	PL
#	- Ventral root fiber (L1-L4)	PL	PL	S	S	S	S	PL	PL
#	- Proximal sciatic nerve (cross and longitudinal sections)	PL	PL	S	S	S	S	PL	PL
#	- Proximal tibial nerve (at knee) (cross and longitudinal sections)	PL	PL	S	S	S	S	PL	PL
#	- Distal tibial nerve (at lower leg) (cross and longitudinal sections)	PL	PL	S	S	S	S	PL	PL

#: only animals/test group scheduled for perfusion fixation
°: only affected animals / test group scheduled for perfusion fixation
P: Paraffin embedding (paraplast), sectioning, staining with hematoxylin-eosin (HE) and histopathological evaluation
PL: Plastic embedding (epoxy resin), semi thin sectioning and staining with Azure II Methylene blue-basic Fuchsine (AMbf) and histopathological evaluation
S: Storage of fixed specimen in buffer solution
F: Preservation in 4 % formaldehyde solution
FT: Preservation in 4 % formaldehyde solution in total

Results:

Concerning clinical signs of toxicity no findings of toxicological concern were seen in male and female animals of all test groups. A tooth anomaly was observed in one female control group animal

from study day 0 until study day 27. This finding occurred only temporarily in a single animal and was assessed as being incidental and not related to treatment.
No mortality was observed throughout the study period.
No ophthalmoscopy examinations were performed in this study.
On study day 1, the mean body weight of female animals before performing the FOB was significantly lower in all test groups (300, 1000 and 4500 ppm). This initial finding might be related to palatability problems before the animals got used to the test material. Therefore, the effect was not assessed as being adverse.
Treatment related effects on body weight development were observed for male and female animals of the 4500 ppm dose group. Although not significantly altered, mean body weights of male animals were lower during the entire study period with a maximum of -5.9 % on study day 28. In addition, on study day 1 the mean body weight of male animals before performing the FOB was significantly lower. Mean body weight change values were significantly lower in this group from study day 0 to 42 (maximum of -24.5 % on study day 7). Significant differences occurred only during the first half of the study: However, the mean body weight change value of these animals was still -7.8 % lower at the end of the administration period. Therefore, the changes were assessed as being test substance related and adverse. In female animals of the high dose group as well as the mean body weight change values were significantly lower from study days 49 onwards (maximum of -7.2 % mean body weight and -15.1 % mean body weight change on study day 77) including study day 85 when body weight was determined before performing the FOB. This effect was regarded to be related to treatment and adverse.
During the weekly performed body weight determination no relevant changes were observed for male and female animals of the low and mid dose group (300 and 1000 ppm).

Table B.6.7-15: Mean body weight, body weight gain and cumulative food consumption data of rats administered dimethenamid-P for at least 91 days

	Males				**Females**			
Dose level [ppm]	0	100	1000	4500	0	100	1000	4500
Body weight [g]								
- Day 0	196.6	198.9	193.7	196.9	154.1	147.7	149.9	150.8
- Day 91	392.7	396.5	387.4	377.7	256.1	245	244.8	238.7
Δ% (compared to control)#		1.0	-1.3	-3.8		-4.3	-4.4	-6.8
Overall body weight gain [g]	196.1	197.6	193.7	180.8	102	97.3	94.9	87.9*
Δ% (compared to control)#		0.8	-1.2	-7.8		-4.6	-7.0	-13.8
Cumulative food consumption [g/animal]§								
- Day 0 to 91	1828	1879	1860	1996	1609	1499	1404	1687
□% (compared to control)		2.8	1.8	9.2		-6.8	-12.8	4.9

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)
§ Values calculated based on group mean daily food consumption

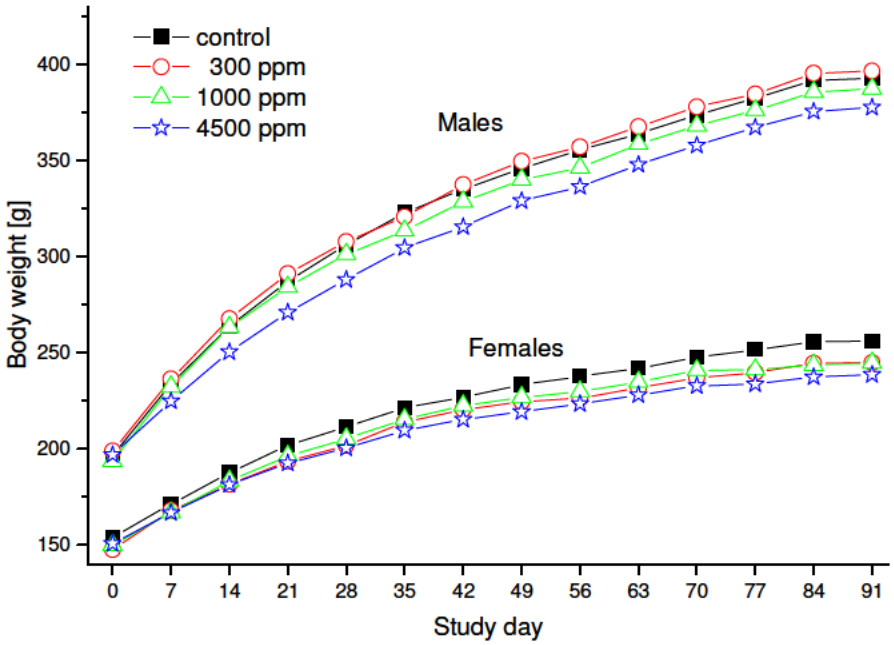


Figure B.6.7-2: Body weight development of rats administered dimethenamid-p for at least 91 days

No test substance related, adverse findings with respect to food consumption or compound intake were observed.
Note: Food spilling was observed in one male and one female of the 4500 ppm group on several days during the administration period. Some values were declared as outliers and were not used for calculation of the mean test substance intake.
The intake of test substance was calculated for the 90-day administration of dimethenamid-P (Table B.6.7-16).

Table B.6.7-16: Calculated intake of dimethenamid-P administered for at least 90-days

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	300	19	23
2	1000	63	71
3	4500	323	390

No haematology, clinical chemistry investigations or urinalysis were conducted in this study.
There were no functional observation battery (FOB) findings of toxicological concern in any of the treatment groups applied 300, 1000 or 4500 ppm to male and female rats. Some deviations from (rank) "zero values" were obtained in several animals.
No treatment related findings were observed in home cage observations.
Teeth anomaly was detected in a female control group animal on study days 1 and 22. This finding was assessed as being incidental.
No treatment related findings were observed in open-field observations.
Deviations from (rank) "zero values" were obtained in some animals. However, all findings were either equally distributed between treated groups and controls or displayed no dose-response relationship or occurred in single animals only, thus these observations were considered incidental.
Concerning sensorimotor tests / reflexes no treatment related findings were observed.

Very frequent vocalisation when touched was detected in one female animal of the (300 ppm) on study day 22. This finding was assessed as being incidental and not related to treatment.
The assessment of quantitative parameters revealed an isolated and not dose-dependent difference, which was considered to be of incidental nature. Landing foot-splay test was significantly higher in male animals of the 300 ppm dose group on study day 50 (+11.8 %).
No treatment related changes of motor activity were noted in treated groups. There were some statistically significant differences between control and treated groups, however, these changes were neither dose related nor consistent over time. Therefore, these changes were considered incidental. These changes consisted of:
- significantly decreased activity in low dose females at interval 9 on study day -7.
- significantly decreased activity in high dose males at interval 4 and significantly increased activity in female high dose animals at intervals 5 and 6 on day 1.
- significantly decreased activity in male animals of the low dose group at intervals 1 and 2 and significantly increased activity in female low dose animals at interval 11 on study day 22.
- significantly decreased activity in male low dose animals at interval 1 and significantly increased activity in female animals of the low dose group at single interval 5 was and significantly increased activity in female high dose animals at intervals 4 and 5 on study day 50.
- significantly increased activity of female low dose animals at single interval 10 on study day 85.

No statistically significant differences on overall motor activity were observed at any dose or any time.
Brain weights in treated animals (Perfusion fixed animals) were not significantly altered from control group animals.
Terminal body weight of animals not subjected to perfusion fixation was not significantly altered in any of the dose groups. However, in both sexes the reduced body weight development of the high dose group (Table B.6.7-15 and Figure B.6.7-2) was reflected by the tendency to reduced terminal body weight.
The increase of kidney weights in male animals of the high dose group as well as the increase of absolute and relative liver weight in male and female animals in the mid and high dose group and relative liver weight in female animals in the low dose group was regarded to be test substance related (see Table B.6.7-17).

Table B.6.7-17: Mean terminal body weights and selected absolute and relative organ weights of rats administered dimethenamid-P for at least 91 days

Sex	Males				Females			
Dose [ppm]	0	300	1000	4500	0	300	1000	4500
[mg/kg bw/day]		19	63	323		23	71	390
Terminal body weight [g]	381.62	375.94	369.54	362.0	237.84	231.7	229.5	225.42
[% of control]	100	99	97	95	100	97	96	95
Liver, absolute [g]	8.164	8.798	8.814	10.844**	5.406	5.77	6.08	7.3**
[% of control]	100	108	108	133	100	107	112	135
Liver, relative [%]	2.139	2.342	2.385	2.996**	2.269	2.494*	2.647**	3.243**
[% of control]	100	110	112	140	100	110	117	143
Kidneys, absolute [g]	2.246	2.336	2.276	2.58*	1.596	1.584	1.686	1.686
[% of control]	100	104	101	115	100	99	106	106
Kidneys, relative [%]	0.589	0.622	0.618	0.712**	0.673	0.686	0.734	0.748
[% of control]	100	106	105	121				

* p≤0.05; ** p≤0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)
Values may not calculate exactly due to rounding of figures

No histopathological examination of the liver and kidneys was performed in this study. In the subchronic 90-day study in rats conducted with dimethenamid-P and the racemic dimethenamid which

were performed at comparable dose levels, as well as other rat studies with dimethenamid-P or racemic dimethenamid it was reported that liver weight increases were accompanied at dose levels ≥100 mg/kg bw/day only by hepatocellular hypertrophy and altered clinical chemistry parameters (increase in γ-GT and cholesterol) indicative for liver enzyme induction. There was no indication for adverse histopathological findings (e.g. necrosis, fatty change, degeneration) in these studies.
No gross pathology or neurohistopathological findings were observed.
Four female animals of the high 4500 ppm dose group (not subjected to perfusion fixation) revealed an enlargement of the liver. This finding was seen in relation to the above noted liver weight increases and regarded to be test substance-related.
For the dilation of renal pelvis in male animals of all test groups a test substance related effect could not be excluded. However, no such treatment relation has been determined in any of the other rat toxicity studies conducted with dimethenamid-P or racemic dimethenamid. As no histopathological examination was performed, no further judgment could be made.
No concurrent positive control was employed in this study.
However, in several positive control studies, behavioural and neuropathological sequelae of substances with nervous system effects were evaluated using Functional Observational Batteries (FOB), Motor Activity Measurements and Neuropathology. Clinical signs of peripheral neuropathy (e.g. ataxia, limb weakness), central neuropathy (e.g. tremors) and autonomic signs (e.g. salivation) could be shown. Histopathologically, changes in the peripheral nervous system (e.g. Wallerian-like degeneration) and central nervous system (e.g. neuronal necrosis) were seen. The motor activity device was able to show both increased and decreased activity. The inter-observer reliability of the technicians performing FOBs was proven. Thus, the ability of the methods used to detect signs of neurotoxicity was demonstrated.
Positive control studies employed single or repeated administration of the following neurotoxicants: 3,3'-iminodipriopionitril, carbaryl, nomifensin, diazepam, acrylamide, trimethyltinchloride, triethyltin bromide, methylphenidate hydrochloride, neostigmin bromide and R-(-)-apomorphine hydrochloride. Study summaries are attached to the report.

Conclusion:

This study was submitted with the dossier for the Renewal Assessment Report. The study is considered to be acceptable.
In conclusion, the oral administration of dimethenamid-P to Wistar rats over a period of 3 months revealed no adverse neurobehavioural effects in male and female Wistar rats at any concentration. In addition, no test substance related effects were observed in the neurohistopathology investigation. Under the conditions of this study the NOAEL for neurotoxicity was 4500 ppm for male (323 mg/kg bw/d) and female animals (390 mg/kg bw/d).
Effects indicating a certain level of systemic toxicity were given at least for the high concentration of 4500 ppm, i.e. impairment of body weight development in male and female Wistar rats accompanied by an increase of liver (males and females) and kidney (males only) weights (histopathological examinations were not performed in these organs). Thus, the NOAEL for systemic toxicity was 1000 ppm corresponding to 63 mg/kg bw/day in males and 72 mg/kg bw/day in females.

B.6.7.2 Delayed polyneuropathy studies

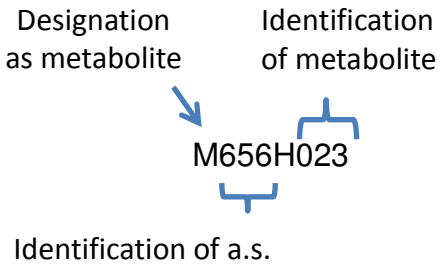
As there was no indication for neurotoxicity and/or neuropathy from any of the studies conducted and as dimethenamid-P does not belong to a chemical class suspected to induce delayed neuropathies, no study was considered necessary and thus no study was conducted.

B.6.8 Other toxicological studies

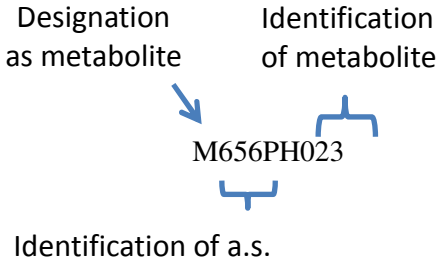
B.6.8.1 Toxicity studies of metabolites and relevant impurities

General explanation on metabolite nomenclature in relation to stereoisomery:

Dimethenamid-P is the S-enantiomere of the racemic dimethenamid. For the active substance a data-package conducted with the racemic mixture was taken into consideration and a bridging concept was applied and accepted for the Annex I inclusion of dimethenamid-P. A comparable situation exists for the metabolite evaluation that partly relies on information where either the source of the metabolite was based on studies conducted with the racemic mixture or where the metabolite evaluated was based on racemic pathway synthesis. Consequently metabolites where the source was the racemic compound and/or where the synthesis could not clearly be attributed to the chiral synthesis pathway were assigned with a code that has the following structure as given for the example M23:

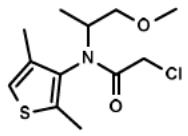
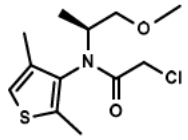
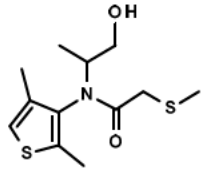
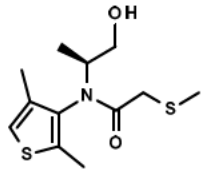
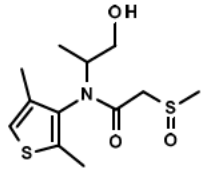
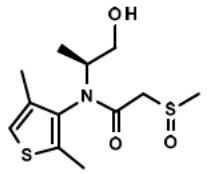
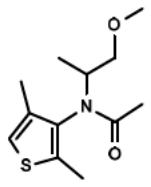
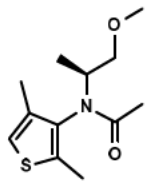


Metabolites were the source of identification and the synthesis route could clearly be attributed to the chiral compound dimethenamid-P were assigned:



The metabolic pathways in soil, water, mammals, and plants are equivalent for the racemic dimethenamid and dimethenamid-P (S-enantiomer). The metabolites derived from either racemic or enantio-enriched source are considered toxicologically equivalent and were taken into account for the assessment below.

Table B.6.8-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
BAS 656 H (275.8)	360720	-	87674-68-8	Rat	
BAS 656 PH	363851	-	163515-14-8	Rat	
M656H001	Not assigned	M1	Not assigned	Rat	
M656PH001	Not assigned	M1	Not assigned	Rat	
M656H002	Not assigned	M2	Not assigned	Rat	
M656PH002	Not assigned	M2	Not assigned	Rat	
M656H003	360717	M3	Not assigned	Rat	
M656PH003	5886782	M3	Not assigned	Rat	

Metabolite designation				Compound found in	Structure/Name
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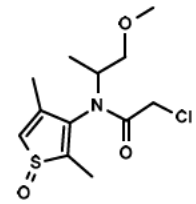
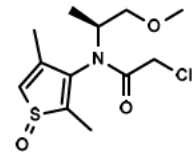
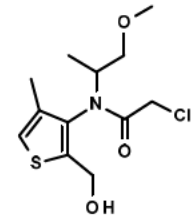
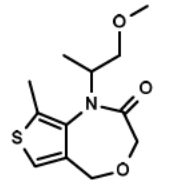
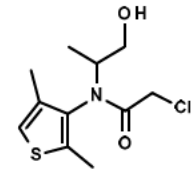
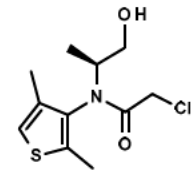
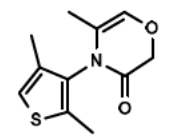
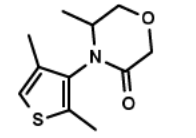
M656H004	Not assigned	M4	Not assigned	Rat Rat Liver Slices	
M656PH004	Not assigned	M4	Not assigned	Rat Liver Slices	
M656H005	Not assigned	M5	Not assigned	Rat	
M656H006	Not assigned	M6	Not assigned	Rat	
M656H007	360718	M7	Not assigned	Rat	
M656PH007	5886783	M7	Not assigned	Soil Rat Human Hepatocytes	
M656H008	Not assigned	M8	Not assigned	Rat	
M656H009	360719	M9	Not assigned	Rat	

Metabolite designation				Compound found in	Structure/Name
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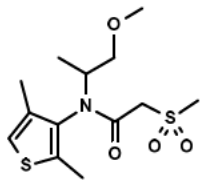
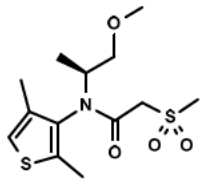
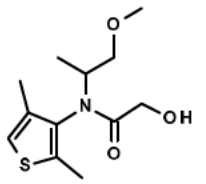
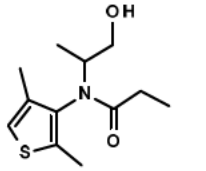
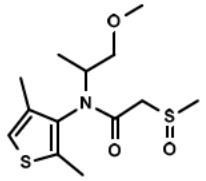
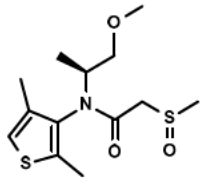
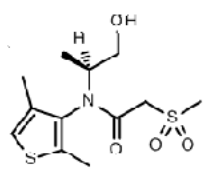
M656H010	Not assigned	M10	Not assigned	Rat	
M656PH010	5931836	M10	Not assigned	Rat	
M656H011	403120	M11	Not assigned	Rat	
M656H012	Not assigned	M12	Not assigned	Rat	
M656H013	Not assigned	M13	Not assigned	Rat	
M656PH013	Not assigned	M13	Not assigned	Rat	
M656H014	Not assigned	M14	Not assigned	Rat	

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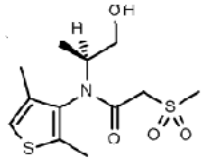
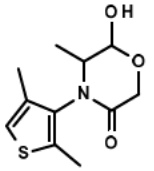
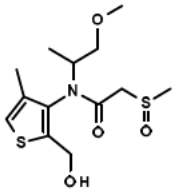
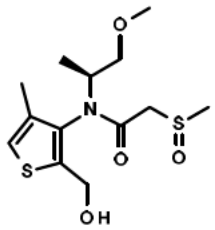
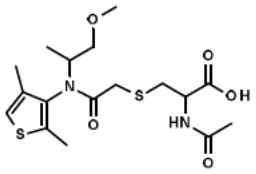
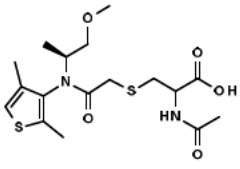
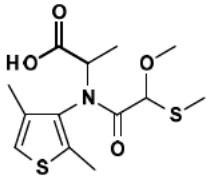
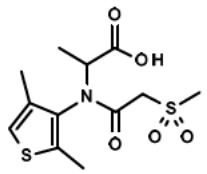
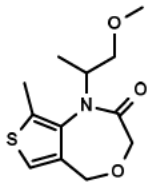
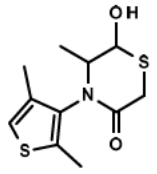
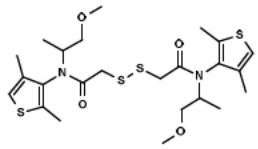
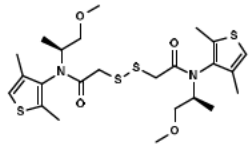
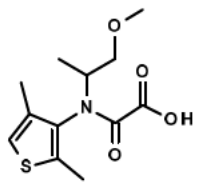
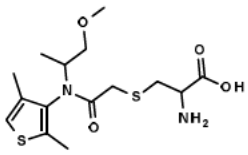
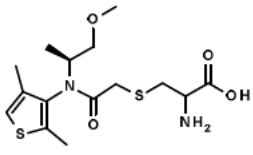
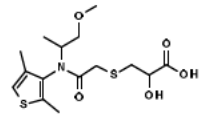
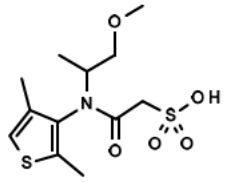
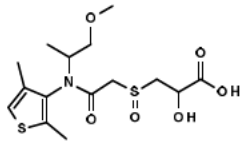
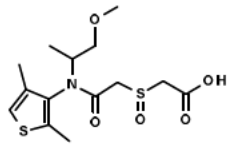
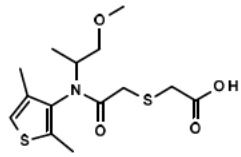
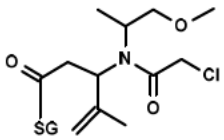
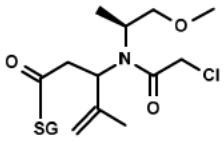
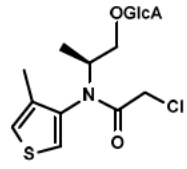
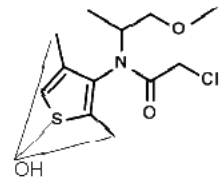
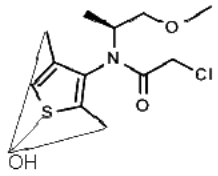
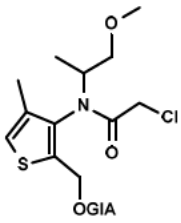
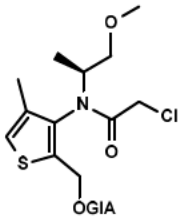
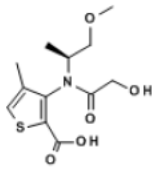
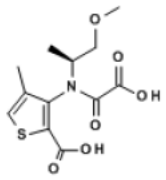
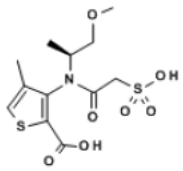
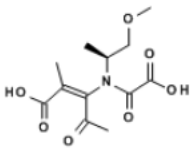
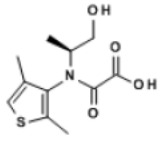
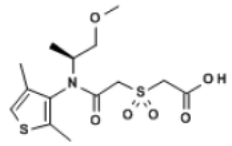
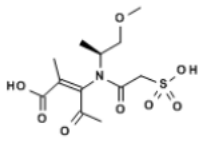
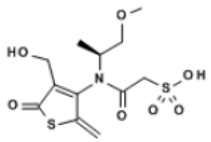
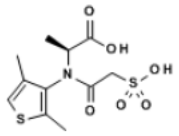
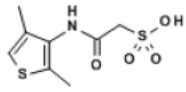
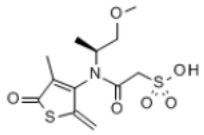
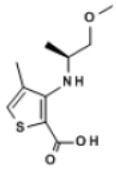
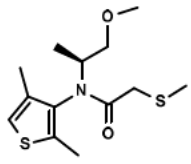
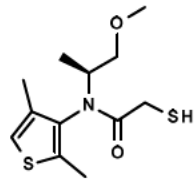
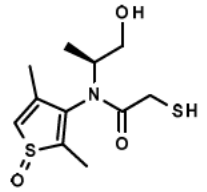
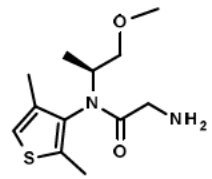
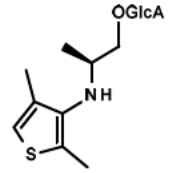
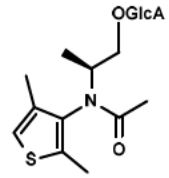
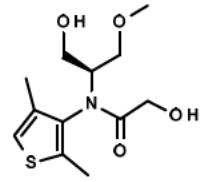
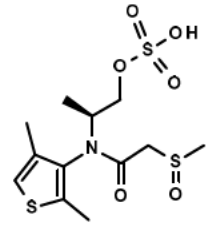
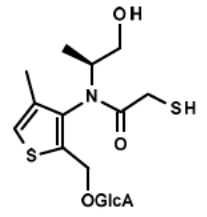
M656PH014	Not assigned	M14	Not assigned	Rat	
M656H015	360711	M15	Not assigned	Rat	
M656H016	Not assigned	M16	Not assigned	Rat	
M656PH016	Not assigned	M16	Not assigned	Rat	
M656H017	Not assigned	M17	Not assigned	Rat	
M656PH017	Not assigned	M17	Not assigned	Rat	
M656H018	Not assigned	M18	Not assigned	Rat	

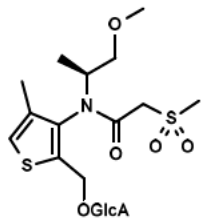
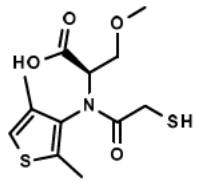
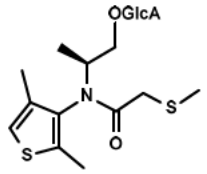
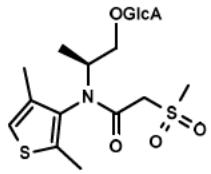
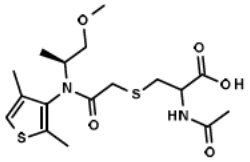
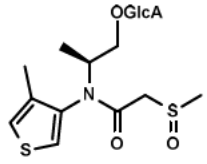
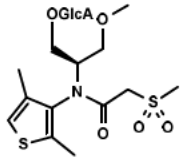
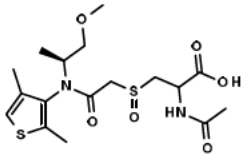
Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656H019	Not assigned	M19	Not assigned	Rat	
M656H020	360713	M20	Not assigned	Rat	
M656H021	Not assigned	M21	Not assigned	Rat	
M656H022	Not assigned	M22	Not assigned	Rat	
M656PH022	Not assigned	M22	Not assigned	Rat	
M656H023	360715	M23	Not assigned	Rat	
M656H025	Not assigned	M25	Not assigned	Rat Liver Slices	
M656PH025	Not assigned	M25	Not assigned	Rat Rat Liver Slices	

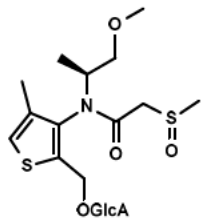
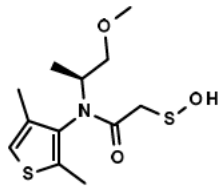
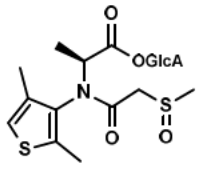
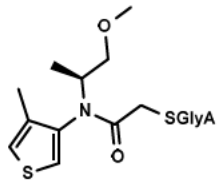
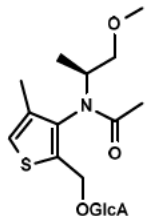
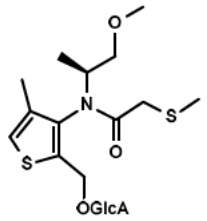
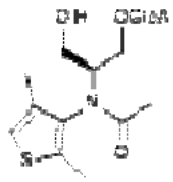
Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656H026	360716	M26	Not assigned	Rat	
M656H027	Not assigned	M27	Not assigned	Rat Mouse	
M656H030	Not assigned	M30	Not assigned	Rat	
M656H031	360712	M31	Not assigned	Rat Mouse	
M656H032	395234	M32	Not assigned	Rat	
M656H033	Not assigned	Not assigned	Not assigned	Rat Liver Slices	
M656PH033	Not assigned	Not assigned	Not assigned	Rat Liver Slices	
M656PH034	Not assigned	Not assigned	Not assigned	Rat Human Hepatocytes	
M656H035	Not assigned	Not assigned	Not assigned	Rat Liver Slices	

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH035	Not assigned	Not assigned	Not assigned	Rat Liver Slices	
M656H036	Not assigned	Not assigned	Not assigned	Rat Liver Slices	
M656PH036	Not assigned	Not assigned	Not assigned	Rat Rat Liver Slices	
M656PH043	5917262	M43/M44	Not assigned	Groundwater	
M656PH045	5917261	M45/M46	Not assigned	Groundwater	
M656PH047	5917260	M47/M48	Not assigned	Groundwater	
M656PH049	Not assigned	M49	Not assigned	Groundwater	
M656PH050	Not assigned	M50	Not assigned	Groundwater Soybean	

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH051	5931835	M51	Not assigned	Groundwater Rotational Crop Soybean	
M656PH052	Not assigned	M52	Not assigned	Groundwater	
M656PH053	Not assigned	M53/M57	Not assigned	Groundwater	
M656PH054	Not assigned	M54/M58	Not assigned	Groundwater	
M656H055	5749263	M55	Not assigned	Groundwater	
M656PH059		M59/M60/M61	Not assigned	Groundwater	
M656H062	5742710	M62	Not assigned	Groundwater	
M656PH067	Not assigned	Not assigned	Not assigned	Rat	
M656PH080	Not assigned	Not assigned	Not assigned	Rat	

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH082	Not assigned	Not assigned	Not assigned	Rat	
M656PH083	Not assigned	Not assigned	Not assigned	Rat	
M656PH085	Not assigned	Not assigned	Not assigned	Rat	
M656PH086	Not assigned	Not assigned	Not assigned	Rat	
M656PH087	Not assigned	Not assigned	Not assigned	Rat	
M656PH088	Not assigned	Not assigned	Not assigned	Rat	
M656PH091	Not assigned	Not assigned	Not assigned	Rat	

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH092	Not assigned	Not assigned	Not assigned	Rat	
M656PH093	Not assigned	Not assigned	Not assigned	Rat	
M656PH095	Not assigned	Not assigned	Not assigned	Rat	
M656PH096	Not assigned	Not assigned	Not assigned	Rat	
M656PH097	Not assigned	Not assigned	Not assigned	Rat	
M656PH098	Not assigned	Not assigned	Not assigned	Rat	
M656PH099	Not assigned	Not assigned	Not assigned	Rat	
M656PH100	Not assigned	Not assigned	Not assigned	Rat	

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH101	Not assigned	Not assigned	Not assigned	Rat	
M656PH102	Not assigned	Not assigned	Not assigned	Rat	
M656PH103	Not assigned	Not assigned	Not assigned	Rat	
M656PH105	Not assigned	Not assigned	Not assigned	Rat	
M656PH106	Not assigned	Not assigned	Not assigned	Rat	
M656PH107	Not assigned	Not assigned	Not assigned	Rat	
M656PH108	Not assigned	Not assigned	Not assigned	Rat	

Strategy to assess metabolite relevance:
Dimethenamid-P showed a complex metabolism in terms of the number of its metabolites, their chemical structure and the metabolic relationship between them. The retrospective characterisation and quantification of the dimethenamid-P breakdown products obtained in the lysimeter study using racemic dimethenamid included a re-evaluation of the original data from the pertinent study as well as additional mock studies (mini-lysimeter and soil degradation studies). The results of this analysis showed that a multitude of breakdown products exist in various quantities. Thus, in accordance with the SANCO guidance document, Sanco/221/2000-rev.10-final (2003) (ASB2012-3097), the question of their toxicological relevance has to be addressed including toxicity testing.
With respect to further toxicity testing, it is proposed - in order to avoid unnecessary animal studies - not to test all degradates, but only a selection of representative key structures. The applicant proposed a strategy for testing of selected key structures (Jilderda, 2014, ASB2014-8610). For this purpose the detected metabolites are compiled into groups and the key metabolites are identified within each group. The identification of these metabolites is based on their chemical structure and/or their expected metabolism to other metabolites in the same group in mammals. Overlap exists between the metabolism of dimethenamid-P in soil, plant and animal, which will also be taken into consideration for the grouping proposal. The grouping proposed is mainly referring to soil and groundwater metabolites, but also includes testing strategies for crop metabolites. Finally, the extent of testing for each key metabolite is proposed based on its approximate concentration in the lysimeter studies in conjunction with the already available information.

Metabolite grouping strategy:
Thus a stepwise approach was conducted to group the metabolites, and to select the metabolites for toxicological testing as described below.

Proposal for metabolite grouping:
The grouping proposal takes into account:

- Chemical similarity,
- Coverage by mammalian toxicity studies conducted with parent dimethenamid-P or racemic dimethenamid.
- Information on chemical reactivity (structural alerts)

In a further step key structures were selected for toxicological relevance assessment within every metabolite group.

Selection of key structures for hazard assessment:
Chemical Similarity:
With regard to evaluation of chemical similarity the general proposals given by e.g. the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment (EFSA Journal 2012;10(07):2799; ASB2012-10281) were followed. The following general molecular modifications were considered to probably not cause higher toxicity of the metabolites:

- Simple demethylation of the ring or side chain,
- Simple hydroxylation of the ring system without any cleavage of the ring,
- Hydroxylation of another ring position,
- Conjugation of metabolites with amino acids,

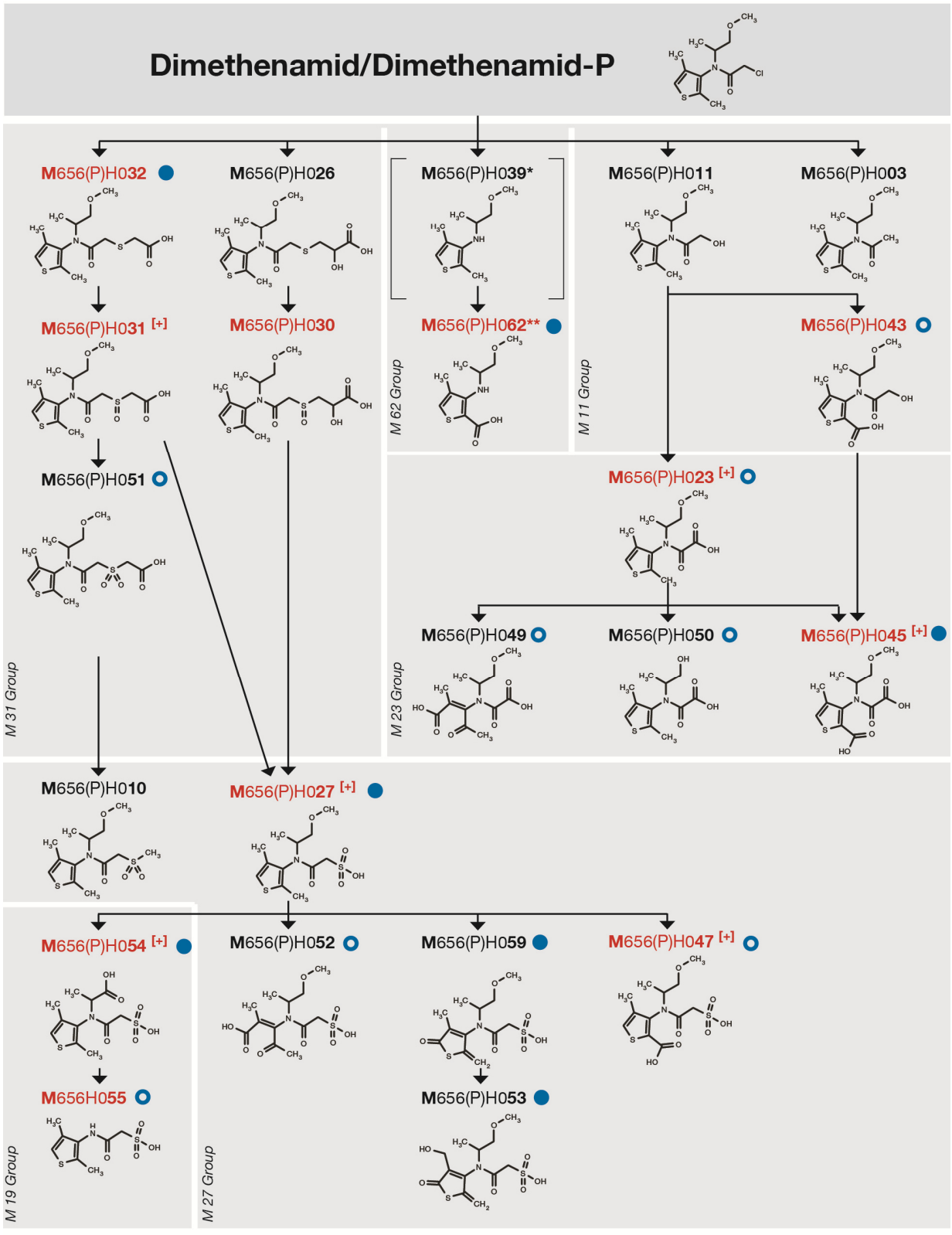
Comparison was made to parent as well as to the grouped metabolites in order to select key metabolites for testing. In addition consideration of increased hydrophilicity and thus considered faster excretion of the grouped metabolites as compared to the tested key metabolites and/or parent was taken into account.
Generally there is some overlap between functional groups covered by metabolites of different metabolite groups. Therefore on a case by case basis only one of the metabolites with this functional group was considered for testing and considered suitable to allow evaluation of the metabolite with the same functional group included in another group, where such consideration was taken into account this is indicated in the respective grouping table.

Coverage of metabolites of concern by mammalian toxicity studies:
Although not comparable some overlap exists in the metabolism of dimethenamid-P in animals, plant and soil. Thus general conclusions drawn from the animal metabolism of dimethenamid-P as well as overlaps of metabolite structures were taken into consideration when grouping the metabolites with regard to human exposure from the source of groundwater. It was considered whether either the metabolites under consideration or similar structures were formed in the metabolisms studies conducted in mammals.
Moreover, it is taken into consideration that groundwater metabolites after uptake into the body could be transformed by known metabolic pathways into structures that have been identified in the mammalian metabolism studies conducted.

Presence of Structural alerts:
For all metabolites identified with potential relevance or as corresponding group members presence for potential structural alerts was evaluated with different SAR/QSAR models. Models used, were the OECD toolbox, OASIS TIMES, DEREK (partly) and VEGA. These evaluations were in particular taken into account for those metabolites in the grouping approach where toxicological data are not available. However, the QSAR predictions obtained are limited by the reliability as most of the structures evaluated were not in the prediction domain. Thus, given the structural relationship of the metabolites evaluated inter alia and in relation to the parent molecule dimethenamid-P, the predicted alerts were compared to those for the parent and those metabolites where toxicological data were available in order to overcome the limitations of the predictions made.
Moreover, the systems used do not distinguish chiral structures. Thus, any prediction made applies generally to the racemic molecules as well as to the S-enantiomere metabolites considered for dimethenamid-P.
OECD Toolbox (Profiling module):
The OECD toolbox version 3.2 as downloadable via link of the ECHA webpage (http://www.qsartoolbox.org/download.html) was used for the evaluation. The outcome of the OECD toolbox profiling conducted for the metabolites was exported and collected (ASB2014-8407) as the report function of the current OECD toolbox version did not work properly. The profiling module provided structural alerts for different endpoints. Of particular interest were the modules dealing with protein- or DNA-binding capacity as well as genotoxicity and/or carcinogenicity predictions. It should be noticed that the profiles just provide structural alerts without consideration on probability that these alerts may become active or inactive due to chemical reactivity and/or sterical hindrance. The current version of the OECD toolbox does not allow to generate reports out of the conducted evaluations, instead the toxicological profiles obtained with the different modules were exported to EXCEL. These exported profiles however, do not include the explanation that are available for the different alerts identified, these can be obtained when running the evaluation with the OECD toolbox as available and have been included for evaluation of the individual compounds below.
OASIS TIMES:
OASIS TIMES is a hybrid statistical and knowledge-based model for toxicity prediction. The Tissue Metabolism Simulator (TIMES), developed by LMC (Bourgas University, Bulgaria; http://oasis-lmc.org/) integrates on the same platform a metabolic simulator and QSAR models for predicting toxicity of selected metabolites. The metabolic simulator generates plausible metabolic maps from a comprehensive library of biotransformations and abiotic reactions. It allows prioritisation of chemicals according to toxicity of their metabolites. Of OASIS TIMES the prediction models for Ames test and *in vitro* chromosome aberration were considered and therefore predictivity is limited to these test systems only. The reports for the evaluations made are available under ASB2014-8408, ASB2014-8409, ASB2014-8410 and ASB2014-8411. Q(SAR) Model Reporting Formats (QMRF) for both endpoints are provided in ASB2014-8489 and ASB2014-8490.
The reactivity model describing interactions of chemicals with DNA is based on an alerting group approach. Only those toxicophores extracted from the training set having clear interpretation for the molecular mechanism causing the ultimate effect included in the model. The mechanistic interrelation between alerts and related parametric ranges generalising the effect of the rest of the molecules on the alert is also considered. The structural component of the model is based on the structural similarity between chemicals in the training set which were correctly predicted by the model. The structural neighbourhood of atom-centered fragments is used to determine this similarity. The training set

consists of 1514 chemicals for Ames and 808 chemicals for chromosomal aberration.
The derived model is combined with metabolic simulator TIMES used for predicting metabolic activation of chemicals with the S9 mix. The metabolic simulator is trained to reproduce documented maps for mammalian liver metabolism for 261 chemicals. Parent chemicals and each of the generated metabolites are submitted to a battery of models to screen for a general effect and mutagenicity mechanisms. Thus, chemicals are predicted to be mutagenic as parents only, parents and metabolites, and metabolites only. Mutagenicity could be due to the parent chemical only or as a result of its metabolic activation (i.e., the parent is inactive but it is transformed to a mutagenic metabolite), or both parent structure and metabolites could be mutagenic.
This OASIS QSAR system is also included in the OECD Toolbox (but not in combination with TIMES), in order to make use of (Q)SAR approaches also in the assessment of chemicals under REACH (OECD, 2008). The applicant reported that the BASF-internal version has the advantage that it is capable to consider metabolic transformation.
DEREK:
DEREK is a predictive computer program, which is an expert system for the identification of toxic potential from chemical structure. The evaluation conducted for metabolites of dimethenamid-P is presented in ASB2014-8491. Further details of the system can be found on the supplier's web site http://www.lhasalimited.org/ under the section for DEREK. The QMRFs for mutagenicity, chromosomal damage and carcinogenicity are available on the ECP-JMPR homepage (http://qsardb.jrc.it/qmrf/). DEREK Nexus uses a knowledge base, which contains alerts describing structure-toxicity relationships, with an emphasis on the understanding of mechanisms of toxicity and metabolism. During an interactive session, DEREK Nexus identifies any toxophores or substructures associated with toxicity, and highlights these to the user with a brief statement about the hazard it represents. The user can access additional information concerning the structure-toxicity relationship including literature references and supporting examples. It is well known that the physicochemical properties of a compound play an important role in determining potential toxicity. DEREK Nexus calculates LogKp (by the Potts & Guy equation), logP (by the Moriguchi estimation) and Molecular weight –by LPS). These values are used in the DEREK assessment where appropriate (e.g. when skin penetration is a factor in assessing the significance of a finding). The knowledge base covers a wide variety of important toxicological end points, which include carcinogenicity, mutagenicity, skin sensitisation, reproductive toxicity, irritation, and respiratory sensitisation.
VEGA:
Using the VEGA platform, access to a series of QSAR (quantitative structure-activity relationship) models for regulatory purposes was obtained. Of the models offered by VEGA [http://www.vega-qsar.eu/] only the two independent statistical prediction models for mutagenicity (Ames) were selected. The data obtained for dimethenamid-P and its metabolite can be found in ASB2014-8412 and ASB2014-8413. The first one is an implementation of CAESAR, which makes predictions based on the comparison of the structure of interest to the CAESAR database of mutagenicity data of substances in the structure database. A score is provided for the match of the structures, and the mutagenicity data of the closest related substances compared to the structure of interest. Consequently, if a structure is not adequately presented in the database, the prediction is only of very limited validity. It is important to note, that although the chemical space of any moiety of chloroacetanilid herbicides is covered no significant match to dimethenamid-P was identified.
The second algorithm SarPy searches for isolated structural alerts of substructures in the molecule. Again this is based on the mutagenicity data provided in the structure database.
CAVEAT on reliability of QSAR modules implied:
With regard to the QSAR evaluations as implied in the OECD toolbox, in OECD TIMES, in DEREK and in VEGA it should be noted that for nearly all analysis the algorithms reported "structural domain error". As a consequence the predictivity is solely based on the proposed DNA-interaction via the structural alert, not (OECD toolbox and VEGA) or not appropriately (OASIS TIMES, DEREK) taking into account possible functional group interaction and stereochemical hindrance. It is well acknowledged that these structural activity predictions are therefore of limited validity. To overcome these limitations the evaluation was conducted mainly in comparison to the parent compound dimethenamid-P or metabolites with available toxicological data, in order to assess whether same or other predictions than for the compared compound were made.
Based on the general considerations illustrated above the following grouping proposal was developed

by the applicant (K. Jilderda 2014, ASB2014-8610) as shown in Figure B.6.8-1 below.



Concentration

0.1 µg/l ≤ ○ < 0.75 µg/l ≤ ●

metabolites tested = Level I: genotox

metabolites tested [+] = Level II: genotox + repeated dose

* Presumed metabolism intermediate and tentative live-stock metabolite

** Ethylester derivative was used as surrogate for toxicological testing

Figure B.6.8-1: Grouping proposal for relevance assessment of groundwater metabolites of dimethenamid-P

The groups were named as follows based on either already identified rat or key metabolites considered members of these groups:

1. M31-group
2. M11-group
3. M19-group
4. M62-group
5. M23-group
6. M27-group

Selection of key structures hazard assessment and toxicological testing strategy:

As provided by the EU SANCO guidance document on relevance assessment of groundwater (ASB2012-3097) the predicted exposure levels are taken into consideration. Principally all metabolites above 0.1 µg/L require a relevance assessment but the stage to be followed are based on the exposure levels. The steps included are:

Stage 1 of Step 3:	Screening for biological activity (all metabolites >0.1 µg/L)
Stage 2 of Step 3:	Screening for genotoxicity (all metabolites >0.1 µg/L)
Stage 3 of Step 3:	Screening for toxicity (provided there is no relevant toxicological alert from the parent molecule; all metabolites >0.75 µg/L)

The exposure thresholds provided in the SANCO guidance document of 2003 (SANCO/221/2000 – rev.10- final 25 February 2003; ASB2012-3097), are based on the threshold of toxicological concern (TTC) concept (Cramer et al., 1978, CHE2006-1120; Kroes et al., 2004, TOX2004-1275; Munro et al., 1996, TOX2004-1274), which has been further developed since then. Consequently in the group evaluations below these developments have been taken into consideration for the overall relevance assessment.

In 2010 for metabolites in groundwater a TTC value of 3.0 µg/L in drinking water has been proposed (Melching-Kollmuß et al.; 2010, ASB2011-12734) for non-genotoxic compounds in Cramer-Class III. This proposal took into consideration that reprotoxic compounds might not have been adequately covered in the original database the TTC-concept was developed on (Cramer et al., 1978, CHE2006-1120; Kroes et al., 2004, TOX2004-1275; Munro et al., 1996, TOX2004-1274).

Based on the original TTC concept proposal, it is assumed that non-relevant metabolites in drinking water would be assigned to Cramer class III (most toxic group). This TTC value is used to calculate a maximum tolerable drinking water concentration of 4.5 µg/L on basis of the WHO rules for determination of drinking water guidance values from ADIs (WHO, 2011; Guideline for drinking-water quality, 4th edition, World Health Organisation, Geneva). This is based on the assumption of an average consumption of 2 L water per day of a person with 60 kg body weight, with 10 % of exposure to the metabolite being derived from drinking water consumption. Recent assessments of toxicological endpoints for prenatal developmental effects of chemicals have shown that the TTC value of 4.5 µg/L (calculated from the Cramer class III value) is protective regarding developmental effects. More recently a re-evaluation of the Cramer classification for 824 NOAELs from repeated dose toxicity studies of industrial chemicals concluded that the existing TTC values are indeed protective.

Also the European Food Safety Authority (EFSA) has in its recent evaluation on the TTC concept (EFSA, 2012b. Scientific Opinion: Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). European Food Safety Authority (EFSA) Scientific Committee, Parma, Italy; EFSA Journal, 10(7), 2750, (ASB2015-2796) and its applicability on the assessment of plant metabolites (EFSA, 2012a, Scientific opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment, EFSA Panel on Plant Protection Products and their Residues (PPR), European Food Safety Authority (EFSA), Parma, Italy; EFSA Journal, 10(07), 2799 (ASB2012-10281)) come to the conclusion that the TTC values based on Cramer classes are protective against reproduction and developmental toxicity.

Therefore, a consideration may be helpful whether the metabolites in the leachate are expected to occur below or above this TTC value of 4.5 µg/L drinking water equivalent to the proposed EFSA TTC value of 1.5 µg/kg bw/day for the evaluation of plant metabolites.

All metabolite exposure predictions for dimethenamid-P groundwater metabolites have clearly been shown to be below this level applicable for compounds without evidence for genotoxicity since even the entire fractions in which they were detected are ≤4.5 µg/L. Provided it could be demonstrated that

there is no evidence for genotoxicity, the metabolites could thus considered not to be toxicologically relevant.
When the higher threshold level as discussed above could be applied this approach would allow to avoid toxicological screening testing as described in the Stage 3 of Step 3 of the relevance assessment in combination with the Step 4 exposure assessment. However, the current guidance document of 2003 has not yet been adapted to the more recent developments.
Thus, for the testing approach out of each group with more than one ground-water metabolite with relevant predicted ground-water exposure levels at least two representative group members were selected for hazard assessment.
For the stage 3 of the hazard assessment as a pragmatic approach the toxicological properties of the parent molecule are taken into consideration. Dimethenamid-P is not classified to be toxic either by acute or by subchronic to chronic exposure. As recently evaluated by ECHA (http://echa.europa.eu/web/guest/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling) dimethenamid-P is neither classified for genotoxicity, carcinogenicity nor reprotoxicity. Thus metabolites would not require a toxicological testing for these endpoints. However, in order to obtain certain information of toxicological properties and to allow to derive a hazard based exposure level it was agreed upon with the Rapporteur Member State Germany to test selected structures in a 28-day rat study.
In conclusion out of each group that contained more than two groundwater metabolites two representatives were selected to assess the potential hazard of the entire group. For groups where exposure levels for none of the group members were above 0.75 µg/L the testing was limited to genotoxicity testing, while for groups that contained structures with predicted exposure levels equal to or above 0.75 µg/L a 28-day rat study was conducted in addition.

M31-group

For the M31-group the following molecules were taken into consideration: M656PH025, **M656PH026**, M656PH028, **M656PH030**, M656PH031, **M656PH032** and **M656PH051**. Metabolites highlighted in bold are the considered significant metabolites determined in groundwater or in edible commodities at relevant exposure levels for humans.

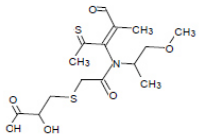
M31-group - Chemical Similarity:
The metabolite **M656PH032** (thioglycolic acid conjugate) formed by glutathione-conjugation to M656PH024 and subsequent hydrolysis of the glutathione conjugate to the cysteine conjugate, M656PH025. Rapid replacement of the amine group with a hydroxyl group gives **M656PH026**. This was identified in plants (maize), soil and in groundwater.
M656PH030 is formed by facile oxidation of the thioether of **M656PH026** to a sulfone.
Oxidation of **M656PH032** leads to formation of M656PH031 (sulfoxide of thioglycolic acid conjugate).
And further oxidation gives **M656PH051**. Both metabolites M656PH031 and **M656PH051** were identified in soil and water and M656PH031 was found in maize and soybean metabolism. **M656PH051** was found in a confined rotational crop study but they were not determined in the rat metabolism studies.
The metabolites **M656PH026** and **M656PH030** are soil metabolites with no detectable leachate concentrations. **M656PH026** was found in goat dosed with **M656PH030**, maize and soybean while **M656PH030** was also identified in all plant metabolism studies as well as a goat metabolism study dosed with **M656PH030**. They were found in some forage or tops samples destined as animal feed. Taking the most recent discussions on the definition of the relevant residue for dietary exposure assessment (OECD, EFSA) into account, they need to be considered for toxicological relevance assessment of consumers. The metabolites **M656PH026** and **M656PH030** were included in the residue analytical method for plants and also quantified during supervised field trials. The limit of quantitation for both components is at 0.01 mg/kg. As expected from the plant metabolism studies, their presence was confirmed in some feed items (highest levels of **M656PH030** at approximately 2.9 mg/kg in rape forage) and they were also found in edible commodities. The calculated exposure levels for humans are 0.0025 µg/kg bw/day (threshold for genotoxic compounds) < **M656PH026** or **M656PH030** ≤ 1.5 µg/kg bw/day (threshold for non-genotoxic Cramer Class III compounds). Both

metabolites are tentative rat metabolites determined in a rat metabolism studies conducted with racemic dimethenamid (previously reviewed under Annex I). In the new goat metabolism study (Ferguson, 2014, ASB2014-8342) dosed with M6565PH030, **M656PH026** was the major metabolite in all tissues with ~69 % of the applied dose excreted in urine and ~20 % excreted in faeces. **M656PH030** was also observed in the goat at ~4 % in bile and lower levels in all other matrices.
The precursor rat metabolite M656PH025 (cysteine conjugate) and the plant metabolite M656PH028 (sulfoxide of the cysteine conjugate) are also considered to be members of this group although not identified as being groundwater metabolites or plant metabolites with relevant human exposure.

M31-group - Coverage of metabolites of concern by mammalian toxicity studies:
The metabolite M656PH025 is a major rat metabolite (3.2 - 20.7 % of applied dose in bile) in the new rat metabolism study, while **M656PH026** and **M656PH030** are tentative rat metabolites that were only determined in trace amounts of the applied dose in the original rat metabolism studies conducted (<0.1 - 0.4 % of applied dose or <0.1 - 0.2 % of applied dose). In the new goat metabolism study (Ferguson, 2014, ASB2014-8342) dosed with M6565PH030, **M656PH026** was the major metabolite in all tissues with ~69 % of the applied dose excreted in urine and ~20 % excreted in faeces. **M656PH030** was also observed in the goat at ~4 % in bile and lower levels in all other matrices. Thus in principle metabolites of this group could be considered adequately covered by the rat and goat toxicity studies. However, the groundwater metabolites with relevant exposure levels (**M656PH032** and **M656PH051**) have not been identified as rat metabolites per se, while the plant metabolites **M656PH026** and **M656PH030** found in edible commodities were only covered in trace amounts in the rat studies. However, **M656PH026** and **M656PH030** were measured in significant amounts in the goat. **M656PH051** is a hypothetical mammalian metabolite of M656PH031 since it is known from many examples that aliphatic sulfoxides are easily converted to the respective sulfones.
Comparable structural alerts for chromosome aberration *in vitro* had been identified for **M656PH026**, M656PH028, **M656PH030**, M656PH031, **M656PH032** and **M656PH051** by presumed formation of ring-opened transformation products.

1.22

Metabolite

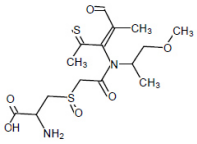


Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

Presumed metabolite of **M656PH026**

4.9

Metabolite

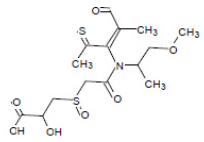


Predicted CA with S9	in vitro CA negative
Predicted Mechanism	
Alert info	Alfa,Beta-Unsaturated Aldehydes,Alpha,beta-carbonyls polarised double bonds
ModelReliability	
Total Domain	N/A

Presumed metabolite of M656PH028

5.16

Metabolite

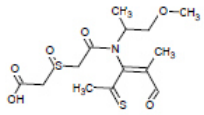


Predicted CA with S9	in vitro CA negative
Predicted Mechanism	
Alert info	Alfa,Beta-Unsaturated Aldehydes,Alpha,beta-carbonyls polarised double bonds
ModelReliability	
Total Domain	N/A

Presumed metabolite of **M656PH030**

6.9

Metabolite

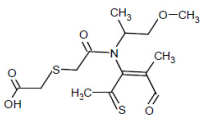


Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

Presumed metabolite of M656PH031

7.12

Metabolite

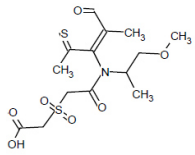


Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

Presumed metabolite of **M656PH032**

8.7

Metabolite



Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

Presumed metabolite of **M656PH051**

Figure B.6.8-2: Comparison of structural alerts for presumed degradates of M656PH026, M656PH028, M656PH030, M656PH031, M656PH032 and M656PH051 in OASIS-TIMES

Thus it is reasonable to assume that the toxicological testing conducted with **M656PH030**, and M656PH031 (see Table B.6.8-2) covers potentially structural alerts of **M656PH026**, **M656PH032** and **M656PH051**.

M31-group - Presence of Structural alerts:
The structure activity evaluation conducted for the metabolites of the M31-group is summarised in the Table below.

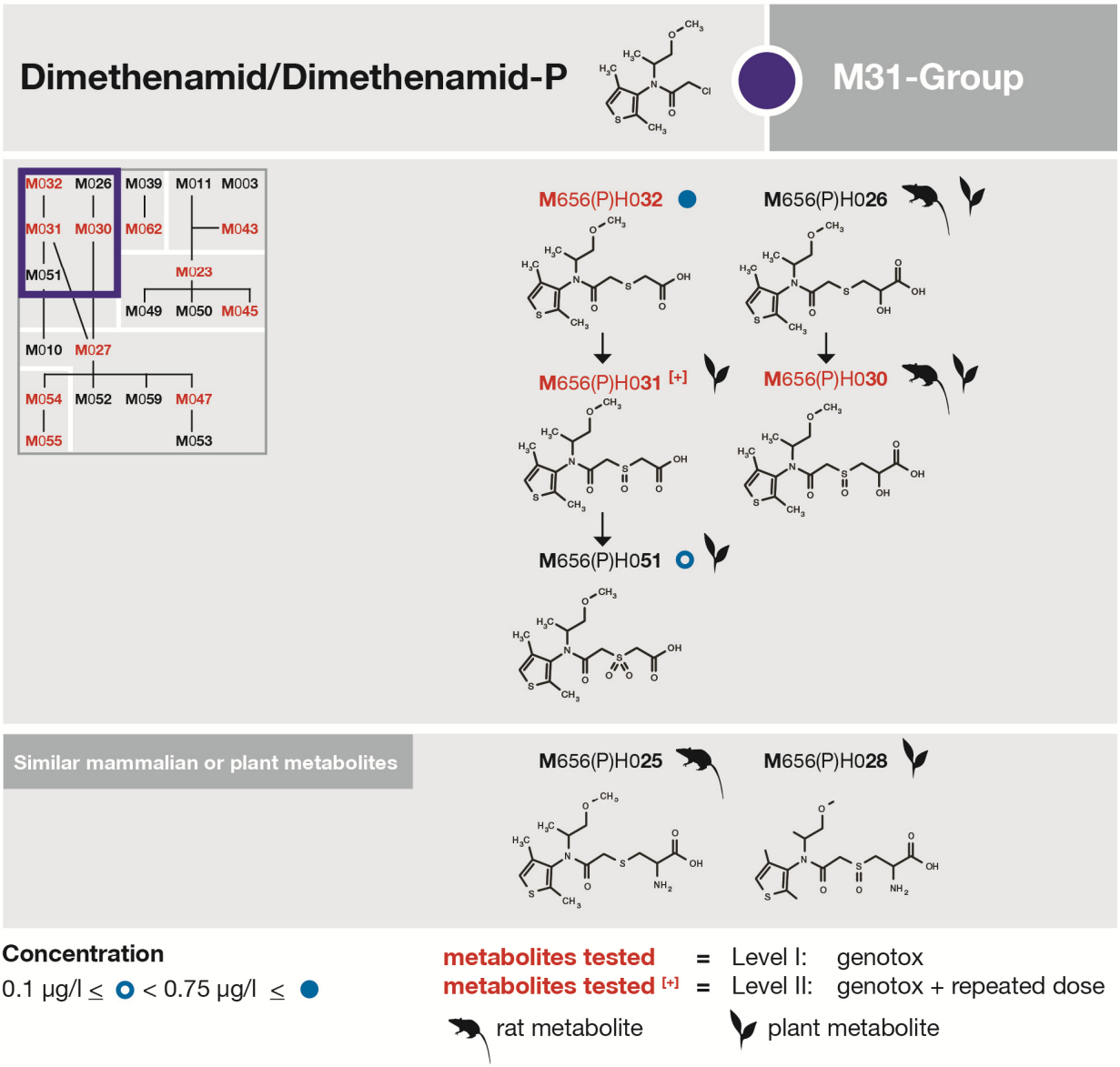
Table B.6.8-2: Structure activity evaluation of metabolites in M31-group

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M656PH025	OE	No other alert than parent	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
M656PH026	OE	No other alert than parent	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	VE	Not mutagenic	Low	
M656PH028	OE	No other alert than parent	Low	No conclusive alert for genotoxicity Weight of evidence: not mutagenic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH030	OE	No other than parent	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
	VE	Not mutagenic	Low	
M656PH031	OE	No other than parent	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Low	
M656PH032	OE	No other than parent	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Low	

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M656PH051	OE	No other than parent	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	DE	No alert for genotoxicity	Low	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	

OE = OECD-toolbox
OA = OASIS-times
DE = DEREK
VE = Vega

Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, 2014, ASB2014-8610).
For some group members (**M656PH026**, M656PH031, **M656PH032** and **M656PH051**) there was a structural alert given for transformation into a potentially genotoxic degradate. The structural alert was that of unsaturated carbonyl compounds. They are known to present toxicological concerns in biological systems *in vitro*. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl building a reactive centre for nucleophilic addition and is mostly characterised for small, low molecular weight molecules. This alert is known to become active in *in vitro* systems but in particular for greater complex molecules like the structures presented in Figure B.6.8-3 above the evidence to become effective *in vivo* is lacking.
Based on the evaluation in Step A to C (see above) it was proposed to include the following groundwater metabolites and their presumed precursors into the group as illustrated below (see Figure B.6.8-3), in addition similar metabolites determined in the rat were added to that group as was a plant metabolite with potential relevance for human exposure via feed item.



Concentrations of potential ground-water contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

Figure B.6.8-3: M31-group proposal

M31-group - Selection of key structures for hazard assessment:

Based on the threshold concern concept except for genotoxicity no toxicological testing would be required for any groundwater metabolite of that group as all exposure levels are well below 4.5 µg/L, which is based on most recent publications and evaluations the relevant threshold for non-genotoxic compounds of Cramer Class III (see explanation in the section above). Thus the predicted exposure levels for groundwater and plant metabolites suggest no toxicological concern provided that there is no genotoxicity alert. However, although this approach has been taken into consideration by EFSA for the evaluation of plant metabolites the EU guidance for groundwater metabolites of 2003 still provides a threshold of 0.75 µg/L which would be exceeded by the metabolite **M656PH032**. The predicted exposure levels in groundwater and edible commodities are summarised in the Table below.

M656PH032 has a thioether structure and, as demonstrated with numerous other aliphatic dialkyl sulfides, most likely would be rapidly metabolised to the sulfoxide M656PH031 by rats and other mammals. Therefore it was decided to rather use M656PH031 for toxicity testing (mutagenicity and 28 days oral toxicity). Degradate **M656PH051** is the corresponding sulfone of M656PH031. The toxicity of **M656PH051** can be extrapolated from the structurally very similar M656PH031. In

addition, **M656PH051** is a hypothetical mammalian metabolite of M656PH031 since it is known from many examples that aliphatic sulfoxides are easily converted to the respective sulfones. **M656PH032** was chosen as second group representative for additional testing in an Ames test to support the conclusion on lacking genotoxicity of the entire group. Furthermore following the available guidance on plant metabolite relevance assessment the metabolite **M656PH030** (determined in edible commodities) was additionally selected for genotoxicity testing aiming to address the toxicological non-relevance of this plant metabolite as well as of the other plant metabolite with potential human exposure **M656PH026**. As for the pair **M656PH032** and M656PH031 it is expected that **M656PH026** is rapidly metabolised to the sulfoxide **M656PH030**.

Table B.6.8-3: M31-group: Metabolite source and exposure levels

Metabolite	**Source**				**Relative concentration in rat**	**Concentration in groundwater**	**Consumer exposure via residues**	**Selected group representative for biological activity screening**	**Selected group representative for toxicity testing**
	Rat	Plant	Soil	Groundwater	[% of administered dose]	[µg/L]			
M656PH025	X				3.2-20.7 in bile				
M656PH026	X*	X	X	X	<0.1-0.4 in urine and faeces		>0.0025 µg /kg bw/day ≤1.5 µg/kg bw/day		
M656PH030	X*	X	X	X	<0.1-0.2 in urine and faeces		>0.0025 µg /kg bw/day ≤1.5 µg/kg bw/day	B	G
M656PH031		X	X	X		0.06	Not in edible commodities	B	G / S
M656PH032			X	X		0.9		B	A
M656PH051		X	X	X		0.7	Not in edible commodities	B	

B = Biological activity screening
G = Genotoxicity battery
A = Ames test
S = Systemic toxicity: 28-day rat
* Tentatively identified metabolites in rat

Concentrations of potential ground-water contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

M11-group

For the M11-group the following molecules where taken into consideration: M656PH003, M656PH011, **M656PH043** and M656PH087. Metabolites highlighted in bold are the considered significant metabolites determined in groundwater or in edible commodities at relevant exposure

levels for humans.

Chemical Similarity:

- M656PH011 the dechlorinated, hydroxylated metabolite of dimethenamid-P is considered an intermediate in the formation of **M656PH043** and was not determined in the lysimeter. M656PH011 is however, a plant and a rat metabolite determined in the studies conducted with racemic dimethenamid (previously reviewed under annex I) and dimethenamid-P (see ASB2014-8383).
- The relevant groundwater metabolite assigned to that group is **M656PH043**, which has not been identified in the rat. The degradate **M656PH043** is derived from M656PH011 by oxidation of one of the methyl groups attached to the thiophene ring. The structure of the resulting carboxylic acid is still closely related to M656PH011. However, it can be considered less toxic than M656PH011 because of their increased polarity which makes it less bioavailable and easily excretable.
- M656PH003 the decarboxylated **M656PH043** has also been determined in rats.
- M656PH087 is another related rat metabolite identified in the recent rat metabolism study (see ASB2014-8383). This metabolite shows hydroxylation at the isopropyl ether side chain in comparison to M656PH011.

Coverage of metabolites of concern by mammalian toxicity studies:

The rat metabolites of this group M656PH003, M656PH011 and M656PH087 were present at trace levels in the studies conducted. Thus the groundwater metabolite **M656PH043** of this group could not considered to be adequately covered by the rat toxicity studies.

Presence of Structural alerts:

The structure activity evaluation conducted for the metabolites of the M11-group is summarised in Table B.6.8-4 below.

Toxicological data on metabolites:

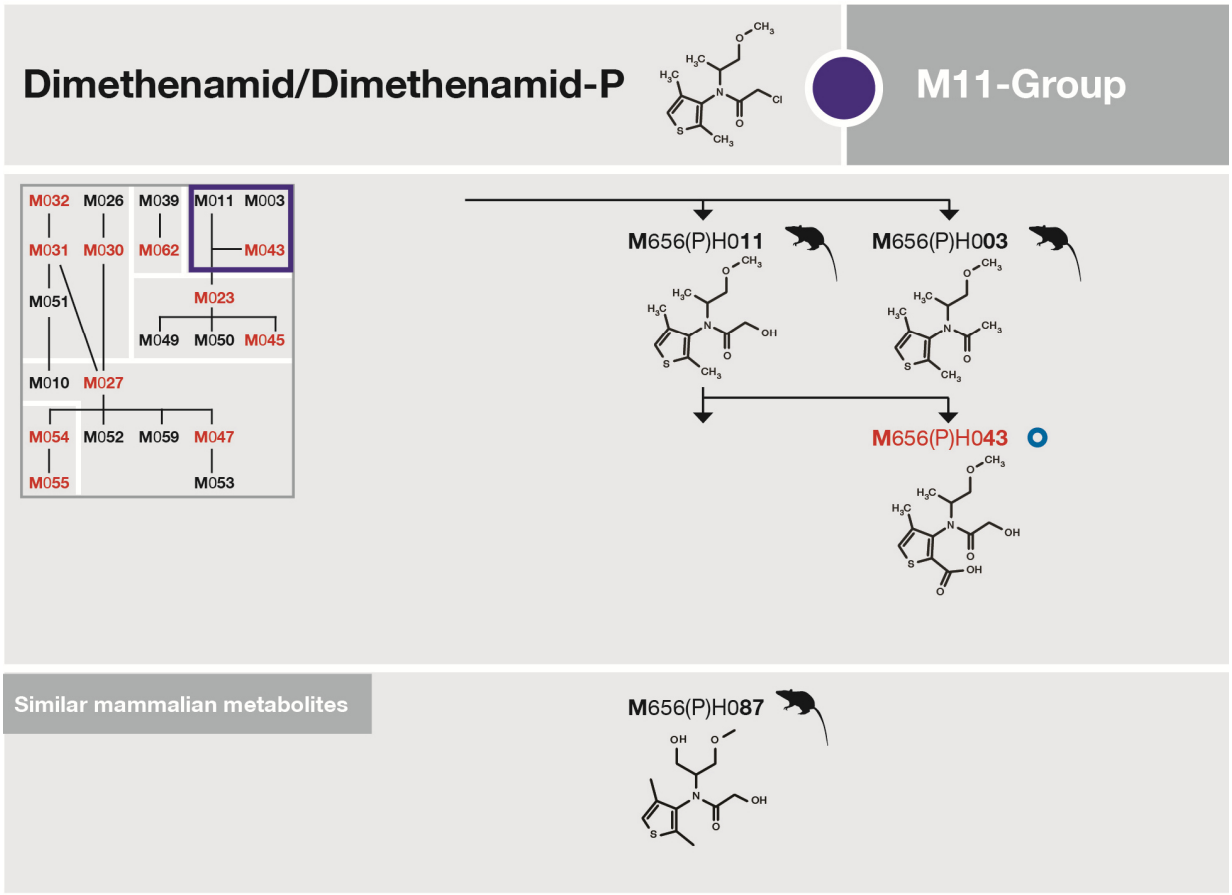
Studies (acute oral toxicity, Ames test and micronucleus test *in vitro*) on plant metabolites M656H023 (former assigned M23) and sodium salt of M656H037 (former assigned M27) have already been evaluated for Annex I inclusion of dimethenamid-P. For the convenience of the reviewer, these are summarised below as extracted from the monograph including addenda together with the extended studies conducted meanwhile for these metabolites.

Table B.6.8-4: Structure activity evaluation of metabolites in M11-group

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M656PH003	OE	No other alert than parent	Low	Alert for chromosomal aberration *in vitro*, covered by toxicological testing of dimethenamid-P
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low Reasonable	
	DE	No alert for genotoxicity	Low	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH011	OE	No other alert than parent	Low	Alert for chromosomal aberration *in vitro*, covered by toxicological testing of dimethenamid-P
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low Reasonable	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH043	OE	No other alert than parent	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (Chromosome aberration *in vitro*)	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Low	
M656PH087	OE	No other alert than parent	Low	Alert for chromosomal aberration *in vitro*, covered by toxicological testing of dimethenamid-P
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low Reasonable	

OE = OECD-toolbox
OA = OASIS-times
DE = DEREK
VE = Vega
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

For the parent molecule dimethenamid, M656PH003 and M656PH011 a structural alert has been identified for chromosomal aberration *in vitro* based on formation of transformation products containing the structural alert for alpha, beta polarised carbonyls. The structural alert characterised and considered in the prediction domain, was an aldehyde formation at the methyl-rest of the thiophene ring. Thus the prediction was considered reasonable. As the structures of concern were all rat metabolites they were considered covered intrinsically by the toxicological testing of racemic dimethenamid and dimethenamid-P. For the groundwater metabolite **M656PH043** this alert was not predicted as it contained a carboxyl rest instead of a methyl rest.
Based on the evaluation in Step A to C (see above) it was proposed to include the following ground-water metabolites and their presumed precursors into the group as illustrated in the figure below, in addition structurally similar metabolites determined in the rat were added to that group.



Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

Figure B.6.8-4: M11-group proposal

Selection of key structures for hazard assessment**:**
Several similar structures were identified in the rat metabolism studies conducted leading to the suggestion that this structural group is covered by the toxicity studies conducted with racemic dimethenamid and dimethenamid-P. However, the exposure levels for the rat metabolites of that group are in sum below 10 %. The predicted exposure levels in groundwater for **M656PH043** is below 2.2 µg/L, while it is <0.2 µg/L for M656PH003 and <0.1 µg/L for M656PH011 (see Table below).
M656PH043 was selected as key metabolite and evaluated for genotoxicity only (see Table below).

Table B.6.8-5: M11-group: Metabolite source and exposure levels

Metabolite	Source				Relative concentration in rat	Concentration in groundwater	Consumer exposure via residues	Selected group representative for biological activity screening	Selected group representative for toxicity testing
	Rat	Plant	Soil	Groundwater					
					[% of administered dose]	[µg/L]			
M656PH003	X			X	0.26-2 in urine and faeces	0.2			
M656PH011	X	X	X	X	<0.1-0.4 in urine and faeces	not detected in lysimeter	Not in edible commodities, occurrence in animal feed		
M656PH043			X	X		2.2		B	G
M656PH087	X				Combined peak with M656PH097 0.4 in urine				

B = Biological activity screening
G = Genotoxicity battery
A = Ames test
S = Systemic toxicity: 28-day rat
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

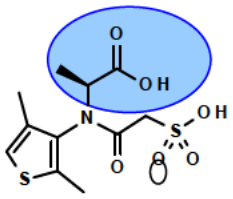
M19-group

For the M19-group the following molecules where taken into consideration: M656H019, **M656PH054**, **M656H055** and M656PH103. Metabolites highlighted in bold are the considered significant metabolites determined in groundwater or in edible commodities at relevant exposure levels for humans.

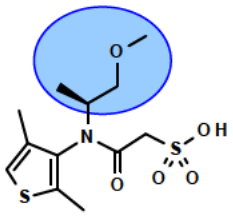
Chemical Similarity:

- The structurally closely related metabolite M656H019 was identified in urine and faeces in quantities of up to 1.1 % of the dose after oral administration of racemic dimethenamid to male and female rats. The potential toxicity of M656H019 is therefore covered by studies on the toxicity of the parent active substance.
- **M656PH054** bears a sulphonic acid group instead of the methylsulfone moiety present in M656PH019. Moreover, **M656PH054** is also closely related to M656PH027 (please refer to M27-group below). In comparison to M656PH027 this structure bears an additional carboxylic acid group instead of the methyl-ether group (see Figure B.6.8-5 below). Being more polar **M656PH054** is considered to be less bioavailable and easily excretable and thus presumably less toxic.
- The structure of degradate **M656H055** may be regarded as a dealkylation (removal of the propionic acid side chain) product from M656PH054, forming the secondary amide.
- M656PH103 is another related rat metabolite identified in the recent rat metabolism study [see

DocID 2012/1194996]. This metabolite shows a glucuronate-conjugation at the propionic acid side chain and a methylsulfone group instead of the sulfonic acid group in comparison to **M656PH054**.



M656PH054



M656PH027

Figure B.6.8-5: Comparison of M656PH054 and M656PH027

Coverage of metabolites of concern by mammalian toxicity studies:
M656PH054 can be considered less toxic than M656PH019 since it is more polar, considering that M656PH019 bears a methylsulfone moiety instead of the sulphonic acid group present in **M656PH054**. The rat metabolites of this group M656H019 and M656PH103 add up to at most 1.6 % of the applied dose in the rat metabolism studies conducted. Thus the structurally similar groundwater metabolite M656PH054 could not be considered adequately covered by the toxicological testing conducted with racemic dimethenamid or dimethenamid-P.
Due to the structural similarity to M656PH027, **M656PH054** could be considered to be covered by the toxicological testing of M656PH027, in particular as formation of the carboxylic acid moiety from the methyl-ether had originally been suggested in the rat metabolism (i.e. formation of M656H019 and M656H018). However, more recent investigations on the metabolism of dimethenamid-P in rats have raised some doubts on the formation of reasonable amounts of M656PH019 and M656PH018 in the rat. Thus, it was concluded that **M656PH054** is also not adequately covered by the toxicological testing of M656PH027.
M656H055 is not considered to be adequately covered by the rat toxicity studies as it seems that the formation of a secondary amide by cleavage of the methyl-ether-isopropal group is not happening in the rat metabolism. Moreover, it cannot be excluded that the secondary amide will have a different genotoxic profile than the tertiary amides represented by the other group members.

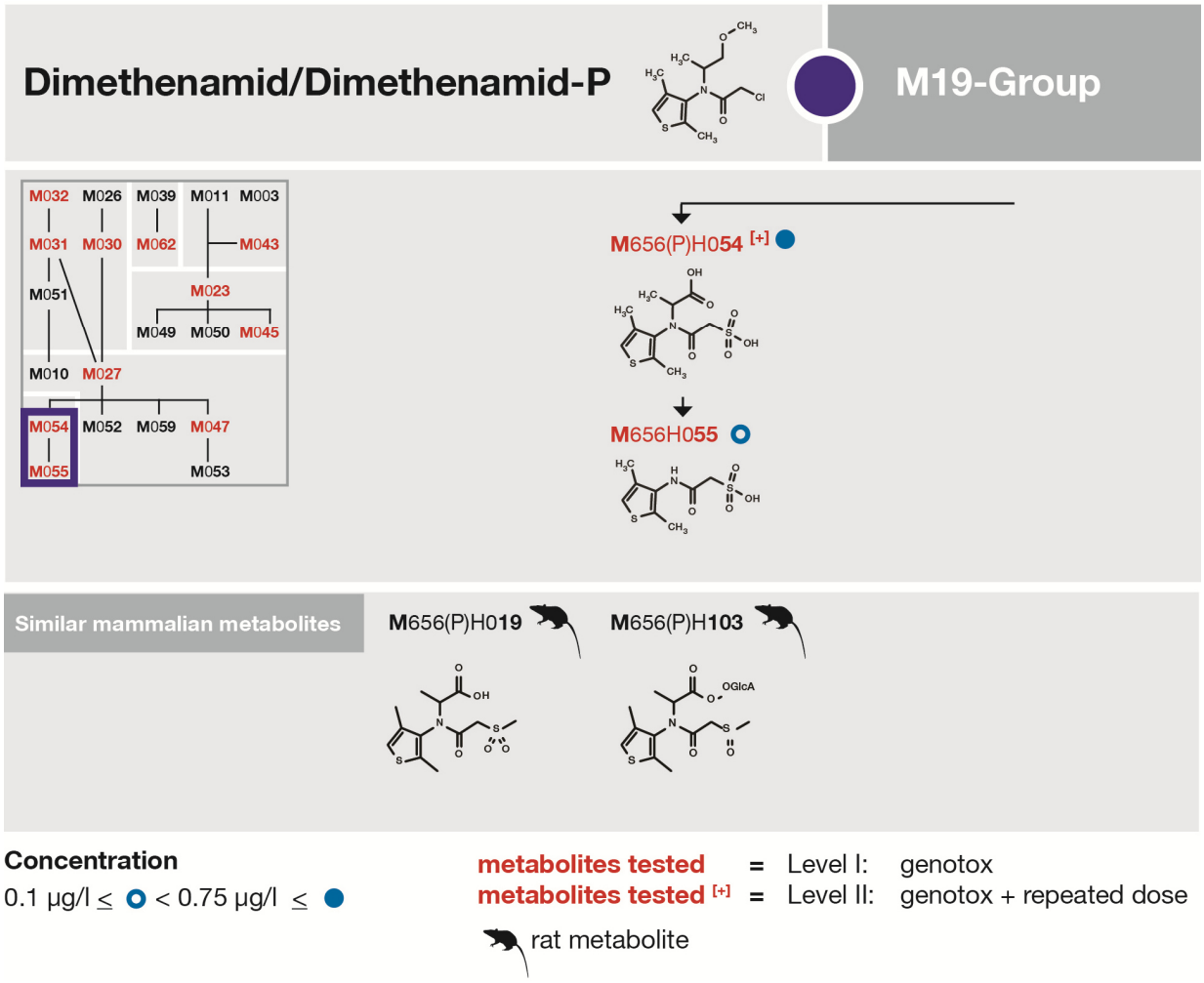
Presence of Structural alerts:
The structure activity evaluation conducted for the metabolites of the M19-group is summarised in the Table below.

Table B.6.8-6: Structure activity evaluation of metabolites in M19-group

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M656H019	OE	No other alert than parent	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH054	OE	No other than parent	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Reasonable	
M656H055	OE	No other than parent	Low	Alert for chromosomal aberration *in vitro* not confirmed by genotoxicity testing conducted
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Reasonable	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Reasonable	
M656PH103	OE	No other than parent	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	

OE = OECD-toolbox
OA = OASIS-times
DE = DEREK
VE = Vega
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

For **M656PH054** a structural alert has been identified for chromosomal aberration *in vitro* based on formation of transformation products containing the structural alert for alpha, beta polarised carbonyls. This structural is known to present toxicological concerns in biological systems *in vitro*. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl building a reactive center for nucleophilic addition and is mostly characterised for small, low molecular weight molecules. This alert is known to become active in *in vitro* systems but in particular for greater complex molecules like the structures presented in Figure B.6.8-2 above or Figure B.6.8-6 and Figure B.6.8-7 below the evidence to become effective *in vivo* is lacking.
Based on the evaluation in Step A to C (see above) it was proposed to include the following rat metabolites and groundwater metabolites and their presumed precursors into the group as illustrated the figure below.



Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

Figure B.6.8-6: M19-group proposal

Selection of key structures for hazard assessment:

Some similar structures were identified in the rat metabolism studies conducted leading to the suggestion that this structural group is covered by the toxicity studies conducted with racemic dimethenamid and dimethenamid-P. However, the exposure levels for the rat metabolites of that group is low and the structural similarity is given to **M656PH054** only as it seems that the formation of a secondary amide is not happening in the rat metabolism. Based on the threshold concern concept except for genotoxicity no toxicological testing would be required for any groundwater metabolite of that group as all exposure levels are well below 4.5 µg/L, which is based on most recent publications and evaluations the relevant threshold for non-genotoxic compounds of Cramer Class III (see explanation in the section above). However, although this approach has been taken into consideration by EFSA for the evaluation of plant metabolite the EU guidance for groundwater metabolites of 2003 still provides a threshold of 0.75 which would be exceeded by the metabolite **M656PH054**. Thus **M656PH054** was selected for genotoxicity and systemic toxicity testing. As it cannot be excluded that the secondary amide moiety of **M656H055** will exhibit a deviating genotoxic profile than **M656PH054**, **M656H055** was in addition selected for genotoxicity testing.

Table B.6.8-7: M19-group: Metabolite source and exposure levels

Metabolite	Source				Relative concentration in rat	Concentration in groundwater	Consumer exposure via residues	Selected group representative for biological activity screening	Selected group representative for toxicity testing
	Rat	Plant	Soil	Groundwater					
					[% of administered dose]	[µg/L]			
M656PH019	X				<0.1-0.8 in urine and faeces	-	-		
M656PH054				X		2.0	-	B	G / S
M656H055				X		0.4	-	B	G
M656PH103	X				Combined peak with M656PH010 0.8 in urine	-	-		

B = Biological activity screening
G = Genotoxicity battery
A = Ames test
S = Systemic toxicity: 28-day rat
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

M62-group

For the M62-group the following molecules where taken into consideration: M656PH039, **M656PH062** and M656PH085. Metabolites highlighted in bold are the considered significant metabolites determined in groundwater or in edible commodities at relevant exposure levels for humans.

Chemical Similarity:

- M656PH039 (former named PL 1588, [redacted] is a tentative live-stock metabolite. Like **M656PH062** it is the secondary amide obtained by loss of the chloroaceto-group of dimethenamid-P. Although not determined in soil or groundwater it is the presumed precursor in formation of **M656PH062**. The methyl-group of M656PH039 is oxidised to a carboxylic acid in M656PH062.
- The only relevant groundwater metabolite assigned to that group is **M656PH062**.
- M656PH085 is a related rat metabolite that also lost the chloro-aceto-group. The difference to M656PH039 is the glucuronidation of the methyl-ether-isopropal group.

Coverage of metabolites of concern by mammalian toxicity studies:
As stated above the only similar rat metabolite identified is M656PH085, which was however only quantified by levels of 0.598 % of the applied dose in urine. Thus, it was concluded that M656PH062 is not adequately covered by the toxicological testing of racemic dimethenamid or dimethenamid-P.
Some limited toxicological information is available for M656PH039 [redacted] for acute and limited repeated dose toxicity. However, no genotoxicity studies were conducted. Thus, the available information is not considered to be adequate to evaluate toxicological relevance of **M656PH062**.

Presence of Structural alerts:
The structure activity evaluation conducted for the metabolites of the M19-group is summarised in the table below.

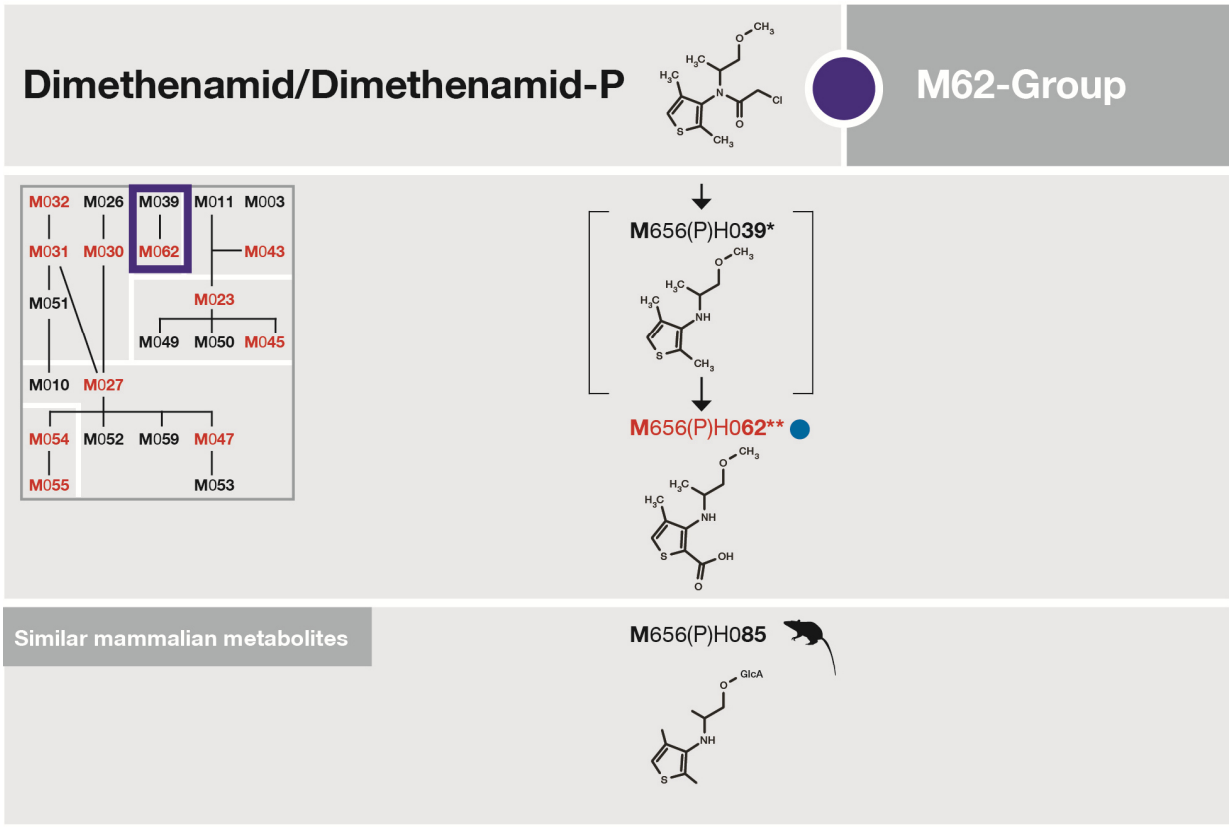
Table B.6.8-8: Structure activity evaluation of metabolites in M62-group

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M656PH039	OE	No other alert than parent for genotoxicity (however, DNA-binding alert of OECD module differs being a secondary amine (nitrenium ion formation) instead of a tertiary amine (iminium ion formation)	Low	Alert for chromosomal aberration *in vitro*, covered by toxicological testing of dimethenamid-P
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low Reasonable	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH062	OE	No other alert than parent for genotoxicity (however, DNA-binding alert of OECD module differs being a secondary amine (nitrenium ion formation) instead of a tertiary amine (iminium ion formation)	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (Chromosome aberration *in vitro*)	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Low	
M656PH085	OE	No other alert than parent for genotoxicity	Low	
	OA	Not mutagenic (Ames) Not genotoxic (Chromosome aberration *in vitro*)	Low	

OE = OECD-toolbox
OA = OASIS-times
DE = DEREK
VE = Vega
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

As for dimethenamid and some rat metabolites a structural alert has been identified for M656PH039 for chromosomal aberration *in vitro* based on formation of transformation products containing the structural alert for alpha, beta polarised carbonyls. The structural alert characterised and considered in the predictivity domain, was an aldehyde formation at the methyl-rest of the thiophene ring. Thus the prediction was considered reasonable. As the same alert was identified for dimethenamid it was considered covered intrinsically by the toxicological testing of racemic dimethenamid and dimethenamid-P. For the groundwater metabolite **M656PH062** this alert was not predicted as it contained a carboxyl rest instead of a methyl rest.
Based on the evaluation in Step A to C (see above) it was proposed to include the following rat

metabolites and groundwater metabolites and their presumed precursors into the group as illustrated the figure below.



Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

Figure B.6.8-7: M62-group proposal

Selection of key structures for hazard assessment:

Only one similar structure (M656PH039) a tentative hen metabolite and assumed pathway intermediate was identified. Thus the groundwater metabolite **M656PH062** was identified as key structure for hazard assessment (see Table below).

The predicted exposure via groundwater was 0.75 µg/L ≤ **M656PH062** ≤ 4.5 µg/L, thus **M656PH062** was selected for genotoxicity and systemic toxicity testing.

When synthesis of the metabolite was initiated it turned out that it was not possible to obtain **M656PH062** as stable test item for testing as the compound was rapidly decarboxylated at the thiophene ring. Thus, decision was taken to test the ethylester derivate of this metabolite as a surrogate taking into account that ester-bonds are easily cleaved in metabolic capable test systems leading to the formation of the intended metabolite.

Table B.6.8-9: M62-group: Metabolite source and exposure levels

Metabolite	Source				Relative concentration in rat	Concentration in groundwater	Consumer exposure via residues	Selected group representative for biological activity screening	Selected group representative for toxicity testing
	Rat Hen	Plant	Soil	Groundwater					
					[% of administered dose]	[µg/L]			
M656PH039	H				In hen:	-	-		
M656PH062			X	X		1.2	-	B¥	G/S¥
M656PH085	X				0.6 in urine		-		

B = Biological activity screening
G = Genotoxicity battery
A = Ames test
S = Systemic toxicity: 28-day rat
H = tentative metabolite in hen
¥Due to instability of M656PH062 the ethylester-derivative was chosen as surrogate for biological and toxicological testing.
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

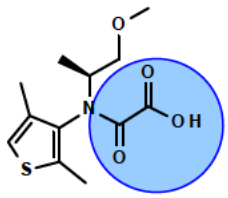
M23-group

For the M23-group the following molecules were taken into consideration: **M656PH023**, **M656PH045**, **M656PH049** and **M656PH050**. Metabolites highlighted in bold are the considered significant metabolites determined in groundwater or in edible commodities at relevant exposure levels for humans.

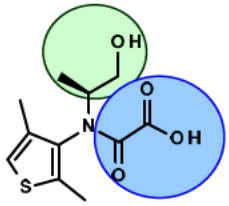
Chemical Similarity:
The metabolite **M656PH023** represents the dehalogenated and carboylated derivative of dimethenamid-P considered to be built from the precursor M656PH011 (see M11-group above).
The degradation product **M656PH045** is derived from **M656PH023** by oxidation of one of the methyl groups attached to the thiophene ring. The structures of the resulting carboxylic acids are still closely related to **M656PH023** but considered to be more polar.
Metabolite **M656PH049** is the ring opening product of **M656PH023** formed by several consecutive oxidation steps. It is a very polar acidic compound.
Metabolite **M656PH050** is derived from **M656PH023** by cleavage of the methoxy group to the respective alcohol. **M656PH050** is therefore structurally closely related to **M656PH023** but slightly more polar.

Coverage of metabolites of concern by mammalian toxicity studies:
No similar metabolites could be identified in the rat metabolism studies conducted except for the precursor M656PH011 (see M11-group above). However, the levels for these metabolites in the rat were low and thus, the metabolites of concern are not considered to be adequately covered by the toxicity testing of racemic dimethenamid or dimethenamid-P.
M656PH023 had already been identified as a key metabolite for the Annex I inclusion of dimethenamid-P thus acute and genotoxicity testing had already been conducted and evaluated.
Formation of **M656PH045** from **M656PH023** in mammals is not considered a reasonable metabolic pathway as carboxylation of a methyl group of the thiophene ring had not been observed in the rat metabolism studies conducted.
M656PH050 is derived from **M656PH023** by cleavage of the methoxy group to the respective alcohol. **M656PH050** is therefore structurally closely related to **M656PH023** but slightly more polar

and therefore potentially less toxic. In addition, it is very likely that in mammals this additional OH-group will be further oxidised to a carboxylic acid as similar metabolic transformations were found in rat metabolism studies with racemic dimethenamid (e.g. M656H001 → M656H013, M656H014 → M656H019). The resulting (hypothetical) carboxylic acid would show similar toxicological properties as M656PH054 (see M19-group above and figure below).



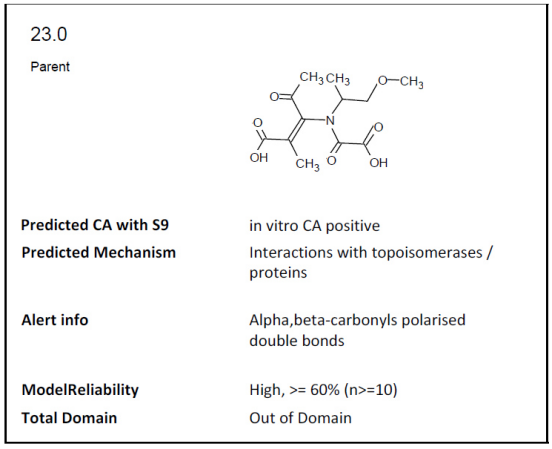
M656PH023



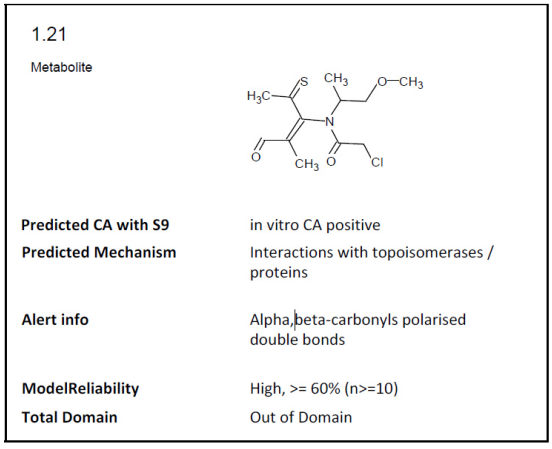
M656PH050

Figure B.6.8-8: Coverage of functional groups of M656PH050 by toxicological testing of other metabolites

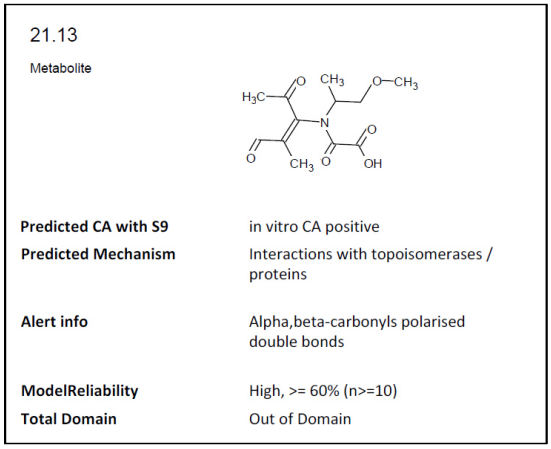
Consequently **M656PH050** is considered adequately covered by the toxicological testing of **M656PH023** and M656PH054.
Degradation product M656PH049 is the ring opening product of **M656PH023** formed by several consecutive oxidation steps. It is a very polar acidic compound. As indicated by the TIMES prediction on S9-induced metabolisation formation of such ring opening structures is a plausible metabolic pathway in mammals and thus several similar ring-open alpha-beta unsaturated carbonyl degradates have been predicted for e.g. dimethenamid-P, **M656PH023**, M656PH030, M656PH031, **M656PH032**, M656PH043, M656PH054 (see ASB2014-8410). The identity of considered closest related degradates is shown in Figure B.6.8-9.



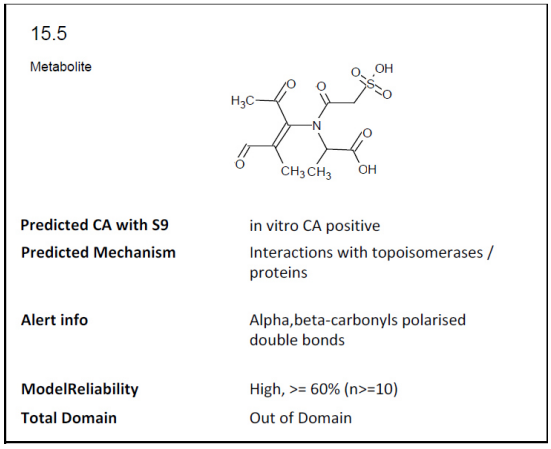
M656PH049



Degradate of dimethenamid-p



Degradate of **M656PH023**



Degradate of M656PH054

Figure B.6.8-9: Coverage of M656PH049 by presumed degradates of tested compounds

Thus it is reasonable to assume that the toxicological testing conducted with dimethenamid-P, **M656PH023** and M656PH054 covers potentially structural alerts of M656PH049.

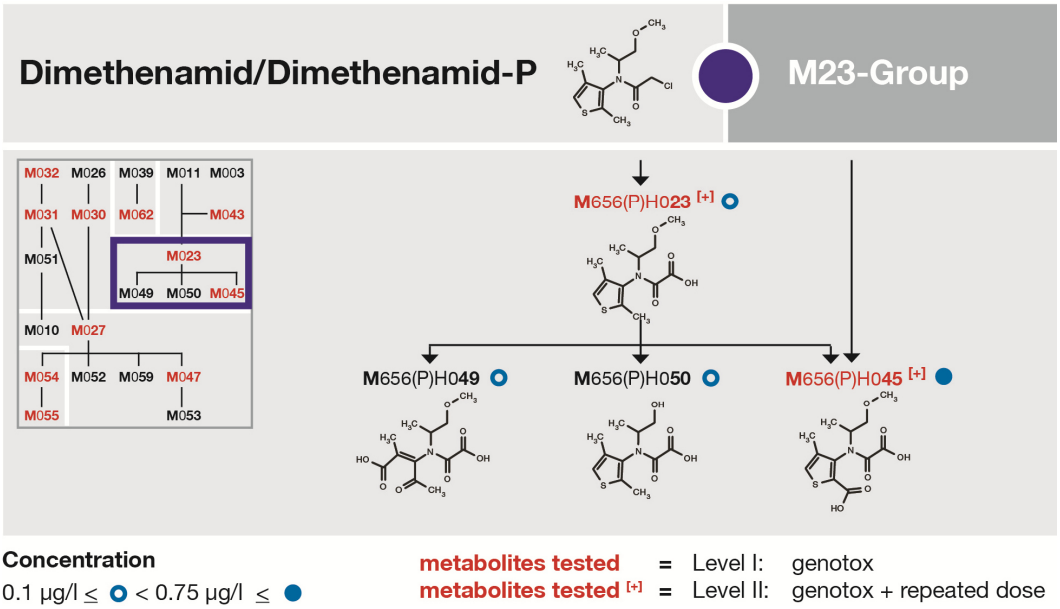
Presence of Structural alerts:
The structure activity evaluation conducted for the metabolites of the M23-group is summarised in the Table below.

Table B.6.8-10: Structure activity evaluation of metabolites in M23-group

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M656PH023	OE	No other than parent	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Low	
M656PH045	OE	No other than parent	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Low	
M656PH049	OE	Genotoxic in ISS modules for Ames and chromosomal aberration Not genotoxic in OASIS modules for Ames and chromosomal aberration	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	DE	Chromosomal aberration *in vitro* not considered relevant for *in vivo*	Low	
	VE	Not mutagenic	Low	
M656PH050	OE	No other than parent	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
	DE	No alert for genotoxicity	Low	
	VE	Not mutagenic	Low	

OE = OECD-toolbox
OA = OASIS-times
DE = DEREK
VE = Vega
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

A structural alert for **M656PH023** based on formation of transformation products and for **M656PH049** for the metabolite structure per se *in vitro* chromosomal aberration was identified. The structural alert identified was that of unsaturated alpha-beta carbonyl compounds. This structural is known to present toxicological concerns in biological systems *in vitro*. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl building a reactive centre for nucleophilic addition and is mostly characterised for small, low molecular weight molecules. This alert is known to become active in *in vitro* systems but in particular for greater complex molecules like the structures presented above or below the evidence to become effective *in vivo* is lacking.
Based on the evaluation in Step A to C (see above) it was proposed to include the following ground-water metabolites and their presumed precursors into the group as illustrated in the Figure below.



Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

Figure B.6.8-10: M23-group proposal

Selection of key structures for hazard assessment:
The relevant group information and the selected key structures are summarised in the Table below.

Table B.6.8-11: M23-group: Metabolite source and exposure levels

Metabolite	Source				Relative concentration in rat	Concentration in groundwater	Consumer exposure via residues	Selected group representative for biological activity screening	Selected group representative for toxicity testing
	Rat	Plant	Soil	Groundwater					
					[% of administered dose]	[µg/L]			
M656PH023		X	X	X		0.6	Not in edible commodities	B	G/S
M656PH045			X	X		1.2		B	G/S
M656PH049			X	X		0.6			
M656PH050		X	X	X		0.3	Not in edible commodities		

B = Biological activity screening
G = Genotoxicity battery
A = Ames test
S = Systemic toxicity: 28-day rat
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

The metabolite **M656PH023** is considered a key metabolite and had thus already been selected for toxicological testing for the Annex I inclusion of dimethenamid-P. Further toxicological studies have been conducted meanwhile to complete the toxicological profile.
Given the predicted exposure level in groundwater of 0.75 µg/L < M656PH045 ≤ 4.5 µg/L and taking into account that formation of **M656PH045** from **M656PH023** in mammals is not considered reasonable M656PH045 was identified as the second key metabolite. **M656PH050** is considered to be adequately covered by the genotoxicity testing conducted with M656PH023 and M656PH054 (see M19-group above). **M656PH049** is considered to be covered by the toxicological testing of **M656PH023**. The structural alert for genotoxicity *in vitro* identified for **M656PH049** with considered low relevance *in vivo* is presumed to be intrinsically covered by the testing of dimethenamid-P, **M656PH023** and M656PH054 as similar metabolites were identified in the metabolic pathway prediction of these molecules in OASIS times. A further aspect taken into consideration for the selection of key structures was the failure of synthesis efforts for **M656PH049** (see above).

M27-group

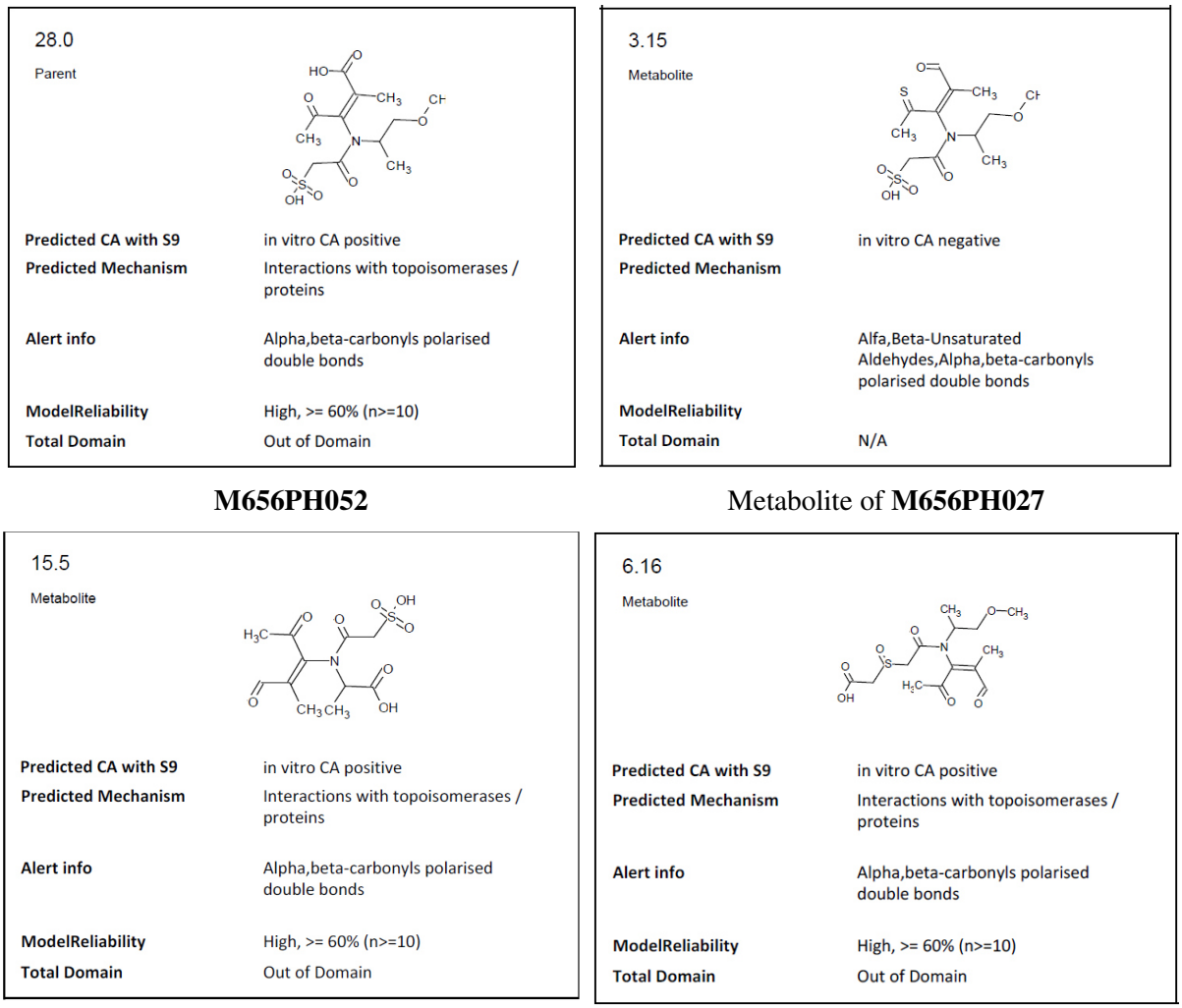
For the M27-group the following molecules were taken into consideration: M656PH010, M656PH16, **M656PH027**, **M656PH047**, **M656PH052**, **M656PH053, M656PH059** and M656PH095. Metabolites highlighted in bold are the considered significant metabolites determined in groundwater or in edible commodities at relevant exposure levels for humans.

Chemical Similarity:

- The metabolite M656PH027 is produced by the dealkylation of M656PH031 and the successive oxidation of the sulphur group to a sulphonate (see M31-group above).
- M656PH047 is derived from **M656PH027** by oxidation of the methyl group of the thiophene ring. The structure of the resulting carboxylic acid is still closely related to **M656PH047**.
- Degradate **M656PH052** is similar to M656PH049 (see M23-group) the ring opening product of **M656PH027** formed by several consecutive oxidation steps and differing only by a sulfonic acid group in the position of the carboxylic acid moiety. It is a very polar acidic compound.
- **M656PH059** is also derived from **M656PH027** by oxidation, forming an unsaturated 2-thiophenone/vinylogous amide structure. It is a very polar compound.
- **M656PH053** built from **M656PH059** by a further hydroxylation step of the methyl at the 3 position. It is a very polar compound.
- M656PH095 is an observed rat metabolite glucuronidated at the isopropylalcohol side chain and containing a methyl thioether on the amide side chain. M656PH016 is a related rat metabolite that only differs in being hydroxylated at the methyl-group of the thiophene the thioether oxidised to the sulfone. This suggests that the later metabolite is closely related in the oxidative metabolism scheme.

Coverage of metabolites of concern by mammalian toxicity studies:
Similar metabolites were identified in the rat metabolism studies conducted namely M656PH010 M656PH016 and M656PH095. M656PH016 gives indication that the oxidative pathway in rats is followed. However, the levels for these metabolites in the rat were in sum not more than 4 % of the applied dose. As a consequence the metabolites of concern are not considered to be adequately covered by the toxicity testing of racemic dimethenamid or dimethenamid-P.
M656PH027 had already been identified as a key metabolite for the Annex I inclusion of dimethenamid-P thus acute and genotoxicity testing had already been conducted and evaluated.
M656PH047 is a presumed metabolite of M656PH027 as predicted in the OASIS-TIMES metabolic pathway (molecule 3.18). Thus in principle this structure could be considered covered by the toxicological testing of **M656PH027**.
The ring-opened **M656PH052** similar to M656PH049 of the M23-group (see section above) is a very polar acidic compound. As indicated by the TIMES prediction on S9-induced metabolisation formation of such ring opening structures is a plausible metabolic pathway in mammals and thus several similar ring-open thiols and alpha-beta unsaturated carbonyl degradates have been predicted

for e.g. dimethenamid-P, M656PH023, **M656PH027**, **M656PH047**, M656PH030, M656PH031, **M656PH032**, M656PH043, M656PH054 (ASB2014-8410).
The identity of considered closest related transformation products is shown in the Figure below.



M656PH052

Metabolite of **M656PH027**

Metabolite of M656PH054

Presumed metabolite of M656PH031

Figure B.6.8-11: Coverage of M656PH052 by presumed degradates of tested compounds

Thus it is reasonable to assume that the toxicological testing conducted with **M656PH027**, M656PH054 and M656PH031 (see section below) covers intrinsically potentially structural alerts of **M656PH052**.
M656PH053 and **M656PH059** are very polar compounds. Structures related to **M656PH053** and **M656PH059** have been predicted for the metabolic transformation of **M656PH027** and **M656PH047** (see figure below). These structures also contain the unsaturated cyclic 2-thiophenone moiety.

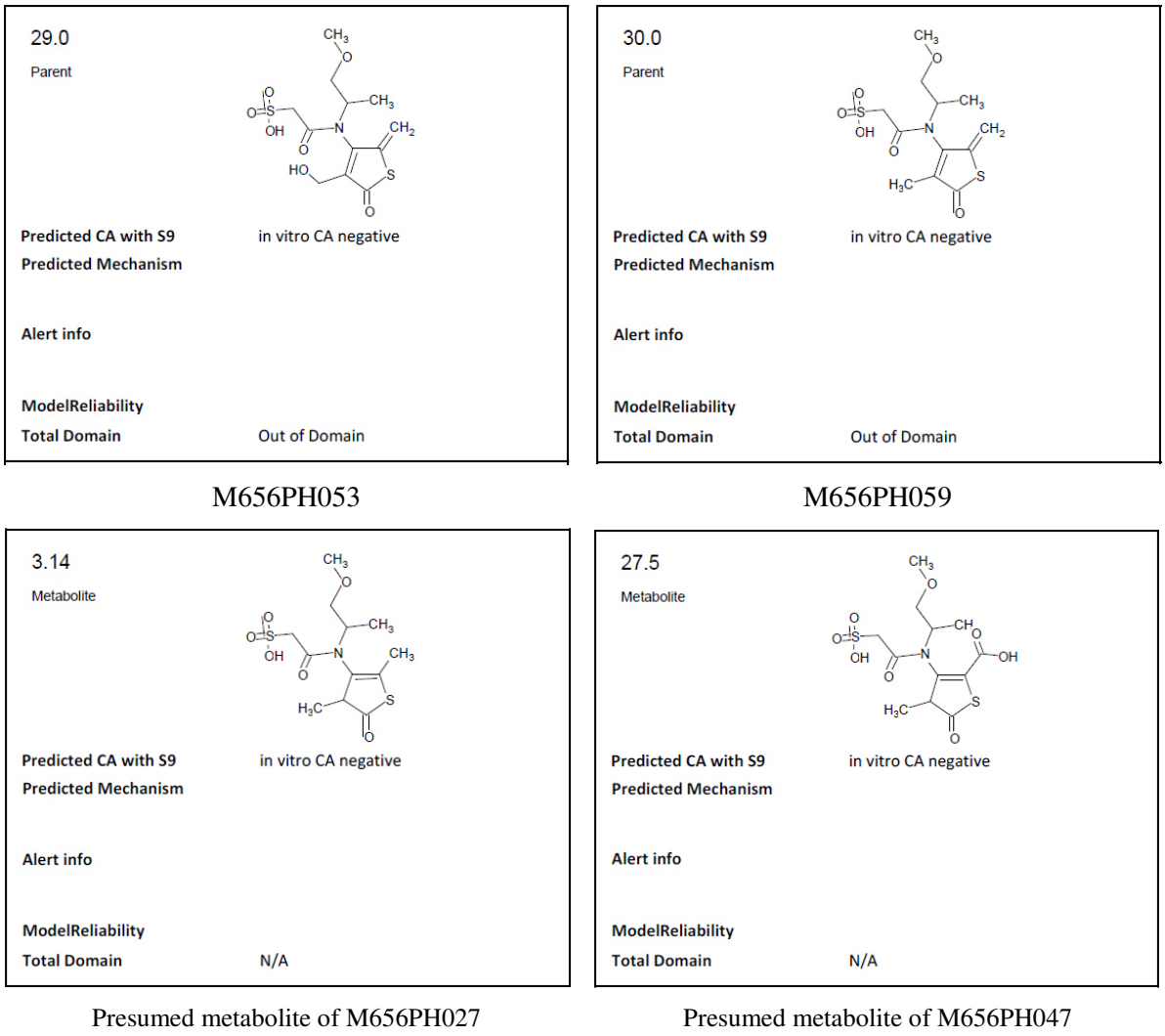


Figure B.6.8-12: Coverage of M656PH053 and M656PH059 by presumed degradates of tested compounds

Thus it is reasonable to assume that the toxicological testing conducted with **M656PH027**, and **M656PH047** (see section below) intrinsically covers potentially structural alerts of M656PH053 and M656PH059.

Presence of Structural alerts:
The structure activity evaluation conducted for the metabolites of the M27-group is summarised in the Table below.

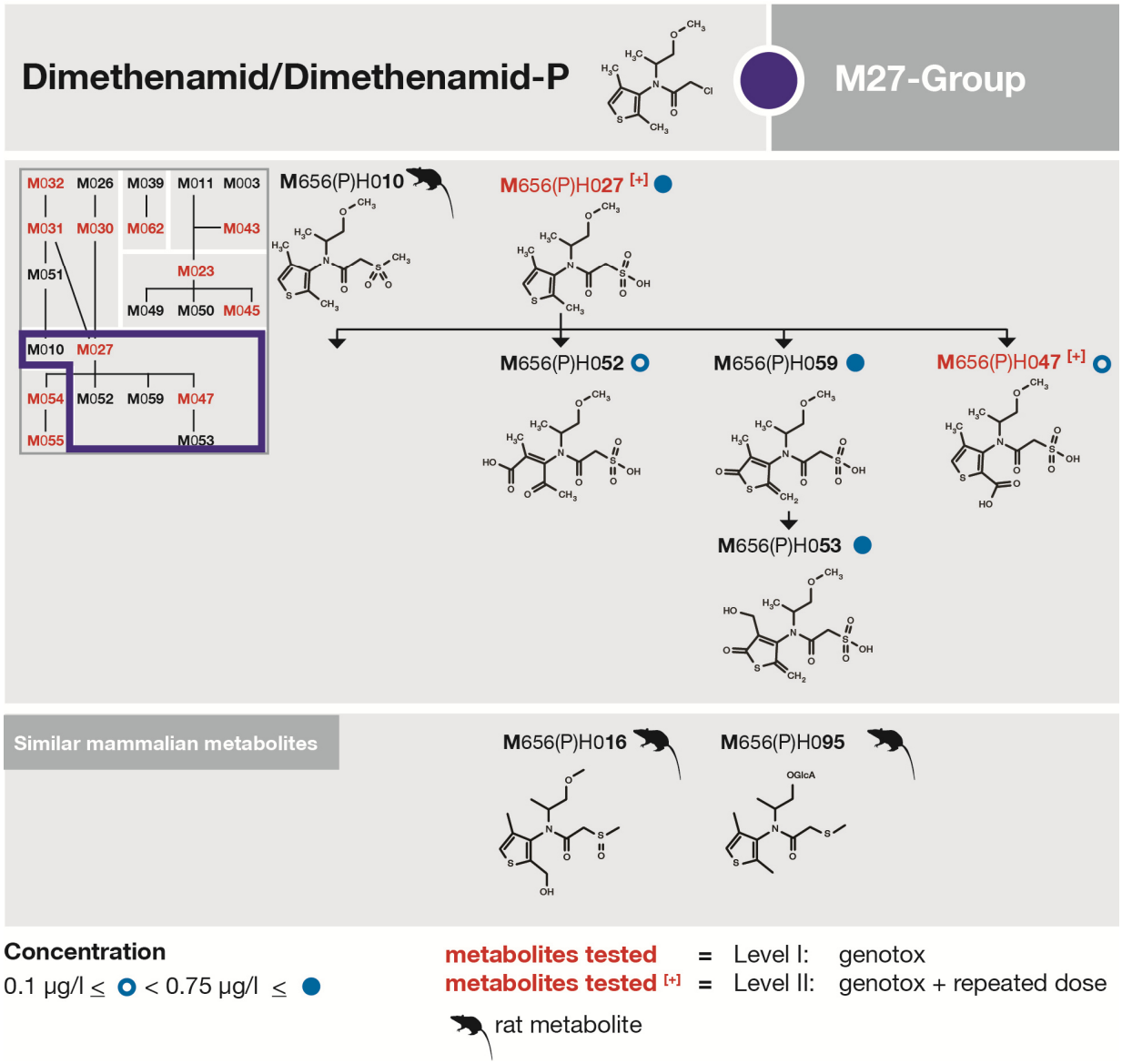
Table B.6.8-12: Structure activity evaluation of metabolites in M27-group

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M656PH010	OE	No other than parent	Low	Alert for chromosomal aberration *in vitro* same as for M656PH027 and thus not confirmed by genotoxicity testing conducted covered by toxicological testing of dimethenamid-P
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low Reasonable	
M656PH016	OE	No other alert than parent	Low	Alert for chromosomal aberration *in vitro*, same as for M656PH027 and thus not confirmed by genotoxicity testing conducted covered by toxicological testing of dimethenamid-P
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low Reasonable	
M656PH27	OE	No other than parent	Low	Alert for chromosomal aberration *in vitro* not confirmed by genotoxicity testing conducted
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low Reasonable	
	DE	No alert for genotoxicity	Low	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH047	OE	No other than parent	Low	No conclusive alert for genotoxicity Weight of evidence: not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
	DE	No alert for genotoxicity	Low	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH052	OE	Genotoxic in ISS modules for Ames and chromosomal aberration Not genotoxic in OASIS modules for Ames and chromosomal aberration	Low	By weight of evidence structural alert for chromosomal aberration *in vitro*, with low relevance *in vivo*
	OA	Not mutagenic (Ames) Alert for chromosomal aberration *in vitro*	Low	
	DE	Chromosomal aberration *in vitro* not considered relevant for *in vivo*	Low	

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH053	OE	Genotoxic in ISS modules for Ames and chromosomal aberration Not genotoxic in OASIS modules for Ames and chromosomal aberration	Low	No conclusive alert for genotoxicity Weight of evidence: not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
	VE	Not mutagenic (Ames)	Low	
M656PH059	OE	Genotoxic in ISS modules for Ames and chromosomal aberration Not genotoxic in OASIS modules for Ames and chromosomal aberration	Low	No conclusive alert for genotoxicity Weight of evidence: not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	
	VE	Inconclusive mutagenic in CAESAR module not mutagenic in SarPy module	Low	
M656PH095	OE	No other than parent	Low	Not genotoxic
	OA	Not mutagenic (Ames) Not genotoxic (chromosomal aberration *in vitro*)	Low	

OA = OASIS-times
DE = DEREK
VE = Vega
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

For M656PH010, M656PH016 and **M656PH027** a structural alert has been identified for chromosomal aberration *in vitro* based on formation of transformation products containing the structural alert for alpha, beta carbonyls with polarised double bonds. The structural alert characterised was an aldehyde formation at the methyl-rest of the thiophene ring, following the oxidative pathway. The presumed transformation products were in the prediction domain. Thus the prediction was considered reasonable. M656PH010 and M656PH016 were rat metabolites and thus were considered covered intrinsically by the toxicological testing of racemic dimethenamid and dimethenamid-P. M656PH027 was chosen as key metabolite for toxicological testing and the study conducted (see below) gave no evidence for chromosomal aberration *in vivo*.
Based on the evaluation in Step A to C (see above) it was proposed to include the following metabolites groundwater metabolites into the group as illustrated below, in addition similar metabolites determined in the rat were added to that group.



Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

Figure B.6.8-13: M27-group proposal

Selection of key structures for hazard assessment:

Table B.6.8-13: M27-group: Metabolite source and exposure levels

Metabolite	Source				Relative concentration in rat	Concentration in groundwater	Consumer exposure via residues	Selected group representative for biological activity screening	Selected group representative for toxicity testing
	Rat	Plant	Soil	Groundwater					
					[% of administered dose]	[µg/L]			
M656PH010	X			X	Present but not quantifiable	0.04			
M656PH016	X				0.9-3.3 in urine and faeces				
M656PH027		X	X	X		2.4	Not in edible commodities	B	G/S
M656PH047				X		0.7		B	G/S
M656PH052				X		0.5			
M656PH053				X		1.0 (iso 1) 1.2 (iso 2)			
M656PH059				X		0.5 (iso 1) 0.2 (iso 2) 1.0 (iso 3)			
M656PH095	X				0.369 in urine				

B = Biological activity screening
G = Genotoxicity battery
A = Ames test
S = Systemic toxicity: 28-day rat
Concentrations of potential groundwater contamination given in the table were based on a tiered approach by the applicant to determine the Predicted Environmental Concentrations in groundwater (PEC_{gw}) (Jilderda, BASF – 30/Mar/2014, ASB2014-8610).

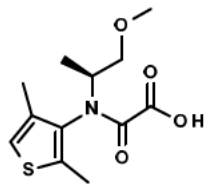
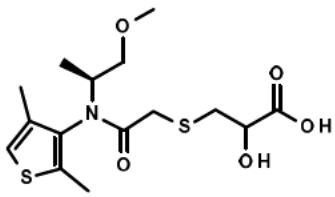
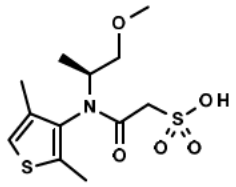
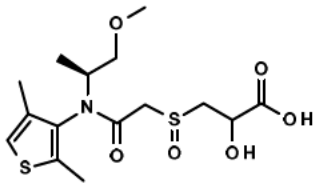
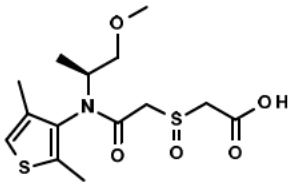
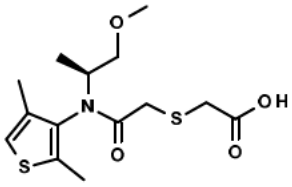
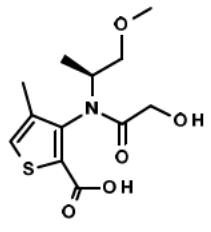
The metabolite **M656PH027** is considered a key metabolite and had thus already been selected for toxicological testing for the Annex I inclusion of dimethenamid-P. Further toxicological studies have been conducted meanwhile to complete the toxicological profile. The structural alert for *in vitro* chromosome aberration identified for this metabolite was considered the same as identified for dimethenamid-P.
Based on the structural alerts identified **M656PH052** would qualify to be the second key structure of this metabolite group. The structural alert for genotoxicity *in vitro* identified for M656PH052 with considered low relevance *in vivo* is presumed to be intrinsically covered by the selection of **M656PH027**, M656PH031 and M656PH054 as key metabolites. The formation of such ring-opened alpha-beta unsaturated carbonyls was identified in the OASIS TIMES presumed metabolic pathways of these molecules (see above). A further aspect taken into consideration for the selection of key structures was the failure of synthesis efforts for **M656PH052** (see above). **M656PH053** and **M656PH059** with predicted exposure levels in groundwater above 0.75 µg/L deemed both not feasible to be synthetised based on the experiences made for M656PH049 and **M656PH052** (see

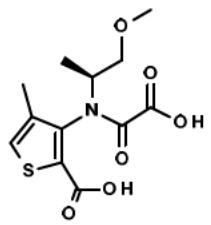
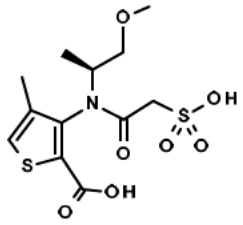
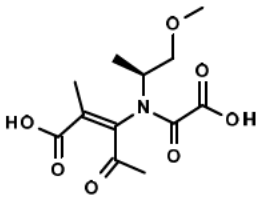
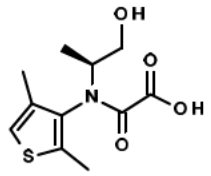
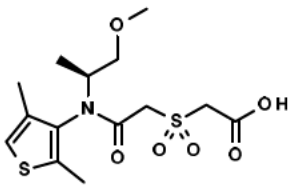
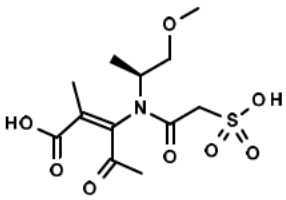
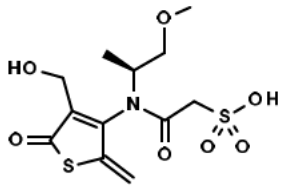
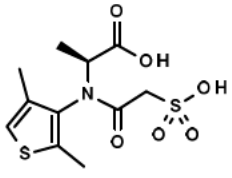
above). Consequently as a further key metabolite **M656PH047** was selected to broaden the database for the M27-group.

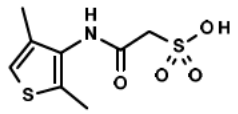
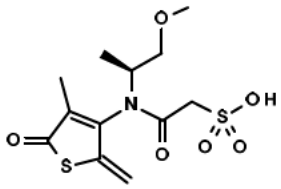
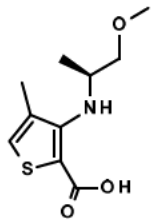
Step 3, Stage 1: screening for biological activity:
The soil metabolites M656PH030, **M656PH023**, M656PH030, M656PH031, M656PH032, M656PH043, M656PH045, **M656PH047**, M656PH054, M656H055, the sodium salt of **M656PH027** and the ethylester derivate for M656PH062 have been screened for biological activity on plants in the greenhouse when applied in pre- and in post-emergence standard tests. The dose rate of the parent dimethenamid-P was the maximum defended dose rate of 864 g a.s./ha. This dose rate was adjusted for the metabolites based on their molar equivalent. The parent compound showed 21 days after application 99 % efficacy on the selected species of the grasses Bromus, Echinochloa, Setaria and Lolium and 90 % efficacy on the broadleaves Chenopodium and Geranium when applied in pre-emergence. The efficacy of the parent applied in post-emergence was 93 and 38 %. The broadleve weed Geranium was controlled with 75 %, Chenopodium was however not controlled in post emergence. All metabolites showed in pre- and in post enmergence not any sign of phytotoxicity. The data conclude that there is no biological activity for the metabolites.
Herbicidal efficacy of BAS 656 H metabolites (Glasshouse efficacy test) - nonGLP:
The biological activity of different metabolites of BAS 656 H was investigated in a plant assay in the glasshouse using monocot and dicot weed species. The results are summarised below. None of the tested metabolites did show biological activity at the tested rates.

With regard to toxicological relevance the following metabolites were taken into consideration and are addressed in this section.

Table B.6.8-14: Dimethenamid-P metabolites considered for potential toxicological relevance

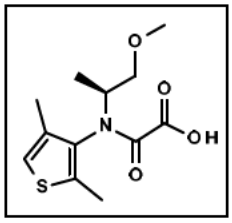
Metabolite	Structure	Reason for relevance assessment
M656PH023		Groundwater metabolite Human exposure: 0.1 µg/L < M656PH023 ≤ 0.75 µg/L
M656PH026		Plant metabolite determined in edible commodities Human exposure: 0.0025 µg/kg bw/day < M656PH026 ≤ 1.5 µg/kg bw/day
M656PH027		Groundwater metabolite Human exposure: 0.75 µg/L < M656PH027 ≤ 4.5 µg/L
M656PH030		Plant metabolite determined in edible commodities Human exposure: 0.0025 µg/kg bw/day < M656PH030 ≤ 1.5 µg/kg bw/day
M656PH031		Groundwater metabolite Human exposure: M656PH031 ≤ 0.1 µg/L
M656PH032		Groundwater metabolite Human exposure: 0.75 µg/L < M656PH032 ≤ 4.5 µg/L
M656PH043		Groundwater metabolite Human exposure: 0.1 µg/L < M656PH043 ≤ 0.75 µg/L

Metabolite	Structure	Reason for relevance assessment
M656PH045		Groundwater metabolite Human exposure: 0.75 µg/L < M656PH045 ≤ 4.5 µg/L
M656PH047		Groundwater metabolite Human exposure: 0.1 µg/L < M656PH047 ≤ 0.75 µg/L
M656PH049		Groundwater metabolite Human exposure: 0.1 µg/L < M656PH049 ≤ 0.75 µg/L
M656PH050		Groundwater metabolite Human exposure: 0.1 µg/L < M656PH050 ≤ 0.75 µg/L
M656PH051		Groundwater metabolite Human exposure: 0.1 µg/L < M656PH051 ≤ 0.75 µg/L
M656PH052		Ground water metabolite Human exposure: 0.1 µg/L < M656PH052 ≤ 0.75 µg/L
M656PH053		Groundwater metabolite Human exposure: 0.75 µg/L < M656PH053 (2 isomers) ≤ 4.5 µg/L
M656PH054		Groundwater metabolite Human exposure: 0.75 µg/L < M656PH054 ≤ 4.5 µg/L

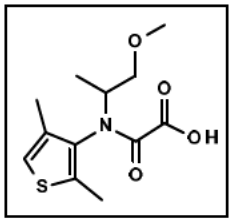
Metabolite	Structure	Reason for relevance assessment
M656H055		Groundwater metabolite Human exposure: 0.1 µg/L < M656H055 ≤ 0.75 µg/L
M656PH059		Groundwater metabolite Human exposure: 0.1 µg/L < M656PH059 (2 isomers) ≤ 0.75 µg/L < M656PH059 (1 isomer) ≤ 4.5 µg/L
M656PH062		Groundwater metabolite Human exposure: 0.75 µg/L < M656PH062 ≤ 4.5 µg/L

B.6.8.1.1 Toxicological testing of metabolites

Metabolite M656H023 former assigned M23 (Reg.No. 360715):
M656PH023 (Reg. No. 5886780) is a metabolite of dimethenamid-P that was determined in soil, surface water, ground-water and plants.



Acute toxicity and genotoxicity studies as already submitted for Annex I inclusion of dimethenamid-P and presented below have been conducted with M656H023 (Reg.No. 360715) representing the respective metabolite of the racemic dimethenamid. The short-term toxicity study in rats was conducted with M656PH023.



Structural alerts for M656PH023:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times (see molecule 21 of report ASB2014-8408) predicted M656PH023 to be not mutagenic in the Ames test neither without nor with metabolic activation but with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (see molecule 21 of report ASB2014-8410) the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure similar to M656PH049. For this alpha-beta unsaturated carbonyl was an alert given for induction of chromosomal aberration by

interaction with topoisomerases/proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (12 in total) gave no alert for chromosomal aberration.
In the DEREK analysis conducted structural alerts for M656PH023 were the thiophene alert for hepatotoxicity and nephrotoxicity which was also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (molecule 6) in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low.
In conclusion in one of the structure activity evaluation tools employed there was a limited alert for chromosomal aberration *in vitro* with metabolic activation considered of low relevance for the *in vivo* situation.

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.8
Report:	[redacted] 1995 (TOX1999-442) Dimethenamid oxalamide: acute oral toxicity study in the rat [redacted] unpublished, 17 March 1995, BASF RegDoc.# 95/11340 (Experimental work from 13 February 1995 – 1 March 1995)
Guideline(s):	OECD Guideline No. 401 (Limit test)
Deviations:	Deviation from Method B.1, Directive 92/69/EEC: unspecified randomisation of rats for allocation to groups ("non-selective" allocation) The deviation is not considered to have compromised the validity of the study.
GLP:	Yes (laboratory certified by The Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid oxalamide (M23); batch No. RS-582OXA-080194; purity: 99.83 %. Test material was formulated in 0.5 % w/v methylcellulose in purified water.
Test Animals:	CD rats; age 5 weeks at start of treatment; pre-fasted body weight: males 101 - 126 g, females 97 - 121 g, Source: Charles River Ltd., Margate, Kent, U.K.

This study was performed to determine the acute oral LD_{50} of the test material in the Sprague-Dawley rat. Groups of ten fasted animals (5/sex) were given a single oral dose of the test material in 0.5 % w/v methylcellulose in water at doses of 2000 or 5000 mg/kg bw. The dosing volume was 20 mL/kg. The animals were observed for 14 d after dosing. Body weights were determined pre-test on study Days 1, 8 and 15. All animals received a gross necropsy.

Results:

Clinical signs observed in animals treated with 5000 mg/kg bw were underactivity, pallor, piloerection, salivation and hunched posture. These signs were seen immediately after exposure and were not seen by day 2 and afterwards. The only clinical sign observed at 2000 mg/kg bw was piloerection which was not observed at day 2 onwards. Overall, the animals gained body weight as expected for their age. There were no treatment related effects on gross pathology.
No deaths occurred at either treatment concentration.

Conclusion:

The oral LD_{50} for dimethenamid oxalamide (M23) in Sprague-Dawley rats was determined to be greater than 5000 mg/kg bw.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.8
Report:	Clare C., 1995 (TOX1999-444) dimethenamid oxalamide (M23): Reverse mutation in 5 histidine requiring strains of *Salmonella typhimurium* Hazleton Laboratories Europe Ltd., Harrogate, Great Britain unpublished, 21 March 1995, BASF RegDoc.# 95/11336 (Experimental work: February- March 1995)
Guideline(s):	OECD-Guideline 471
Deviations:	No deviations
GLP:	Yes (laboratory certified by The Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid oxalamide (M23); batch no. RS-582OXA-080194, purity 99.83 %.
Test System:	*Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537.

Dimethenamid-oxalamide (M23) was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 were exposed to the test substance at various concentrations. In the initial experiment, concentrations ranged from 8 - 5000 µg/plate. Toxicity was observed at the highest dose of 5000 µg/plate in all strains except for TA 100. In a second test, concentrations ranged from 250 - 4000 µg/plate for all strains except TA 100, and from 312.5 - 5000 µg/plate for TA 100. The initial study consisted of a plate incorporation test both with and without metabolic activation (Aroclor induced rat liver S-9 mix). In the second test, a pre-incubation step was used to maximise any potential mutagenic metabolites. The test article (or control solution) was mixed with the bacteria and S-9 for 1 h at 37 °C before the addition of the agar. Three plates were used per dose for each strain in both tests.
For control purposes and to demonstrate the sensitivity of the test system, a negative control and positive controls both without S-9 mix and with metabolic activation were tested.

Results:

Toxicity was observed in the initial study at the highest concentration of 5000 µg/plate in all strains except TA 100. In the second test, toxicity was observed in all strains except TA 100 at the highest concentration of 4000 µg/plate. Also in the second test, toxicity was observed with TA 100 at 5000 µg/plate when tested with S-9 activation.
There were no increases in mutation frequency associated with the test article. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:

According to the results of the study, dimethenamid oxalamide (M23) is not mutagenic in the Ames reverse mutation assay with strains TA 98, TA 100, TA 102, TA 1535 or TA 1537 with or without metabolic activation.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	[redacted] 2000a (TOX2002-1990) Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79/HPRT) with Reg.-No. 360 715/M23 [redacted] unpublished, 5 January 2000, RCC-CCR Project 647601, ZHT-Project-No. 50MO432/979092 (Experimental work: 05 October 1999 – 22 December 1999)
Guideline(s):	EEC 87/302, OECD 476
Deviations:	There were deviations to the test protocol. The ratio of S9 supernatant to cofactor solution was 1:2.0 in the first and 1:9.0 in the second experiment based upon the total volume of S9 mix. The reason for the deviation (ratio of S9 supernatant to cofactor solution in the first experiment) was that the ratio of S9 supernatant to cofactor solution depends on the actual S9 mix charge used in the test. The historical data were updated (1996 – 1998) and the mean values and standard deviations were added on request of the sponsor.
GLP:	Yes (certified by Hessisches Ministerium für Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material	Reg. No. 360 715/M23
Description:	Solid (powder), white
Lot/Batch #:	L59-52
Purity:	99.83 %
Stability of test compound:	The storage stability of the test substance covering the period of the study was guaranteed by the sponsor.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	Untreated cells
Solvent control:	0.5 % (v/v) DMSO in culture medium
Positive control -S9:	Ethylmethane sulfonate (EMS) 300 µg/mL (dissolved in nutrient medium)
Positive control +S9:	7,12-dimethylbenz(a)anthracene (DMBA) 2.5 µg/mL (dissolved in DMSO)
Activation:	S9 was produced from the livers of induced male Wistar [HanIbm] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were

sacrificed.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

The ratio of S9 supernatant to cofactor solution was 1:2.0 in the first and 1:9.0 in the second experiment based upon the total volume of S9 mix.

Test organism: V79 cells with a doubling time of 12 - 16 h, a good cloning efficiency (as a rule more than 50 %) and a modal chromosome number of 22. Stocks of the cell line were maintained in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination, karyotype stability and spontaneous mutant frequency.

Culture medium: Minimal essential medium (MEM) supplemented with 10 % (v/v) foetal calf serum (FCS).

Treatment medium: Serum-free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with "saline G" (containing NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, Na_2HPO_4 x 7 H_2O 290 mg, KH_2PO_4 150 mg; pH 7.2).

Selection medium: no data given

Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

Test concentrations:

Preliminary toxicity assay: Nine concentrations ranging from 26 to 3333 µg/mL

Mutation assay:

1st and 2nd experiment: 84.4, 168.8, 337.5, 675.0, 1350.0 and 2700.0 µg/mL with and without metabolic activation

Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range for the main experiments under the same culturing and experimental conditions as described for the main test. The colony forming ability of approximately 500 single cells (dublicate cultures per concentration level) after treatment with the test substance was observed and compared to the controls. Toxicity of the test substance was indicated by a reduction of the cloning efficiency (CE). PH and osmolarity were determined in the solvent control and in the maximal concentration in the experiment without metabolic activation.

Mutation Assay:

Pretreatment and seeding of cells:

Three days old exponentially grown stock cultures (more than 50 % confluent) were trypsinised at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. A 0.2 % trypsin concentration in Ca-Mg-free salt solution was used which contained NaCl 8000 mg, KCl 400 mg, glucose 1000 mg; $NaHCO_3$ 350 mg. Prior to trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/mL EDTA. Approximately 1.5 x 10^6 (single culture) and 5 x 10^2 cells (in duplicate) were seeded in culture medium for the determination of mutation rate and toxicity, respectively.

Cell treatment:

24 hours after seeding, the medium was replaced with serum-free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with "saline G" (containing NaCl 8000 mg, KCl

400 mg, glucose 1100 mg, Na_2HPO_4 x 7 H_2O 290 mg, KH_2PO_4 150 mg; pH 7.2).
Colonies were stained with 10 % methylene blue in 0.01 % KOH solution. The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope.
Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 4.5 % CO_2, 37 °C.
Statistics:
Since the distribution of mutant cells did not follow known statistical models, an adequate statistical method is not available.
Evaluation criteria:
The test chemical is considered positive if it induces either a concentration related increase of the mutant frequency or a reproducible and positive response at one of the test points.
The test substance producing neither a concentration related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.
A significant response is described as follows:
Test substance is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
The test substance is classified as mutagenic if there is a reproducible concentration related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.
However, in a case by case evaluation this decision depends on the level of the corresponding negative control data. If there is by chance a low spontaneous mutation rate in the range normally found (0.5 - 33.1 mutants per 10^6 cells) a concentration related increase of the mutations within this range has to be discussed.

Results:

The storage stability of the test substance covering the period of the study was guaranteed by the sponsor. The stability of the test substance in solvent was not determined analytically.
In the preliminary experiment, neither precipitation nor toxicity was observed up to the maximal concentration of 3333 µg/mL. Based on these data the highest concentration tested in the mutagenicity experiments was 2700 µg/mL without and with metabolic activation.
Up to the highest investigated concentration, no relevant increase in mutant colony numbers was observed in both independent experiments. No cytotoxicity and no precipitation was observed up to the maximal concentration of 2700 µg/mL (see Table B.6.8-15 and Table B.6.8-16)**.** Low solvent control counts in both cultures of the first and culture I of the second experiment are the reason that the threshold of three times the colony count of the corresponding solvent control was exceeded at some concentrations. The absolute numbers of colonies however, were low and remained well within the range of the historical negative and solvent controls. Therefore, this effect was judged incidental without any biological relevance.
Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Table B.6.8-15: Gene mutation in mammalian cells - 1st experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE1 (survival), relative		CE2 (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	8.0	7.2	100.0	100.0	0.70	0.81
Vehicle control (DMSO)	-	2.0	0.6	100.0	100.0	0.52	0.86
M656H023							
84.4 µg/mL	-	n.c.	n.c.	100.6	105.2	n.c.	n.c.
168.8 µg/mL	-	4.3	5.3	105.6	94.3	0.74	0.84
337.5 µg/mL	-	7.9	6.6	101.4	93.6	0.70	0.67
675.0 µg/mL	-	2.2	1.2	99.7	96.4	0.48	0.85
1350.0 µg/mL	-	4.0	1.2	103.0	95.8	0.62	0.90
2700.0 µg/mL	-	6.4	4.0	100.8	97.3	0.64	0.88
Positive control EMS							
300.0 µg/mL	-	536.3	158.1	75.6	97.9	0.68	0.93
4-hour exposure period							
Negative control	+	1.1	10.5	100.0	100.0	0.87	0.82
Vehicle control (DMSO)	+	8.7	12.1	100.0	100.0	0.86	0.76
M656H023							
84.4 µg/mL	+	n.c.	n.c.	100.6	n.c.	n.c.	n.c.
168.8 µg/mL	+	8.4	2.9	96.2	88.0	0.77	0.71
337.5 µg/mL	+	16.3	5.8	97.3	83.6	0.75	0.81
675.0 µg/mL	+	4.6	3.6	106.5	79.2	0.75	0.64
1350.0 µg/mL	+	4.4	4.7	104.7	91.3	0.82	0.71
2700.0 µg/mL	+	4.3	4.9	108.2	92.9	0.87	0.63
Positive control DMBA							
2.5 µg/mL	+	849.4	974.2	32.4	37.2	0.51	0.61

CE1 : (mean number of found number of cells per flask/corresponding control) x 100
CE2 : (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

Table B.6.8-16: Gene mutation in mammalian cells – 2nd experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE1 (survival), relative		CE2 (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	3.9	0.5	100.0	100.0	0.58	0.77
Vehicle control (DMSO)	-	1.9	7.9	100.0	100.0	0.98	0.87
M656H023							
84.4 µg/mL	-	n.c.	n.c.	103.6	114.6	n.c.	n.c.
168.8 µg/mL	-	16.4	1.3	98.8	100.9	0.69	0.79
337.5 µg/mL	-	1.3	5.2	105.5	104.9	0.82	0.91
675.0 µg/mL	-	0.7	5.4	100.0	100.6	0.78	0.78
1350.0 µg/mL	-	7.3	5.0	104.5	103.0	0.59	0.81
2700.0 µg/mL	-	5.3	2.6	96.7	96.7	0.83	0.78
Positive control EMS							
300.0 µg/mL	-	208.6	208.7	83.3	95.4	0.75	0.78
4-hour exposure period							
Negative control	+	1.6	0.6	100.0	100.0	0.86	0.74
Vehicle control (DMSO)	+	6.0	0.7	100.0	100.0	0.82	0.66
M656H023							
84.4 µg/mL	+	n.c.	n.c.	97.7	114.7	n.c.	n.c.
168.8 µg/mL	+	7.6	4.7	97.7	100.3	0.72	0.72
337.5 µg/mL	+	6.0	5.4	102.5	106.9	0.87	0.56
675.0 µg/mL	+	2.3	2.6	98.8	100.5	0.84	0.53
1350.0 µg/mL	+	4.6	2.6	96.7	112.6	0.64	0.81
2700.0 µg/mL	+	3.3	7.0	101.7	103.6	0.83	0.53
Positive control DMBA							
2.5 µg/mL	+	494.0	673.5	102.0	63.9	0.64	0.55

CE1 : (mean number of found number of cells per flask/corresponding control) x 100
CE2 : (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test, M656H023 did not induce forward mutations in the HPRT locus in V79 cells *in vitro*.

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.8
Report:	[redacted] 1998 (TOX1999-446) Micronucleus assay in bone marrow cells of the mouse with Reg. Nr. 360 715/M23 [redacted] unpublished, 19 January 1998, BASF RegDoc.# 98/10169 (Experimental work: October – December 1997)
Guideline(s):	OECD-Guideline 474 and proposal for updating of guideline 474, ENV/MC/CHEM/TG(96)7
Deviations:	No deviations
GLP:	Yes (laboratory certified by Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen, Gruppe IV C, Postfach 30 06 52, 40190 Düsseldorf, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid oxalamide (M23); batch No. L 59 - 52; purity: 99.83 %.
Test Animals:	NMRI mice

Dimethenamid oxalamide (M23) was tested for clastogenicity and the ability to have a spindle poison effect in NMRI mice using a micronucleus test.
The test substance dissolved in dimethyl sulfoxide (DMSO) was administered once intraperitoneally to 6 male and 6 female animals per group at dose levels of 75, 150 and 300 mg/kg bw (for 24 h observation) and 300 mg/kg bw (48 h observation). A volume of 4 mL/kg bw was used for each dose. These dose levels were selected based on a preliminary toxicity study. The negative control group received the vehicle only. Cyclophosphamide was used as a positive control.
24 or 48 h after the administration of the test substance (as specified above), all surviving animals were sacrificed and the bone marrow of 2 femora was prepared. The positive controls were sacrificed 24 h after administration. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal. 5 surviving animals per sex and group at each time period were evaluated.

Results:

At the high dose level of 300 mg/kg bw, 1 animal in each of the 24 and 48 h observation groups died. The mean number of normochromatic erythrocytes (NCE) was increased after treatment with the test article as compared to the mean value of NCE's of the corresponding vehicle controls, indicating a cytotoxic effect in the bone marrow. This effect was considered slight in all dose groups at 24 h after treatment, but after 48 h the mean frequency of NCE's was substantially increased at 300 mg/kg bw.
The administration of M23 did not lead to an increase in the number of polychromatic erythrocytes containing micronuclei in any of the dose groups at any time period measured after treatment. The administration of the positive control resulted in the expected increase in micronuclei, demonstrating the sensitivity of the assay.

Conclusion:

Under the conditions of this test, dimethenamid oxalamide (M23) does not have a chromosome damaging (clastogenic) effect *in vivo*.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Metabolite M656PH023 (Reg.No. 5886780):

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	[redacted] 2014b (ASB2014-8415) Reg.No. 5886780 (metabolite of BAS 656-PH, Dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats administration via the diet [redacted] unpublished, BASF DocID 2013/1342918 (Experimental work from 11-Jun-2013 - 13-Jan-2014)
Guideline(s):	OECD 407, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
Deviations:	Deviations from Method OECD 407 or (EC) B.7 No. L 142: None that were considered to have compromised the validity of the study.
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Reg. No. 5886780, metabolite of BAS 656-PH (dimethenamid-p)
Description:	Solid/white
Batch/purity #:	L82-104,
Purity:	99.9 %
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor. Expiry date: Jun. 01, 2015
Vehicle and/or positive control:	Rodent diet
Test animals:	
Species:	Rat
Strain:	Wistar Crl:WI (Han) Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 153.3 ± 6.1 g, ♀ 129.3 ± 6.8 g
Source:	Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, *ad libitum*
Housing:	Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment

Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material

Environmental conditions:
Temperature: 20 - 24 °C
Humidity: 30 - 70 %
Air changes: 15 air changes per hour
Photo period: 12 h light/12 h dark
(06:00 - 18:00/18:00 - 06:00)

Animal assignment and treatment:
M656PH023 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomisation list based on body weights.

Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. One diet preparation per dose was performed for this study.
Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 31 days.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analysed. The samples were also used for determination of the test article concentration. For the mid dose level a single sample was analysed. No test article was determined in control diets.

Table B.6.8-17: Analysis of diet preparations for homogeneity and test item content

Dose level [ppm]	Sampling	Concentration Mean ± SD [ppm]	% of nominal concentration	Relative standard deviation [%]
1200 ppm	17-Jun-13	1291 ± 26#	107.6	2.0
4000 ppm	17-Jun-13	4589, 4577¥	114,7, 114.4¥	n.a.
12000 ppm	17-Jun-13	12607 ± 607#	105.1	4.8

n.a.: not applicable;
based on mean values of the three individual samples
¥ values for sample and retain sample
Values may not calculate exactly due to rounding of figures.

Considering the low relative standard deviation in the homogeneity analysis, it can be concluded that M656PH023 was distributed homogeneously in ground Kliba maintenance diet/mouse rat „GLP“ meal.
Generally the mean values of M656PH023 in ground Kliba maintenance diet mouse/rat “GLP” meal were found to be in the range of 90 - 110 % of the nominal concentrations demonstrating the correctness of the concentrations of M656PH023 in the vehicle. There was however a single deviation for the mid dose (4000 ppm) sample as confirmed by the retain sample were the determined concentrations were in the range of 114.4 to 114.7 % of the nominal concentration. These values still in the range of ± 15 % of the target concentration can be regarded as acceptable for analysis of complex matrices like diet also considering that generally all determined concentrations for all dose levels were above 100 %.
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-18: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Table B.6.8-19: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except reticulocytes and differential blood count, urine pH, urine volume, urine specific gravity	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urinalysis, except pH, volume, color, turbidity and specific gravity	Pair wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Table B.6.8-20: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:
Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Food consumption was determined weekly over a period of 1 day and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BWx and BWy body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).
Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted.
Ophthalmoscopy:
Not performed in this study.
Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1.	behaviour when removed from cage	10.	respiration
2.	fur	11.	tremors
3.	skin	12.	convulsions
4.	salivation	13.	abnormal movements
5.	nose discharge	14.	impairment of gait
6.	lacrimation	15.	activity/arousal level
7.	eyes/pupil size	16.	faeces (number of faecal pellets/appearance/ consistency) within two minutes
8.	posture	17.	urine (appearance/quantity) within two minutes
9.	palpebral closure	18.	number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behaviour during "handling"
2.	touch response	9.	vocalisation
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

Motor activity measurement:
Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
Haematology and clinical chemistry:
Blood was withdrawn in the morning from fasted, isoflurane anesthetised animals from the retro-orbital plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomised sequence.

The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
	Red blood cells		White blood cells		Clotting Potential
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Haemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Haematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
Clinical chemistry:					
	Electrolytes		Metabolites and Proteins		Enzymes:
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Urinalysis:
For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analysed in a randomised order.
The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		Semi quantitative parameters		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Colour and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

Sacrifice and pathology:
The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	#	kidneys	✓	✓	#	seminal vesicles with coagulating glans
✓		#	aorta	✓			lachrymal glands, extraorbital	✓			skin
✓		#	bone marrow§	✓		#	larynx	✓		#	spinal cord (3 levels)@
✓	✓	#	brain	✓	✓	✓	liver	✓	✓	#	spleen
✓		#	caecum	✓		#	lung	✓		#	sternum w. marrow
✓		#	colon	✓		#	lymph nodes#	✓		#	stomach (fore- & glandular)
✓		#	duodenum	✓		#	mammary gland (♂ and ♀)	✓	✓	#	testes
✓	✓	#	epididymides¥	✓		#	muscle, skeletal	✓	✓	#	thymus
✓		#	esophagus	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thyroid/parathyroid
✓		#	eyes (with optic nerve)	✓		#	nose/nasal cavity‡	✓		#	trachea
✓			femur (with joint)	✓	✓	#	ovaries and oviduct**	✓		#	urinary bladder
			gall bladder	✓		#	pancreas	✓	✓	#	uterus with cervix
✓		✓	gross lesions	✓			pharynx	✓		#	vagina
✓			Harderian glands	✓		#	pituitary				
✓	✓	#	heart	✓	✓	#	prostate				
✓		#	ileum	✓		#	rectum		✓		body (anesthetised animals)
✓		#	jejunum (w. Payer's plaque)	✓			salivary glands*				

§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; ‡l histopathology at level III, ¥left epididymidis collected for histopathology

The organs or tissues were fixed in 4 % formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.
The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:
• Increased/decreased grade of cortico-medullar ratio (related only to area)
• Increase of starry sky cells
• Changes of cellular density in the cortex
• Changes of cellular density in the medulla
Spleen:
• Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
• Altered cellular composition of follicles
• Altered number of germinal centers

Lymph nodes (mesenteric and axillar lymph nodes):
• Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
• Altered cellular composition of paracortex
• Altered number of germinal centers
• Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):
• Changes of the cellularity of follicles (including mantle zone and germinal centres)
• Changes of the cellularity of interfollicular area
Bone marrow:
• Changes of the cellularity
• Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina. A correlation between gross lesions and histopathological findings was attempted.

Results:

No clinical signs were observed throughout the study. No mortality was observed throughout the study.
Ophthalmoscopy was not performed in this study.
FOB and Motor Activity: Neither home cage nor open field observations revealed any indication of treatment related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
Regarding the overall motor activity as well as single intervals, no test substance related deviations were noted for male and female animals.
There were some statistically significant differences between control and treated groups, however, these changes were isolated findings and not clearly dose related. Therefore, these changes were considered incidental. These changes consisted of:

- Decreased activity at single interval 8 of male animals of the high dose group (12000 ppm)
- Decreased activity at single interval 8 of female animals of the mid dose group (4000 ppm)

No statistically significant differences on overall motor activity were observed at any dose.
No treatment related differences of absolute body weights or body weight gain were noted (see Table B.6.8-21 and Figure B.6.8-14 below). Body weight change values of female animals of all groups dosed with M656PH023 were significantly lower on study day 14, irrespective of the concentration in the diet. However, no significant differences were observed after 21 and 28 days of treatment. A relation to treatment was not assumed.

Table B.6.8-21: Mean body weight of rats administered M656PH023 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1200	4000	12000	0	1200	4000	12000
Body weight [g]								
- day 0	151.0	152.1	153.3	154.7	127	128.8	129	129.6
- day 28	282.2	278.3	290.2	279.2	193.3	185.9	185.4	188.6
Δ% (compared to control) #		-1.4	2.8	-1.1		3.8	-4.1	-2.4
Body weight gain [g]								
- day 0 → day 7	42.5	41.4	45.1	42.9	24.1	16.5	16.5	20.6
- day 0 → day 14	83.4	78.2	85.4	81.6	43.9	36.3 *	31.5 **	35.1 *
- day 0 → day 21	112.6	109.3	117.1	99.5	52.5	49.7	49.8	53.9
Overall body weight gain - day 0 → day 28	131.2	126.2	136.9	124.5	66.3	57.1	56.4	59
Δ% (compared to control)#		-3.8	4.3	-5.1		-13.9	-15	-11

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett test - two sided)
# Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

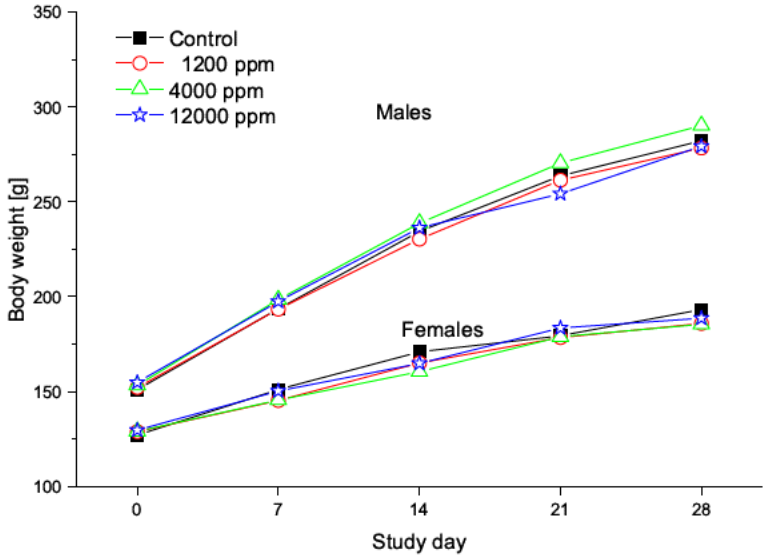


Figure B.6.8-14: Body weight development of rats administered M656PH023 for at least 28 days

No test substance related, adverse food consumption and compound intake findings were observed. All recorded values were within the biological range typical for this strain of rats.
Note: Some values i.e. for male control group animals from day 25 to 28 and for males of the high dose group (12000 ppm) between study days 11 to 14, 18 to 21 as well as 25 to 28 were declared as outlier and thus was not taken into consideration. Increased food spilling was observed for these animals.
The mean daily test substance intake over the entire study period was calculated and is shown in the following table:

Table B.6.8-22: Calculated intake of sodium salt of M656PH023

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1200	106	106
2	4000	357	349
3	12000	1388	1057

No test substance related, adverse changes with regard to water consumption were observed. No treatment related changes among haematological parameters were observed.
No treatment related adverse changes among clinical chemistry parameters were observed.
At the end of the study in males of the high dose group (12000 ppm), triglyceride levels were increased (see Table B.6.8-23), but as this was the only changed clinical pathology parameter in these individuals, this alteration was regarded as possibly treatment related but not adverse following the criteria laid down in the ECETOC Technical Report No. 85, 2002 (ASB2014-8405).

Table B.6.8-23: Selected clinical chemistry findings in rats administered M656PH023 for at least 28 days (group means)

Sex		Males				Females			
Dose	[ppm]	0	1200	4000	12000	0	1200	4000	12000
	[mg/kg bw/day]		106	357	1388		106	349	1057
Triglycerides	[mmol/L]	0.84	0.85	1.01	1.35*	0.37	0.39	0.36	0.41

* $p \leq 0.05$,** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

No treatment related changes among urinalysis parameters were observed.
Terminal body weights of treated rats displayed no statistically significant differences to the controls (see Table B.6.8-24).
Regarding pathology, the only finding was a significant increase (+14 %) of the mean relative liver weight in males of the high dose group (12000 ppm). As there was no histopathological correlate, the liver weight increase was considered to be treatment related but adaptive.
Because there was no dose-response relationship, the increased mean relative kidney weight in males of the mid dose group (4000 ppm) was regarded to be incidental. No other statistically significant changes of absolute or relative organ weights were observed.

Table B.6.8-24: Selected mean absolute and relative organ weights of rats administered M656PH023 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		106	357	1388		106	349	1057
Terminal body weight [g]	254.5	252.74	266.62	251.96	175.74	168.34	169.24	174.02
[% of control]	100	99	105	99	100	96	96	99
Liver, absolute [g]	6.606	6.574	7.092	7.45	4.838	4.69	4.754	4.616
[% of control]	100	100	107	113	100	97	98	95
Liver, relative [%]	2.599	2.599	2.66	2.955**	2.751	2.789	2.811	2.653
[% of control]	100	100	102	114	100	101	102	96
Kidneys, absolute [g]	1.87	1.75	2.096	1.944	1.386	1.344	1.328	1.328
[% of control]	100	94	112	104	100	97	96	96
Kidneys, relative [%]	0.735	0.691	0.786**	0.773	0.788	0.8	0.783	0.764
[% of control]	100	94	107	105	100	102	99	97

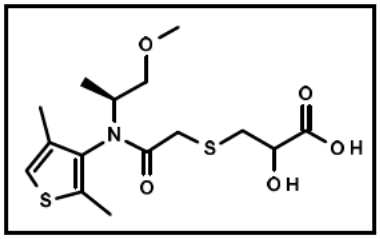
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

No treatment related macroscopic or microscopic alterations were observed. All gross lesions and all histopathological findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered incidental or spontaneous in origin.

Conclusion:

The study is considered to be acceptable.
The administration of M656PH023 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1388 mg/kg bw/d) and in female (1057 mg/kg bw/d) Wistar rats.

Metabolite M656PH026 former assigned M26:
M656PH026 is a metabolite of dimethenamid-P determined in rat, goat (dosed with M565PH030), plant and soil. The determined levels of M656H026 in rats and mice were at trace levels but M656PH026 was up to 68 % of the applied dose in urine when dosed with M656PH030. In plants, the only human consumable it was measured in was bulb onions at a level near the LOQ. It was measured in animal feed items. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment. Exposure for consumer was 0.0025 µg/kg bw/day estimated by the applicant.

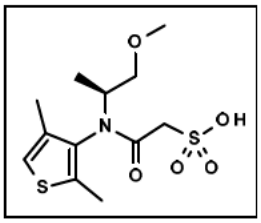


Structural alerts for M656PH026:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered to be of relevance.
OASIS-Times predicted M656PH026 to be not mutagenic in the Ames test without or with metabolic

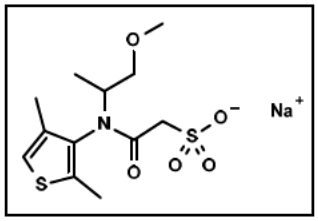
activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure. For this alpha-beta unsaturated carbonyl was an alert given for induction of chromosomal aberration by interaction with topomerases/proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (20 in total) gave no alert for chromosomal aberration.
The Vega prediction (Molecule 7) for Ames mutagenicity in both modules CAESAR and SarPy was negative. However, the reliability of this prediction was low, as no similar compounds with known experimental data were in the database, similar molecules found in the training set disagree with the prediction and the accuracy of the prediction was not optimal. In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.
In conclusion there was a limited alert for chromosomal aberration *in vitro* with metabolic activation in one of the structure activity evaluation tools employed considered of no relevance based on related alert for M656PH031 not confirmed by genotoxicity testing.

Toxicological evaluation of metabolite M656PH026:
The limited alert for chromosomal aberration *in vitro* identified for M656PH026 was considered to be of no relevance *in vivo* in comparison to the parent molecule dimethenamid-P and the closely related metabolites M656PH030, M656PH031 and M656PH032 for which genotoxicity data are available leading to the conclusion M656PH026 not to be genotoxic. Furthermore the determined exposure levels in plant are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 1.5 µg/kg bw/day.
Thus M656PH026 is considered to be of no toxicological relevance by the applicant.

<u>Metabolite M656PH027 former assigned M27:</u>
M656PH027 is a metabolite of dimethenamid-P determined in rat, hen, goat, mice, plant, ground-water and surface water. The determined levels of M656H023 in rats, mice, and goat were at trace levels and were previously reviewed under Annex I.

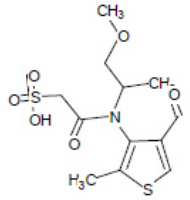


Acute toxicity, genotoxicity studies and short-term toxicity study in rat as already submitted for Annex I inclusion of dimethenamid-P and/or presented below have been conducted with the sodium salt of M656H027 (Reg.No. 360714) representing the respective metabolite of racemic dimethenamid.

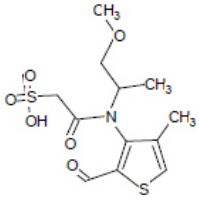


Structural alerts for M656PH027:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times (Molecule 3) predicted M656PH027 to be not mutagenic in the Ames test neither without nor with metabolic activation but with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into structures forming an alpha-beta carbonyl with polarised double bond (see Figure B.6.8-15). For these alpha-beta unsaturated carbonyls was an alert given for induction of chromosomal aberration by interaction with

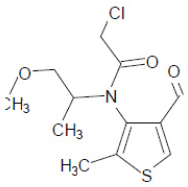
topomerases/proteins (2 transformation products). In this case the prediction was in the domain for this model. Thus the alert was considered principally relevant. Similar structures with the same alerts were also identified for dimethenamid-P. All other presumed transformation products (16 in total) gave no alert for chromosomal aberration.

3.12 Metabolite	
Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	In domain

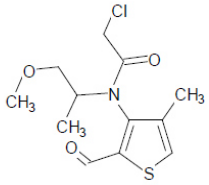
Presumed metabolite of M656PH027

3.13 Metabolite	
Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	In domain

Presumed metabolite of M656PH027

1.9 Metabolite	
Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds,Alpha activated haloalkanes
ModelReliability	High, >= 60% (n>=10)
Total Domain	In domain

Presumed metabolite of dimethenamid-p

1.16 Metabolite	
Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	In domain

Presumed metabolite of dimethenamid-p

Figure B.6.8-15: Presumed mammalian transformation products of M656PH027 and related structures of dimethenamid-P with structural alert for chromosomal aberration *in vitro*

In the DEREK analysis conducted structural alerts for M656PH027 were the thiophene alert for hepatotoxicity and nephrotoxicity which was also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (Molecule 8) for Ames mutagenicity in both modules CAESAR and SarPy was inconclusive. While the CAESAR module predicted M656PH027 to be mutagenic the prediction of the SarPy module was non-mutagenic. However the reliability of these predictions was low.
In conclusion a structural alert for chromosomal aberration *in vitro* after metabolic activation was identified. The genotoxicity testing conducted did however not confirm this alert.

M656H027 former assigned M27 (Reg. No. 360 714):

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.8
Report:	[redacted] 1992 (TOX1999-443) Acute oral toxicity study in rats: SAN 582 H sulfonate metabolite (M-27) [redacted] unpublished, 14 September 1992, BASF RegDoc.# 92/12507 (Experimental work from 27 May 1992 – 10 June 1992)
Guideline(s):	U.S. EPA FIFRA, Subdivision F, Para. 81-1 (November 1984) (Limit Test)
Deviations:	Deviations from Method B.1, Directive 92/69/EEC: None that were considered to have compromised the validity of the study.
GLP:	Yes (laboratory certified by United States EPA Code of Federal Regulations, Title 40, Part 160 Good Laboratory Practices)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	SAN 582H Sulfonate Sodium Salt (dimethenamid metabolite M27); batch No. 4997; purity: 99.45 %
Test Animals:	[Crl: CD®(SD)BR] rats; age: 9 - 12 weeks at start of study, body weights (pretest), males: 284 - 308 g, females: 208 - 223 g, Source: Charles River Laboratories, Kingston, New York.

This study was performed to determine the acute oral LD_{50} of the test material in Sprague-Dawley rats. 10 fasted animals (5/sex) were given a single oral dose of the test material diluted in water at a dose of 5000 mg/kg bw. The animals were observed for 14 d after dosing. Body weights were determined pre-test and on study Days 7 and 14. All animals received a gross necropsy.

Results:

Clinical signs observed were yellow anogenital staining and watery or unformed stool. These signs were not observed on Day 4 and afterwards. Overall, the animals gained body weight as expected for their age. There were no treatment related effects on gross pathology.
No deaths occurred during the study.

Conclusion:

The oral LD_{50} for dimethenamid sulfonate (M27) in Sprague-Dawley rats was determined to be >5000 mg/kg bw.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Data point:	KCA 5.8
Report:	Clare C., 1995 (TOX1999-445) dimethenamid sulfonate (M27): Reverse mutation in 5 histidine-requiring

	strains of Salmonella typhimurium Hazleton Laboratories Europe Ltd., Harrogate, Great Britain unpublished, 21 March 1995, BASF RegDoc.# 95/11338 (Experimental work: February – March 1995)
Guideline(s):	OECD-Guideline 471
Deviations:	No deviations
GLP:	Yes (laboratory certified by The Department of Health and Social Security of the Government of the United Kingdom)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid sulfonate (M27), batch no. RS-582SSS-071494, purity: 97.2 %.
Test System:	*Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537

Dimethenamid-sulfonate (M27) was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 were exposed to the test substance at various concentrations. In the initial experiment, concentrations ranged from 8 - 5000 µg/plate. In a second test concentrations ranged from 312.5 - 5000 µg/plate. The initial study consisted of a plate incorporation test both with and without metabolic activation (Aroclor induced rat liver S-9 mix). In the second test, a pre-incubation step was used to maximise any potential mutagenic metabolites. The test article (or control solution) was mixed with the bacteria and S-9 for 1 h at 37 °C before the addition of the agar. Three plates were used per dose for each strain in both tests.
For control purposes and to demonstrate the sensitivity of the test system, a negative control and positive controls both without S-9 mix and with metabolic activation were tested.

Results:

No toxicity was observed with any strain at any of the dose levels tested. There were no increases in mutation frequency associated with the test article. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:

According to the results of the study, dimethenamid sulfonate (M27) is not mutagenic in the Ames reverse mutation assay with strains TA 98, TA 100, TA 102, TA 1535 or TA 1537 with or without metabolic activation.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	[redacted] 2000b (TOX2002-1991) Gene mutation assay in Chinese Hamster V79 cells *in vitro* (V79/HPRT) with Reg.-No. 360 714/M27 [redacted]

	[redacted] unpublished, 7 January 2000, BASF DocID 2000/10000179; RCC-CCR Project 647602 (Experimental work: 12 October 1999 – 30 December 1999)
Guideline(s):	EEC 87/302, OECD 476
Deviations:	Deviations to the test protocol: The ratio of S9 supernatant to cofactor solution was 1:2.0 in the first and 1:9.0 in the second experiment based upon the total volume of S9 mix. The ratio of S9 supernatant to cofactor solution depends on the actual charge used in the test. The historical data were updated (1996 – 1998) and on request of the sponsor the mean values and standard deviations were added.
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Reg. No. 360 714 (sodium salt of metabolite of BAS 656 H, dimethenamid)
Description:	Solid (powder), white
Lot/Batch #:	L59-50
Purity:	97.2 %
Stability of test compound:	The storage stability of the test substance covering the period of the study was guaranteed by the sponsor.
Solvent used:	deionised water
Control Materials:	
Negative control:	Untreated cells
Solvent control:	10 % v/v deionised water, DMSO
Positive control -S9:	Ethylmethane sulfonate (EMS) 300 µg/mL (dissolved in nutrient medium)
Positive control +S9:	7,12-dimethylbenz(a)anthracene (DMBA) 2.5 µg/mL (dissolved in DMSO)
Activation:	S9 was produced from the livers of induced male Wistar [HanIbm] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

	The ratio of S9 supernatant to cofactor solution was 1:2.0 in the first and 1:9.0 in the second experiment based upon the total volume of S9 mix.
Test organism:	V79 cells with a doubling time of 12 – 16 h, a good cloning efficiency (as a rule more than 50 %) and a modal chromosome number of 22. Stocks of the cell line were maintained in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contami nation, karyotype stability and spontaneous mutant frequency.

Culture media:

Culture medium:	Minimal essential medium (MEM) supplemented with 10 % (v/v) foetal calf serum (FCS).
Treatment medium:	Serum free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with "saline G" (containing NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, Na_2HPO_4 x 7 H_2O 290 mg, KH_2PO_4 150 mg; pH 7.2).
Selection medium:	no data given
Locus examined:	hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)
Test concentrations:	
Preliminary toxicity assay:	Eight concentrations ranging from 26.6 to 3400 µg/mL
Mutation assay:	
1st and 2nd experiment:	106.3, 212.5, 425.0, 850.0, 1700.0 and 3400.0 µg/mL with and without metabolic activation

Preliminary cytotoxicity assay:
A pre-test was performed in order to determine the concentration range for the main experiments under the same culturing and experimental conditions as described for the main test. The colony forming ability of approximately 500 single cells (dublicate cultures per concentration level) after treatment with the test substance was observed and compared to the controls. Toxicity of the test substance is indicated by a reduction of the cloning efficiency (CE). PH and osmolarity were determined in the solvent control and in the maximal concentration in the experiment without metabolic activation.
Mutation Assay:
Pretreatment and seeding of cells:
Three days old exponentially grown stock cultures (more than 50 % confluent) were trypsinised at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. A 0.2 % trypsin concentration in Ca-Mg-free salt solution was used which contained NaCl 8000 mg, KCl 400 mg, glucose 1000 mg; $NaHCO_3$ 350 mg. Prior to trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/mL EDTA. Approx. 1.5 x 10^6 (single culture) and 5 x 10^2 cells (in duplicate) were seeded in culture medium for the determination of mutation rate and toxicity, respectively.
Cell treatment:
24 hours after seeding, the medium was replaced with serum free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with "saline G" (containing NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, Na_2HPO_4 x 7 H_2O 290 mg, KH_2PO_4 150 mg; pH 7.2).
Colonies were stained with 10 % methylene blue in 0.01 % KOH solution. The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope.
Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 4.5 % CO_2, 37 °C.
Statistics:
Since the distribution of mutant cells did not follow known statistical models, an adequate statistical method is not available.
Evaluation criteria:
The test chemical is considered positive if it induces either a concentration related increase of the mutant frequency or a reproducible and positive response at one of the test points.
The test substance producing neither a concentration related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.
A significant response is described as follows:

- Test substance is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
- The test substance is classified as mutagenic if there is a reproducible concentration related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding negative control data. If there is by chance a low spontaneous mutation rate in the range normally found (0.5-33.1 mutants per 10^6 cells) a concentration related increase of the mutations within this range has to be discussed.

Results:

The storage stability of the test substance covering the period of the study was guaranteed by the sponsor. The stability of the test substance in solvent was not determined analytically.
In the preliminary experiment, neither precipitation nor toxicity was observed up to the maximal concentration of 3400 µg/mL.
Based on these data the highest concentration tested in the mutagenicity experiments was 3400 µg/mL without and with metabolic activation.
In the mutagenicity assays up to the highest investigated concentration, no relevant increase in mutant colony numbers was observed in both independent experiments. No cytotoxicity and no precipitation was observed up to the maximal concentration of 3400 µg/mL (see Table B.6.8-25 and Table B.6.8-26).
Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Table B.6.8-25: Gene mutation in mammalian cells - 1st experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE1 (survival), relative		CE2 (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	8.1	6.2	100.0	100.0	0.75	0.75
Vehicle control (deio.water)	-	11.5	5.0	100.0	100.0	0.77	0.79
Sodium salt of M656H027							
106.3 µg/mL	-	n.c.	n.c.	95.8	99.0	n.c.	n.c.
212.5 µg/mL	-	13.6	0.7	83.2	108.9	0.73	0.75
425.0 µg/mL	-	3.8	8.7	88.6	104.4	0.77	0.79
850.0 µg/mL	-	5.8	11.8	80.6	89.5	0.84	0.80
1700.0 µg/mL	-	5.0	3.9	85.5	106.5	0.71	0.69
3400.0 µg/mL	-	5.8	6.5	78.6	81.6	0.76	0.76
Positive control EMS							
300.0 µg/mL	-	143.7	163.9	85.4	74.7	0.70	0.67
4-hour exposure period							
Negative control	+	6.8	3.1	100.0	100.0	0.65	0.67
Vehicle control (deio.water)	+	9.0	3.1	100.0	100.0	0.77	0.67
Vehicle control (DMSO)	+	4.3	5.3	96.2	103.9	0.67	0.72
Sodium salt of M656H027							
106.3 µg/mL	+	n.c.	n.c.	98.8	n.c.	n.c.	n.c.
212.5 µg/mL	+	7.8	4.9	98.5	110.7	0.76	0.76
425.0 µg/mL	+	2.8	8.0	100.8	104.1	0.79	0.76
850.0 µg/mL	+	11.1	4.3	97.7	109.8	0.62	0.63
1700.0 µg/mL	+	17.2	1.5	100.8	108.8	0.58	0.58
3400.0 µg/mL	+	2.3	5.5	97.3	107.9	0.67	0.68
Positive control DMBA							
2.5 µg/mL	+	1102.7	1063.3	49.3	57.0	0.46	0.47

CE1: (mean number of found number of cells per flask/corresponding control) x 100
CE2: (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

Table B.6.8-26: Gene mutation in mammalian cells – 2nd experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE1 (survival), relative		CE2 (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	2.9	3.3	100.0	100.0	0.68	0.75
Vehicle control (deio.water)	-	5.7	6.8	100.0	100.0	0.77	0.86
Sodium salt of M656H027							
106.3 µg/mL	-	n.c.	n.c.	98.1	82.0	n.c.	n.c.
212.5 µg/mL	-	3.2	3.6	90.2	83.3	0.74	0.72
425.0 µg/mL	-	3.8	1.3	99.5	92.0	0.82	0.71
850.0 µg/mL	-	6.9	3.7	81.7	80.5	0.68	0.79
1700.0 µg/mL	-	11.0	3.1	91.0	68.0	0.70	0.79
3400.0 µg/mL	-	1.2	3.6	81.2	66.6	0.80	0.83
Positive control EMS							
300.0 µg/mL	-	224.7	330.8	75.2	104.2	0.72	0.56
4-hour exposure period							
Negative control	+	6.9	1.4	100.0	100.0	0.79	1.03
Vehicle control (deio.water)	+	3.6	4.7	100.0	100.0	0.92	1.00
Vehicle control (DMSO)	+	6.0	1.0	109.2	101.2	0.85	0.97
Sodium salt of M656H027							
106.3 µg/mL	+	n.c.	n.c.	100.6	94.8	n.c.	n.c.
212.5 µg/mL	+	4.9	7.2	111.9	99.7	0.82	0.86
425.0 µg/mL	+	3.1	1.0	106.2	87.0	0.79	0.86
850.0 µg/mL	+	1.6	3.6	118.9	93.0	0.93	0.88
1700.0 µg/mL	+	1.3	1.2	108.6	103.0	0.78	0.89
3400.0 µg/mL	+	1.9	2.2	113.7	101.5	0.89	0.94
Positive control DMBA							
2.5 µg/mL	+	321.0	322.6	89.7	94.6	0.87	0.91

CE1: (mean number of found number of cells per flask/corresponding control) x 100
CE2: (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test sodium salt of M656H027 did not induce forward mutations in the HPRT locus in V79 cells *in vitro*.

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point:	KCA 5.8
Report:	[redacted] 1998 (TOX1999-447) Micronucleus assay in bone marrow cells of the mouse with reg. nr. 360714/M27 [redacted] unpublished, 19 January 1998, BASF RegDoc.# 98/10168 (Experimental work: October – December 1997)
Guideline(s):	OECD-Guideline 474 and proposal for updating of guideline 474, ENV/MC/CHEM/TG(96)7
Deviations:	No relevant deviations
GLP:	Yes (laboratory certified by Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen, Gruppe IV C, Postfach 30 06 52, 40190 Düsseldorf, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Dimethenamid sulfonate (M27); batch No. L 59-50; purity: 97.2 %
Test Animals:	NMRI-mice

M27 was tested for clastogenicity and the ability to have a spindle poison effect in NMRI mice using a micronucleus test.
The test substance dissolved in 0.9 % NaCl solution was administered once intraperitoneally to 6 male and 6 female animals per group at dose levels of 500, 1000 and 2000 mg/kg bw (for 24 h observation) and 2000 mg/kg bw (48 h observation). A volume of 20 mL/kg bw was used for each dose. These dose levels were selected based on a preliminary toxicity study which indicated the limit dose of 2000 mg/kg bw could be tested. The negative control group received the vehicle only. Cyclophosphamide was used as a positive control.
24 or 48 h after the administration of the test substance (as discussed above), all surviving animals were sacrificed and the bone marrow of 2 femora was prepared. The positive controls were sacrificed 24 h after administration. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal. Five surviving animals per sex and group at each time period were evaluated.

Results:

No mortality was observed at any dose level or sacrifice time. The mean number of normochromatic erythrocytes (NCE) was not increased after treatment with the test article as compared to the mean value of NCEs of the corresponding vehicle controls indicating a cytotoxic effect in the bone marrow had not occurred. However, testing was conducted to the guideline limit dose of 2000 mg/kg bw.
The administration of M27 did not lead to an increase in the number of polychromatic erythrocytes containing micronuclei in any of the dose groups at any time period measured after treatment. The administration of the positive control resulted in the expected increase in micronuclei, demonstrating the sensitivity of the assay.

Conclusion:

Under the conditions of this test, dimethenamid sulfonate (M27) does not have a chromosome damaging (clastogenic) effect *in vivo*.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	[redacted] 2014a (ASB2014-8416) Reg.No. 360714 (metabolite of BAS 656 H, Dimethenamid) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet [redacted] Germany unpublished, 03 February 2014, BASF RegDoc.# 2013/1342917 (Experimental work: 25-June-2013 – 09-January-2014) Class T., 2013b (ASB2014-8450) Analytical report - Homogeneity and concentration control of dimethenamid metabolite M27 (Reg.No. 360714) in vehicle 2013/1413980 PTRL Europe, 89081 Ulm, Germany unpublished, 19 July 2013, BASF RegDoc # 2013/1413980 (Experimental work: 10-July-2013 – 19-July-2013)
Guideline(s):	OECD 407, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany) Yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Reg. No. 360 714 (sodium salt of metabolite of BAS 656 H, dimethenamid)
Description:	Solid/white
Batch/purity #:	L82-97,
Purity:	92.3 %
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.
Expiry date:	Sep. 09, 2014
Vehicle and/or positive control:	Rodent diet
Test animals:	
Species:	Rat
Strain:	Wistar Crl:WI (Han)
Male and female	
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 155.0 ± 6.8 g, ♀ 125.2 ± 5.7 g
Source:	Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*

Water: Tap water in bottles, *ad libitum*

Housing: Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment
Motor activity measurements were conducted in polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material

Environmental conditions:

Temperature: 20 - 24 °C

Humidity: 30 - 70 %

Air changes: 15 air changes per hour

Photo period: 12 h light/12 h dark
(06:00-18:00/18:00-06:00)

Animal assignment and treatment:
Sodium salt of M656H027 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomisation list based on body weights.

Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. One diet preparation per dose was performed for this study.
Analyses performed prior to the start of the administration period revealed that the test substance was stable in the diet for at least 31 days.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analysed. The samples were also used for determination of the test article concentration. For the mid dose level a single sample was analysed. No test article was determined in control diets.

Table B.6.8-27: Analysis of diet preparations for homogeneity and test item content

Dose level [ppm]	Sampling	Concentration Mean ± SD [ppm]	% of nominal concentration	Relative standard deviation [%]
1200 ppm	03-Jul-13	1235 ± 152#	103.3	12.3
4000 ppm	03-Jul-13	4170	104.0	n.a.
12000 ppm	03-Jul-13	11765 ± 878#	98.3	7.5

n.a.: not applicable;
based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Homogeneity tested for the lower level of 1200 µg/g (ppm) indicated a slightly lower concentration of about 1064 ppm or about 11 % lower than nominal in the sample aliquot collected from the lower portion of the vessel and slightly higher concentrations (≤13 % higher than nominal) in sample aliquots collected from the middle and upper portions of the vessel.
Homogeneity tested for the highest level of 12000 µg/g (ppm) indicated a slightly lower concentration of about 10755 ppm or about 10 % lower than nominal in the sample aliquot collected from the upper

portion of the vessel.
Relative standard deviations (RSD) were found to be in the range of 12 % to 7 % for the lowest (1200 ppm) and highest concentration (12000 ppm). In general, a RSD of ≤10 % can be regarded as acceptable for diet analysis. This very slight deviation for the lowest concentration (RSD = 12 %) from the acceptance criterion is, however, considered acceptable taking into account that the highest concentration was determined in the mid sample and thus not indicating a systematical inhomogeneity.
The mean values of sodium salt of M656H027 in ground Kliba maintenance diet mouse/rat "GLP" meal were found to be in the range of 90 - 110 % of the nominal concentrations. These results demonstrated the correctness of the concentrations of sodium salt of M656H027 in the vehicle.
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-28: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians.

Table B.6.8-29: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except reticulocytes and differential blood count, urine pH, urine volume, urine specific gravity	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians. For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urinalysis, except pH, volume, colour, turbidity and specific gravity	Pair wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Table B.6.8-30: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.

Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behaviour during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. faeces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Food consumption was determined weekly over a period of 1 day and calculated as mean food consumption in grams per animal and day.
Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BWx and BWy body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).
Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted.
Ophthalmoscopy:
Not performed in this study.
Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of

25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1.	behaviour when removed from cage	10.	respiration
2.	fur	11.	tremors
3.	skin	12.	convulsions
4.	salivation	13.	abnormal movements
5.	nose discharge	14.	impairment of gait
6.	lacrimation	15.	activity/arousal level
7.	eyes/pupil size	16.	faeces (number of faecal pellets/appearance/ consistency) within two minutes
8.	posture	17.	urine (appearance/quantity) within two minutes
9.	palpebral closure	18.	number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behaviour during "handling"
2.	touch response	9.	vocalisation
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

Motor activity measurement:
Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
Haematology and clinical chemistry:
Blood was withdrawn in the morning from fasted, isoflurane anesthetised animals from the retro-orbital plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomised sequence.
The following haematological and clinical chemistry parameters were determined for all animals:

	Haematology:				
✓	Red blood cells		White blood cells		Clotting Potential
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Haemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Haematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
	Clinical chemistry:				
	Electrolytes		Metabolites and Proteins		Enzymes:
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Urinalysis:
For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analysed in a randomised order.
The following parameters were determined for all animals:

	Urinalysis				
	Quantitative parameters:		Semi quantitative parameters		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

Sacrifice and pathology:
The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).											
C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	#	kidneys	✓	✓	#	seminal vesicles with coagulating glans
✓		#	aorta	✓			lachrymal glands, extraorbital	✓			skin
✓		#	bone marrow§	✓		#	larynx	✓		#	spinal cord (3 levels)@
✓	✓	#	brain	✓	✓	✓	liver	✓	✓	#	spleen
✓		#	caecum	✓		#	lung	✓		#	sternum w. marrow
✓		#	colon	✓		#	lymph nodes#	✓		#	stomach (fore- & glandular)
✓		#	duodenum	✓		#	mammary gland (♂ and ♀)	✓	✓	#	testes
✓	✓	#	epididymides¥	✓		#	muscle, skeletal	✓	✓	#	thymus
✓		#	esophagus	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thyroid/parathyroid
✓		#	eyes (with optic nerve)	✓		#	nose/nasal cavity‡	✓		#	trachea
✓			femur (with joint)	✓	✓	#	ovaries and oviduct**	✓		#	urinary bladder
			gall bladder	✓		#	pancreas	✓	✓	#	uterus with cervix
✓		✓	gross lesions	✓			pharynx	✓		#	vagina
✓			Harderian glands	✓		#	pituitary				
✓	✓	#	heart	✓	✓	#	prostate				
✓		#	ileum	✓		#	rectum		✓		body (anesthetised animals)
✓		#	jejunum (w. Payer's plaque)	✓			salivary glands*				
§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; ‡l histopathology at level III, ¥left epididymidis collected for histopathology											

The organs or tissues were fixed in 4 % formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.
The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:
• Increased/decreased grade of cortico-medullar ratio (related only to area)
• Increase of starry sky cells
• Changes of cellular density in the cortex
• Changes of cellular density in the medulla
Spleen:
• Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
• Altered cellular composition of follicles
• Altered number of germinal centers

Lymph nodes (mesenteric and axillar lymph nodes):
• Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
• Altered cellular composition of paracortex
• Altered number of germinal centers
• Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):
• Changes of the cellularity of follicles (including mantle zone and germinal centers)
• Changes of the cellularity of interfollicular area
Bone marrow:
• Changes of the cellularity
• Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

Results:

Clinical signs of toxicity: No clinical signs were observed throughout the study.
Mortality: No mortality was observed throughout the study.
Ophthalmoscopy: Not performed in this study.
FOB and Motor Activity:
Neither home cage nor open field observations revealed any indication of treatment related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
No statistically significant differences of overall motor activity between control and treated animals were observed for males of the other test groups (1200 and 4000 ppm) as well as for the female animals of all test groups.
Body weights or body weight gain:
No statistically significant differences of absolute body weights or body weight gain were noted (Table B.6.8-31, Figure B.6.8-16).

Table B.6.8-31: Mean body weight of rats administered sodium salt M656H027 for at least 28 days

	Males				**Females**			
Dose level [ppm]	0	1200	4000	12000	0	1200	4000	12000
Body weight [g]								
- Day 0	151.7	155.2	156.8	157.0	125.5	127.6	124.6	123.3
- Day 28	291.8	282.0	292.2	293.9	180.1	188.4	179.1	175.2
Δ% (compared to control) #		-3.3	0.2	0.7		4.6	-0.5	-2.7
Overall body weight gain [g]	138.8	126.9	135.5	136.9	54.7	60.8	54.6	51.9
Δ% (compared to control)#		-8.6	-2.4	-1.3		11.2	-0.1	-5

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

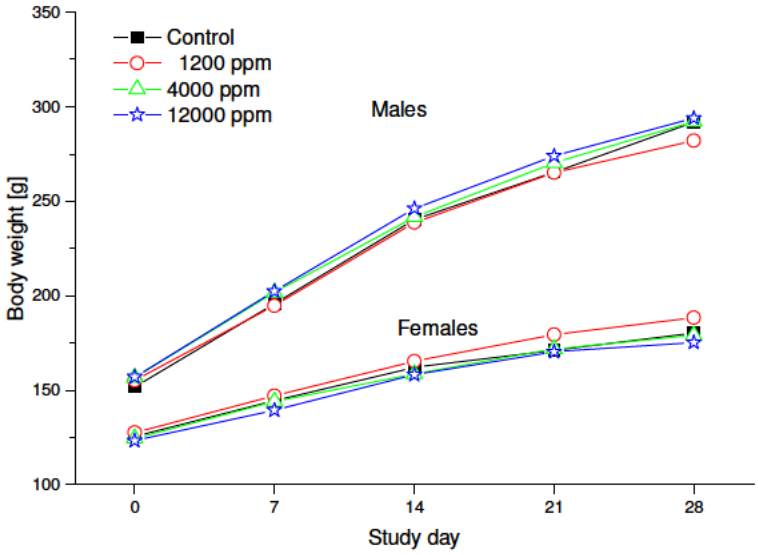


Figure B.6.8-16: Body weight development of rats administered sodium salt of M656H027 for at least 28 days

Food consumption and compound intake:
No test substance related, adverse findings were observed. All recorded values were within the biological range typical for this strain of rats.
Note: The value for male animals of the high dose group (12000 ppm) between study days 11 to 14 was declared as outlier and thus was not taken into consideration. Increased food spilling was observed for these animals.
The mean daily test substance intake over the entire study period was calculated and is shown in the following table:

Table B.6.8-32: Calculated intake of sodium salt of M656H027

Test group	Concentration in the vehicle (ppm)	Mean daily test substance intake (mg/kg bw/d)	
		Males	Females
1	1200	99	144
2	4000	364	341
3	12000	1064	1247

No test substance related, adverse changes with regard to water consumption were observed. No treatment related changes among haematological parameters were observed. No treatment related changes among clinical chemistry parameters were observed.
At the end of the study in females of test groups 1, 2 and 3 (1200, 4000 and 12000 ppm), ALP activities were decreased (in test group 2 not significantly). However, all means were within the historical control range (see Table B.6.8-33). Therefore, this alteration was regarded as incidental and not treatment related.

Table B.6.8-33: Selected clinical chemistry findings in rats administered sodium salt of M656H027 for at least 28 days (group means)

Sex		Males				Females			
Dose	[ppm]	0	1200	4000	12000	0	1200	4000	12000
	[mg/kg bw/day]		99	364	1064		144	341	1247
ALP	[µkat/L]	2.48	2.64	2.38	2.54	1.43	1.25*	1.18	1.10**
Historical control range [µkat/L]						0.71-2.01 (mean 1.25)			

* $p \leq 0.05$, **$p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

No treatment related changes among urinalysis parameters were observed.
The lower specific gravity of the urine in males of the 4000 ppm group (see Table B.6.8-34) was considered to be incidental as there was no dose response relationship.

Table B.6.8-34: Selected findings in urinalysis in rats administered sodium salt of M656H027 for at least 28 days (group means)

Sex		Males				Females			
Dose	[ppm]	0	1200	4000	12000	0	1200	4000	12000
	[mg/kg bw/day]		99	364	1064		144	341	1247
Specific gravity	[g/L]	1.054	1.052	1.039*	1.056	1.064	1.056	1.077	1.073

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Terminal body weights of treated rats displayed no statistically significant differences to the controls (see Table B.6.8-35).
When compared to control group the mean absolute thymus weights of in the male 1200 ppm group and the mean absolute ovary weights in the 12000 ppm group were significantly decreased. In the male 12000 ppm group the mean relative heart weights were significantly decreased. The significant thymus weight decrease in males of test group 1 (1200 ppm) occurred without any dose dependency and was regarded as incidental. The significant weight decrease of the ovaries in females of the 12000 ppm group and the significant relative heart weight decreases in males of the 12000 ppm group occurred without any histopathological findings and, therefore, were considered to be incidental.

Table B.6.8-35: Selected mean absolute and relative organ weights of rats administered sodium salt of M656H027 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		99	364	1064		144	341	1247
Terminal body weight [g]	264.84	258.66	264.66	268.26	164.2	169.1	163.34	160.02
[% of control]	100	98	100	101	100	103	99	97
Thymus, absolute [mg]	470	403.6**	534.8	514.6	456.8	398.2	430.4	374.8
[% of control]	100	86	114	109	100	87	94	82
Thymus, relative [%]	0.178	0.156	0.202	0.192	0.277	0.235	0.265	0.234
[% of control]	100	88	114	108	100	85	96	85
Ovaries, absolute [g]					95.2	96.4	92	81*
[% of control]					100	101	97	85
Ovaries, relative [%]					0.058	0.057	0.056	0.051
[% of control					100	99	97	88
Heart, absolute [g]	0.886	0.864	0.886	0.818	0.592	0.620	0.598	0.612
[% of control]	100	98	100	92	100	102	96	93
Heart, relative [%]	0.334	0.334	0.334	0.305*	0.360	0.366	0.367	0.382
[% of control	100	100	100	91	100	102	102	106

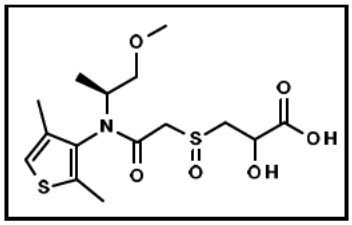
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

No other statistically significant changes of absolute or relative organ weights were observed.
No treatment related macroscopic or microscopic alterations were observed. All gross lesions and all histopathological findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered incidential or spontaneous in origin.

Conclusion:

The study is considered to be acceptable.
The administration of sodium salt of M656H027 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1064 mg/kg bw/d) and in female (1247 mg/kg bw/d) Wistar rats.

Metabolite M656PH030 former assigned M30:
M656PH030 a metabolite identified in rat, hen, and goats dosed with M656PH030. The determined levels of M656H030 in rats and mice were at trace levels but M656PH030 reached 4 % of the applied dose in goats that were dosed with 12 mg/kg of M656PH030. In plants it was only determined in edible commodities of bulb and spring onions, kale, Chinese cabbage, and head cabbage as well as several animal feed items. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.



Structural alerts for M656PH030:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times (molecule 5) predicted M656PH030 to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. There was no structural alert for *in vitro* chromosomal aberration for the structure itself and the only structural alert identified for a transformation products was predicted not become active. Again the reliability of the predictions was limited as the molecule was out of the prediction domain.
The Vega prediction (molecule 10) of both modules CAESAR and SarPy was non-mutagenic. However the reliability of these predictions was low as no similar compounds with known experimental data were in the database, as some similar molecules found have experimental values that disagree with the predicted value, as the accuracy of prediction for similar molecules is not optimal and as some atom centred fragments of the compound have not been found or are rare fragments in the database.
Overall no conclusive, relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

Genotoxicity studies of M656PH030:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Woitkowiak C., 2014d (ASB2014-8451) Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2014/1018061
Guideline(s):	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656H030 (Reg.No. 5296352) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and a pre-incubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

Test Material	Reg.No. 5296352 (Metabolite of BAS 656 PH, dimethenamid-P)
Description:	Solid, white to slightly off grey
Lot/Batch #:	L82-138

Purity:	98.7 % (tolerance +/- 1.0 %, (see Certificate of Analysis, study code ASAP13_228))
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2015 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control:	The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation:
S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate. The optical density of the fresh thawed bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 10^9 cells per mL).

Test concentrations:
Plate incorporation assay
(1st experiment): Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay:
(2nd experiment): The test substance/vehicle/positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 22-Jan-2014 to 07-Feb-2014.

Plate incorporation assay:
To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates).
Pre-incubation assay:
100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9-mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.
Statistics:
No special statistical tests were performed.
Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

A dose related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and *E.coli* WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolising system.
A test substance is generally considered non-mutagenic in this test if:
The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

Results:

Analytical determination:
The test substance was stable over the study period under the storage conditions.
The stability of the test substance in the vehicle dimethyl sulfoxide (DMSO) was verified analytically (see separate BASF study no. 01Y0009/13Y055).
Toxicity:
A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
A weak bacteriotoxic effect (slight decrease in the number of his+ revertants) was observed in the standard plate test from about 2500 µg/plate onward only in tester strain TA 1537 without S9-mix. In the pre-incubation assay bacteriotoxicity (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants) was observed depending on the strain and test conditions from about 2500 µg/plate onward.
Mutation assay:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-36).
Test substance precipitation was found in the standard plate test at 5000 µg/plate without S9-mix and from about 2500 µg/plate onward after adding the metabolising system.

Table B.6.8-36: Bacterial gene mutation assay with M656H030 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		*E. coli*	
1st experiment: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	23.3	19.3	55.3	36.7	11.0	14.0	8.0	7.3	49.3	57.7
M656H030										
33 µg/plate	23.7	14.0	48.3	47.3	8.7	9.3	6.3	7.7	57.3	55.0
100 µg/plate	24.3	17.0	59.7	33.3	13.3	9.7	5.7	5.7	52.7	57.3
333 µg/plate	27.0	21.3	49.0	40.0	12.0	10.7	7.7	5.7	49.0	51.3
1000 µg/plate	25.0	12.7	52.0	48.3	11.0	9.3	6.3	9.7	58.3	64.3
2500 µg/plate	25.3[P]	15.0[P]	62.0[P]	43.7[P]	9.0[P]	10.0[P]	8.0[P]	4.7[P]	67.7[P]	45.7[P]
5000 µg/plate	29.0[P]	16.0	56.3[P]	40.7	9.3[P]	8.0	6.7[P]	3.7	93.3[P]	58.7
Pos. control[§]	2254.3	383.0	3148.7	4949.3	220.3	4110.7	250.3	2023.3	253.7	817.0
2nd experiment: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	19.3	17.0	47.0	34.3	10.3	9.3	8.7	7.7	46.7	58.7
M656H030										
33 µg/plate	22.3	17.0	44.3	32.0	10.7	8.7	7.3	6.7	44.7	54.7
100 µg/plate	19.7	12.3	48.3	29.7	9.0	7.3	7.3	5.3	49.7	48.3
333 µg/plate	20.0	12.7	53.0	36.3	7.7	7.7	7.0	6.0	49.7	55.7
1000 µg/plate	20.3	16.0	33.0	43.3	10.0	7.7	7.0	6.0	52.7	53.7
2500 µg/plate	12.7[B]	18.7[B]	0.0[B]	22.0[B]	8.7[B]	5.7[B]	4.3[B]	6.0[B]	45.7[B]	59.7[B]
5000 µg/plate	0.0[B]	0.0[B]	0.0[B]	0.0[B]	0.0[B]	1.3[B]	0.0[B]	0.0[B]	14.0[B]	0.0[B]
Pos. control[§]	473.0	404.7	349.7	922.3	197.3	1497.7	259.3	1172.0	232.3	951.3

[B]= reduced background growth
[P] = Precipitation
[§] = Compound and concentrations see Material and Methods (I.A.2.) above

Conclusion:

The study is considered to acceptable.
According to the results of the present study, the test substance M656H030 is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point:	KCA 5.8
Report:	[redacted] 2014b (ASB2014-8458) Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P): *In vitro* cell mutation assay at the Thimidine Kinase Locus (TK+/-) in mouse lymphoma L5178Y cells 2014/1018062
Guideline(s):	OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300, EPA 712-C-98-221
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656PH030 (Reg.No. 5296352) was tested *in vitro* for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the TK+/- locus. Two independent experiments were conducted in the presence and absence of metabolic activation.

Test Material	Reg. No. 5296352 (Metabolite of BAS 656-PH, dimethenamid-p)
Description:	Solid, white to slightly off grey
Lot/Batch #:	L82-138
Purity:	98.7 % (tolerance ± 1.0 %) for details see Certificate of Analysis ASAP13_228 (dose calculation adjusted to purity)
Stability of test compound:	Stable in DMSO over 4 hours
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL
Activation:	Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[*a*]pyrene and 2-aminoanthracene in the Ames test.

The protein concentration of the S9 preparation was 29.8 mg/mL (Lot. No.: 050913) in the pre-experiment and experiments I and II. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

Test organism:	The L5178Y cell line, which is characterised by a high proliferation rate (doubling time 10-12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50 %. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with hypoxanthine (5.0×10^{-3} M), aminopterin (2.0×10^{-5} M), thymidine (1.6×10^{-3} M) and glycin (5.0×10^{-3} M) followed by a recovery period of 2 days in RPMI 1640 medium containing hypoxanthine (1.0×10^{-4} M) and thymidine (1.6×10^{-3} M). After this incubation the cells were returned to complete culture medium (see below).
Culture media:	
Complete culture medium:	RPMI 1640 medium supplemented with 15 % horse serum (24 hour treatment, 3 % HS during 4 hour treatment), 1 % of 100 U/100 µg/mL penicillin/streptomycin, 220 µg/mL sodium-pyruvate, and 0.5 - 0.75 % amphotericin used as antifungal agent.
Selection medium:	RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT
Saline G solution:	Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, $Na_2HPO_4 \times 2\ H_2O$ 192 mg, KH_2PO_4 150 mg
Locus examined:	Thymidine Kinase Locus (TK+/-)
Test concentrations:	
a) Preliminary toxicity assay:	Eight concentrations ranging from 29.8 to 3820 µg/mL
b) Mutation assay: 1st and 2nd experiment:	119.4, 238.8, 477.5, 955.0, 1910.0, 3820.0 µg/mL with and without metabolic activation
Dates of experimental work:	02-Dez-2013 to 21-Jan-2014

Preliminary cytotoxicity assay:
A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.
1×10^7 cells (3×10^6 cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

Mutation Assay:
Cell treatment and expression: In the mutation experiment 1×10^7 (3×10^6 during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3 % horse serum (15 % horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied

by the day 2 fold-increase in cell number.
Selection:
After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4 x 10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5 % CO_2/95.5 % humidified air for 10-15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.
Size distribution of the colonies:
Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).
Calculations:

Pre-test:
total suspension growth (4 h treatment):
(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):
(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
relative suspension growth:
total suspension growth x 100/total suspension growth of corresponding control

Main test:
Total suspension growth (4 h treatment): (cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
Total suspension growth (24 h treatment): (cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) x (cell number at 72 h/if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)
Relative suspension growth: total suspension growth x 100/total suspension growth of corresponding control
Relative total growth: relative suspension growth x relative cloning efficiency/100
Cloning efficiency (viability): ln(mean number of empty wells per plate/96)/cells seeded per well
Relative cloning efficiency: cloning efficiency x 100/cloning efficiency of corresponding control
Cells survived: cloning efficiency x cell number seeded in TFT medium
Mutant colonies/10^6 cells: small mutant colonies + large mutant colonies
Threshold: number of mutant colonies per 10^6 cells of each solvent control plus 126
Cloning efficiency (viability): cloning efficiency determined after the expression period to measure viability of the cells without selective agent

Statistics:
A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

Evaluation criteria:
The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6

cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:
- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10 % of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

Results:

Analytical determinations:
Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_228).
Preliminary cytotoxicity assay:
The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 29.8 µg/mL and 3820 µg/mL were chosen with regard to the molecular weight (377.5 g/mol) and the purity of the test item (according to the preliminary information concerning the purity of the test item (98.7 %, tolerance ± 1.0 %). No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.
Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. Only a minor shift of the pH-value and no shift of the osmolarity occurred. The osmolarity was generally high as compared to the normal physiological value of approximately 300 mOsm. This effect was based on DMSO in the culture medium. As the osmolarity is measured by freezing point reduction, a final concentration of 1 % DMSO has a substantial impact on the freezing point of the medium.
The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3820 µg/mL without and with metabolic activation.
Mutagenicity assays:
Relevant and reproducible cytotoxic effects indicated by a relative total growth below 50 % in both parallel cultures solely occurred at the highest concentration of the second experiment without metabolic activation. No precipitation was noted up to the maximum concentration with and without metabolic activation.
No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The threshold of 126 above the corresponding solvent control was not reached (see Table B.6.8-37, Table B.6.8-38).
The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies at acceptable levels of toxicity with at least one of the concentrations of the controls. The relative total growth of the MMS control of the first experiment, culture I fell just short of the lower limit of 10 % (9.5 %). The data are acceptable however, as the deviation was minor and the MMS control of the parallel culture remained above the 10 % limit (17.0 %).

Table B.6.8-37: Gene mutation in mammalian cells - 1st experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/ 10^6 cells	threshold	relative total growth	mutant colonies/ 10^6 cells	threshold
Experiment I/4 h treatment			Culture I			Culture II		
Solv. Control DMSO		-	100.0	110	236	100.0	74	200
Pos. Control MMS	19.5	-	9.5	1407	236	17.0	377	200
M656PH030								
	119.4	-	culture was not continued#			culture was not continued#		
	238.8	-	128.0	36	236	99.1	92	200
	477.5	-	96.2	52	236	98.8	85	200
	955.0	-	101.2	56	236	106.3	84	200
	1910.0	-	79.0	65	236	85.6	72	200
	3820.0	-	141.8	42	236	94.8	79	200
Solv. Control DMSO		+	100.0	83	209	100.0	71	197
Pos. Control CPA	3.0	+	38.7	254	209	44.0	329	197
Pos. Control CPA	4.5	+	25.9	523	209	29.7	498	197
M656PH030								
	119.4	+	culture was not continued#			culture was not continued#		
	238.8	+	100.8	44	209	76.4	119	197
	477.5	+	107.0	69	209	86.8	69	197
	955.0	+	111.3	73	209	86.0	93	197
	1910.0	+	82.3	127	209	90.5	78	197
	3820.0	+	76.8	122	209	117.4	79	197

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Table B.6.8-38: Gene mutation in mammalian cells - 2nd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/ 10^6 cells	threshold	relative total growth	mutant colonies/ 10^6 cells	threshold
Experiment II/24 h treatment			Culture I			Culture II		
Solv. Control DMSO		-	100.0	132	258	100.0	117	243
Pos. Control MMS	13.0	-	22.8	359	258	28.6	388	243
M656PH030								
	119.4	-	culture was not continued#			culture was not continued#		
	238.8	-	78.2	126	258	140.1	95	243
	477.5	-	100.8	94	258	122.2	93	243
	955.0	-	78.5	75	258	116.3	83	243
	1910.0	-	50.9	88	258	86.1	77	243
	3820.0	-	25.4	92	258	27.9	126	243
Experiment II/4 h treatment								
Solv. Control DMSO		+	100.0	109	235	100.0	63	189
Pos. Control CPA	3.0	+	83.7	272	235	49.2	167	189
Pos. Control CPA	4.5	+	36.5	458	235	26.4	258	189
M656PH030								
	119.4	+	culture was not continued#			culture was not continued#		
	238.8	+	111.0	100	235	102.7	61	189
	477.5	+	218.1	65	235	106.6	39	189
	955.0	+	144.7	80	235	92.0	66	189
	1910.0	+	176.0	70	235	102.1	47	189
	3820.0	+	187.7	83	235	100.3	61	189

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test M656PH030 did not induce forward mutations in the TK+/- locus in L5178Y cells *in vitro*.

Data point: KCA 5.8

Report: [redacted] 2014b (ASB2014-8465)
Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P): Micronucleus test in chinese hamster V79 cells *in vitro*
2014/1018063

Guideline(s): OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: *In vitro* Mammalian Cell Micronucleus Test

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

M656PH030 (Reg. No. 5296352) was tested *in vitro* for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments.

Test Material: Reg. No. 5296352 (Metabolite of BAS 656-PH, dimethenamid-P)
Description: Solid, white to slightly off grey
Lot/Batch #: L82-138
Purity: 98.7 % (tolerance ± 1.0 %)
Stability of test compound: Stable in DMSO over 4 hours
Solvent used: Dimethylsulfoxide (DMSO)
Control Materials:
Negative control: A negative control was not employed in this study
Solvent control: DMSO
Positive controls, -S9: Mitomycin C
(MMC, 0.1 and 0.3 µg/mL, dissolved in deionised water)
Griseofulvin (8.0 µg/mL, dissolved in DMSO)
Positive control, +S9: Cyclophosphamide
(CPA, 10.0 µg/mL, dissolved in saline)
Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

Test organisms: Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥70 %).
Culture medium/conditions: About 5×10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) foetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5-1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.
Test concentrations:
a) Preliminary toxicity assay:

7.5-3820.0 µg/mL with and without metabolic activation.
Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment IA. Due to the positive findings in Experiment IA without S9 mix a confirmatory experiment (Exp. IB) was performed. This experimental part was repeated again due to invalid positive controls (see Table B.6.8-39).

b) Cytogenicity assay:

7.5-3820.0 µg/mL without metabolic activation
119.4-3820.0 µg/mL with metabolic activation

Table B.6.8-39: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with M656PH030

Exp.	Prep. interval	Exposure period	Concentrations in µg/mL									
Without S9 mix												
IA	24 h	4 hrs	7.5	14.9	29.8	59.7	119.4	238.8	477.5	955.0	1910.0	3820.0
IB*	24 h	4 hrs	59.7	119.4	238.8	477.5	955.0	1910.0	2387.5	2865.0	3342.5	3820.0
IB	24 h	4 hrs	59.7	119.4	238.8	477.5	955.0	1910.0	2387.5	2865.0	3342.5	3820.0
II	24 h	24 hrs	7.5	14.9	29.8	59.7	119.4	238.8	477.5	955.0	1910.0	3820.0
With S9 mix												
IA	24 h	4 hrs	7.5	14.9	29.8	59.7	119.4	238.8	477.5	955.0	1910.0	3820.0
II	24 h	4 hrs					119.4	238.8	477.5	955.0	1910.0	3820.0

Evaluated experimental points are shown in bold characters
* was repeated due to invalid positive controls

Dates of experimental work: 27-Nov-2013-17-Feb-2014.

Preliminary cytotoxicity assay:
With respect to the molecular weight and the purity (98.8 %, preliminary information at the start of the experiment) of the test item, 3820.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 7.5 and 3820.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

Cytogenicity Assay:
Exposure period 4 hours:
The culture medium of exponentially growing cell cultures was replaced with serum free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.
Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose x H_2O, 192 mg/L Na_2HPO_4 x 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 20 hours.

Exposure period 24 hours:
The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10 % (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.
Preparations of cultures:
For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity:
Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.
Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

Statistics:
Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.
Evaluation criteria:
A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose related and reproducible increase in the number of cells containing micronuclei is observed

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

Results:

Analytical determinations:
Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP13_228).

Preliminary cytotoxicity assay:
In the pre-test no cytotoxicity was observed at the evaluated concentrations in the absence and presence of S9 mix.
Cytogenicity assays:
In Experiment I and II in the absence and presence of S9 mix no clear cytotoxicity was observed up to the highest applied concentration. In addition, no visible precipitation of the test item in the culture medium was observed.
In Experiment IA in the absence of S9 mix one statistically significant increase in micronucleated cells was observed after treatment with 3820.0 µg/mL (3.25 %), clearly exceeding the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells). In Experiment IB in the absence of S9 mix this finding could not be confirmed. However, all evaluated concentrations showed statistical significance due to the low solvent control value. These values were clearly within the historical control range (0.15 - 1.50 % micronucleated cells).
In Experiment IA in the presence of S9 mix one statistically significant increase in micronucleated cells was observed after treatment with 1910.0 µg/mL (1.33 %). The value was within the range of the laboratory historical control data (0.05 - 1.70 % micronucleated cells) and is therefore biologically irrelevant.
In Experiment II in the presence of S9 mix no relevant increase in micronucleated cells was observed.
In Experiment II in the absence of S9 mix statistically significant increases in micronucleated cells were observed after treatment with 1910.0 and 3820.0 µg/mL (1.15 and 4.35 %, respectively). The second value clearly exceeded the range of the laboratory historical control data (0.05 - 1.50 % micronucleated cells) (see Table B.6.8-40).
Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table B.6.8-40: Summary of results of the micronucleus test with M656PH030

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 h without S9 mix				
IA	24 h	Solvent control[1] Positive control[2] 955.0 1910.0 3820.0	2.88 2.71 2.79 2.83 2.42	0.60 4.20S 0.20 0.60 3.25S
IB	24 h	Solvent control[1] Positive control[3] 955.0 1910.0 3820.0	2.69 2.48 2.85 2.90 2.87	0.05 14.95S 0.40S 0.70S 0.60S
Exposure period 24 h without S9 mix				
II	24 h	Solvent control[1] Positive control[4] 955.0 1910.0 3820.0	2.49 2.38 2.43 2.19 1.58	0.10 14.60S 0.40 1.15S 4.35S
Exposure period 4 h with S9 mix				
IA	24 h	Solvent control[1] Positive control[5] 955.0 1910.0** 3820.0	2.32 1.53 2.36 2.31 2.29	0.65 13.25S 0.65 1.33S 0.85
II	24 h	Solvent control[1] Positive control[5] 955.0 1910.0 3820.0	2.43 1.72 2.50 2.46 2.20	0.95 17.45S 1.30 0.80 1.00

* The total number of micronucleated cells was determined in a sample of 2000 cells
** The total number of micronucleated cells was determined in a sample of 4000 cells
s Number of micronucleated cells statistically significantly higher than corresponding control values
1 DMSO 1.0 % (v/v)
2 Mitomycin C 0.03 µg/mL
3 Mitomycin C 0.3 µg/mL
4 Griseofulvin 8.0 µg/mL
5 CPA 10.0 µg/mL

Conclusion:

The study is considered to acceptable.
Based on the results of the study, M656PH030 was mutagenic in this *in vitro* micronucleus test, when tested up to the highest required concentrations.

Data point: KCA 5.8

Report: [redacted] 2014g (ASB2014-8470)
Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1094090

Guideline(s): OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of

	Council on the REACH - Part B No. B.12, EPA 870.5395, EPA 712-C-98-226
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.
Data point:	KCA 5.8
Report:	Becker M., Landsiedel R., 2014d (ASB2014-8477) Analytical report - Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for externa studies 2014/1092435
Guideline(s):	No
Deviations:	No deviations from the described control procedure.
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.
Data point:	KCA 5.8
Report:	Grauert E., Kamp H., 2014c (ASB2014-8482) Analytical report - Reg.No. 5296352 (metabolite of BAS 656-PH, Dimethenamid-P) - Concentration control analyses in dimethylsulfoxide / polyethylenglycol (3+7, v/v) 2014/1101994
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be accepted.

Materials and methods:

M656PH030 (Reg.No. 5296352) was tested for its ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

Test Material	Reg. No. 5296352 (metabolite of BAS 656 PH, dimethenamid-p)
Description:	Solid, white to slightly off grey
Lot/Batch #:	L82-138
Purity:	98.7 % (tolerance ± 1.0 %)
Stability of test compound:	stable in solvent (Confirmed indirectly by dose formulation analytics (see BASF study 04Y009/13Y086)
Solvent used:	DMSO/PEG 400 (3/7)
Control Materials:	
Negative:	No negative control was employed in this study.
Solvent control:	DMSO/PEG 400 (3/7) 30 % DMSO, 70 % PEG 400
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw
Test animals:	
Species:	Albino mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study

Age:	8 - 12 weeks
Weight at dosing:	mean value 35.0 g (SD ± 1.4 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males / 500 and 2000 mg/kg bw; 14 males / 1000 mg/kg bw (24 treatment); 7 males / 1000 mg/kg bw (48 treatment); 5 males per vehicle and control group, respectively
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	The animals were housed in groups in Makrolon Type II/III, with wire mesh top.
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	45 - 65 %
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)
Test compound concentration:	
Range finding test:	2000 (per os) mg/kg bw
Micronucleus assay:	500, 1000 and 2000 mg/kg bw (doses were corrected for purity with a correction factor of 1.01. Test item dose levels as were 505, 1010 and 2020 mg/kg bw.) The test substance was administered once orally using an application volume of 10 mL/kg.
Dates of experimental work:	21-Jan-2014 – 26-Mar-2014.
Preliminary cytotoxicity assay:	
	Male and female NMRI mice were administered the test substance once by oral gavage at a dose of 2000 mg/kg bw.
Micronucleus test:	
Treatment and sampling:	Groups of male mice were treated once with either the vehicle, positive control substance or 500, 1000 or 2000 mg test substance/kg bw by oral administration. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The animals were surveyed for evident clinical signs of toxicity throughout the study. Twenty-four or 48 hours after the administration the mice were killed and the two femora were prepared free of all soft tissue. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with foetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet resuspended.
Slide preparation:	A small drop of the resuspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.

Statistics:
Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.
Evaluation criteria:
A test item is classified as mutagenic if it induces either a dose related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods (nonparametric Mann-Whitney test) were used as an aid in evaluating the results, if necessary. However, the primary point of consideration is the biological relevance of the results.
A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-mutagenic in this system.

Results:

Analytical determinations:
The stability of the test substance in the vehicle was verified in a separate study under the responsibility of the sponsor and the results are reported in a separate report (BASF study code 04Y009/13Y086).
Preliminary range finding test:
None of the male or female mice died after single oral dosing of 2000 mg/kg bw. Ruffled fur was observed in one male after 30 hours post-treatment. On the basis of these data 2000 mg/kg bw were estimated to be suitable as highest dose. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.
Micronucleus assay:
Ruffled fur was observed in some animals treated with test item concentrations of 1000 and 2000 mg/kg bw, respectively. Furthermore, loose faeces were observed in two animals treated with 2000 mg/kg bw. The animals treated with the low dose level did not exhibit any clinical symptoms. The systemic availability of M656PH030 was confirmed by plasma-analytics (ASB2014-8477).
After treatment with the test item at 24 h and 48 h preparation interval the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that the test substance did not induce cytotoxic effects in the bone marrow.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were below or near to the value of the vehicle control group and all values in all dose groups were very well within the historical vehicle control data range (see Table B.6.8-41).
The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (1.93 %), thereby demonstrating the sensitivity of the test system.

Table B.6.8-41: Micronucleus test in mice administered M656PH030

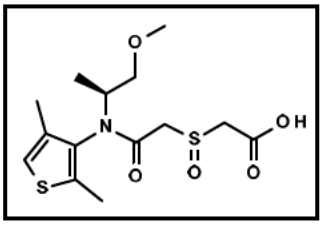
Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
DMSO/PEG 400 (3/7)	24	0.080	0-5	1196
M656PH030*				
500 mg/kg bw	24	0.121	0-7	1211
1000 mg/kg bw	24	0.171	1-8	1238
Positive control				
Cyclophosphamide	24	1.930	20-63	1283
48 h sampling				
DMSO/PEG 400 (3/7)	48	0.100	0-4	1139
M656PH030*				
1000 mg/kg bw	48	0.064	0-4	1183

* Doses were corrected for purity with a correction factor of 1.01. Test item dose levels as actually were 505, 1010 and 2020 mg/kg bw.

Conclusion:

The study is considered to acceptable.
Based on the results of this study, M656PH030 did not induce the formation of micronuclei in mouse polychromatic erythrocytes under *in vivo* conditions. By weight of evidence M656PH030 is not considered to be genotoxic based on the study results available.

Metabolite M656PH031 former assigned M31:
M656PH031 is a metabolite of dimethenamid-P determined in maize and soybean metabolism studies but not observed in edible commodities as well as in soil, surface water and groundwater.



Structural alerts for M656PH031:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times (molecule 6) predicted M656PH031 to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure. For this alpha-beta unsaturated carbonyl (transformation predicted by 2 different pathways) was an alert given for induction of chromosomal aberration by interaction with topomerases / proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (14 in total) gave no alert for chromosomal aberration.
In the DEREK analysis conducted structural alerts for M656PH031 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction in both modules (CAESAR and SarPy) was non-mutagenic, however the reliability of this prediction was low and thus not taken into further consideration.
In conclusion there was a limited alert for chromosomal aberration *in vitro* with metabolic activation in one of the structure activity evaluation tools employed considered of no relevance as not confirmed by the genotoxicity testing conducted.

Genotoxicity of M656PH031:
M656H031 was used for toxicological testing and considered toxicologically equivalent to M656PH031.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Schulz M., Landsiedel R., 2008a (ASB2010-6897) Reg.No. 360 712 (metabolite M31 of BAS 656 H) - Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and pre-incubation test) 2008/1064992
Guideline(s):	OECD 471, EEC 2000/32 B.13/B.14, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656H031 (Reg.No. 360 712, using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments.

Test Material	Reg.No. 360 712 (Metabolite M31 of BAS 656 H)
Description:	Solid, white
Lot/Batch #:	L81-46
Purity:	98.7 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed as indicated by the sponsor and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control:	The vehicle control with and without S9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537, *E. coli*: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

Test concentrations:

Plate incorporation assay: Triplicate plates were prepared for each concentration (neg. control;

20, 100, 500, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: The test substance/vehicle/positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 312.5, 625, 1250, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 23-Jul-2008 to 07-Aug-2008.

Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel Bonner agar plates (minimal glucose agar plates). In the experiments with *E. coli* the Vogel Bonner agar plates were replaced by plates containing a SA1 selective agar according to Green and Muriel.

Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

Statistics:

No special statistical tests were performed.

Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

A dose related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

Results:

Analytical determinations:

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

Toxicity:

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (slight decrease in the number of his+ or trp+ revertants, reduction in the titer) was occasionally observed in the standard plate test depending on the strain from about 2500 µg/plate onward. In the pre-incubation assay bacteriotoxicity (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants, reduction in the titer) was observed from about 2500 µg/plate onward.

Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-42).
Precipitation was not observed up to the maximum concentration.

Table B.6.8-42: Bacterial gene mutation assay with M656H031 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	35	25	107	99	14	16	9	8	42	33
M656H031										
20 µg/plate	34	23	94	94	13	15	9	7	39	31
100 µg/plate	31	23	95	92	16	13	10	7	44	33
500 µg/plate	30	26	98	100	13	15	7	6	36	39
2500 µg/plate	23	21	84	94	12	13	6	5	32	32
5000 µg/plate	17	10	67	62	11	12	4	3	18	22
Pos. control[§]	578	490	640	671	183	620	126	507	283	777
Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	29	25	102	101	15	14	8	8	34	29
M656H031										
312.5 µg/plate	32	29	96	93	15	13	8	6	39	35
625 µg/plate	34	24	93	108	14	13	7	7	33	30
1250 µg/plate	31	25	100	94	14	14	6	5	55	32
2500 µg/plate	16	17	71	99	5	15	5	7	33	34
5000 µg/plate	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]
Pos. control[§]	560	481	806	608	151	689	143	142	219	581

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
[B] = reduced background growth

Conclusion:

The study is considered to be acceptable.
According to the results of the present study, the test substance M656H031 was not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point: KCA 5.8

Report: [redacted] 2008b (ASB2008-7224)
Reg.No. 360 712 (metabolite M31 of BAS 656 H) - *In vitro* gene mutation test in CHO cells (HPRT locus assay)
2008/1051510

Guideline(s): OECD 476, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH, EPA 870.5300

Deviations: No relevant deviations

GLP: Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Acceptability: The study is considered to acceptable.

Materials and methods:

M656H031 (Reg.No. 360712) was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation.

Test Material	Reg. No. 360712 (Metabolite M31 of BAS 656 H)
Description:	Solid, white
Lot/Batch #:	L81-46
Purity:	98.7 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Jul 2018 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions. The stability of the test substance at room temperature in the vehicle DMSO and in aqueous formulations was determined analytically.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	A negative control was not employed in this study
Solvent control:	1 % (v/v) DMSO in culture medium
Positive control -S9:	Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9:	Methylcholantrene (MCA) 10 µg/mL
Activation:	S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organism:	Chinese hamster CHO cells (sub-strain K3). Stocks of the CHO cell line were maintained at -196 °C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).
Culture media:	
Culture medium:	Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10 % (v/v) foetal calf serum (FCS).
Pretreatment medium:	("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.
Selection medium:	("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL
All media were supplemented with:	- 1 % (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL) - 1 % (v/v) amphotericin B (250 µg/mL) During pulse exposure (4 hours) to the test substance, Ham‘s F12 medium was used without FCS supplementation. In the case of continuous treatment (24 hours) Ham‘s F12 medium with FCS supplementation was used.
Locus examined:	hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)
Test concentrations:	
a) Preliminary toxicity assay:	Nine concentrations ranging from 13.7 to 3500 µg/mL
b) Mutation assay:	
1st experiment:	218.8, 437.5, 875, 1750 and 3500 µg/mL with and without metabolic activation
2nd experiment:	218.8, 437.5, 875, 1750 and 3500 µg/mL without metabolic activation 250, 500, 1000, 2000 and 3500 µg/mL with metabolic activation
Dates of experimental work:	17-Jul-2008 to 08-Sep-2008.

Preliminary cytotoxicity assay:
Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with 9 test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) or 24 hours (only without metabolic activation) after an attachment period of 20 - 24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 - 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).
Mutation Assay:
Pretreatment of Cells:
Cells with a passage number ≥2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.
Cell treatment:
For each test group, about 1 x 10^6 cells per flask were seeded into 175 cm² flasks containing about 20 mL Ham's F12 medium supplemented with 10 % FCS and incubated for about 20 - 24 hours with 5 % CO_2 at 37 °C and >90 % humidity for cell attachment. 2 flasks were used for each test group.
After the cell attachment period the medium was replaced by the treatment medium. In case of experiments without metabolic activation the treatment medium consisted of 18 mL Ham's F12 medium without FCS plus 2 mL positive control or test substance. For the vehicle control group 20 mL of the treatment medium was supplemented with 0.2 mL of the vehicle. In case of metabolic

activation the treatment medium consisted of 14 mL Ham's F12 medium without FCS, 2 mL positive control or test substance preparation and 4 mL S9-mix. Analogously, for the vehicle control group 16 mL of medium was supplemented with 0.2 mL vehicle and 4 mL S9-mix. For the exposure period of more than 4 hours Hams´s F12 medium with 10 % FCS was used.
Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5 % CO_2, 37 °C and ≥90 % humidity.
Expression:
After incubation for 4 or 24 hours, respectively, the treatment medium was replaced by at least 20 mL Ham's F12 medium with 10 % FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of about 3 days following the 4 hour exposure or 2 days following the 24 hour exposure period. After an entire expression period of 7 - 9 days the cells were transferred into the selection medium (2nd passage).
Selection:
For the mutant selection, six 75-cm^2 flasks each were seeded with 3 x 10^5 cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.
Determination of Cytotoxicity:
Cloning efficiency 1 (survival):
The survival (cloning efficiency 1; CE1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10 % FCS. After a 20 - 24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 or 24 hours as described above. At the end of the treatment period the cells were washed with HBSS and the treatment medium was replaced by Ham's F12 medium with 10 % FCS. After a further incubation for about 5 to 8 days the colonies were fixed, stained and counted.
Cloning efficiency 2 (viability):
The viability (cloning efficiency 2; CE_2) was determined in parallel to the selection of mutants after the expression period under the same conditions as described for cloning efficiency 1.
Calculations:
Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{total\ number\ of\ mutant\ colonies}{number\ of\ seeded\ cells}\ x\ 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\ absolute}}\ x\ 100$$

Cloning efficiency (CE, %) absolute:

$$CE_{absolute} = \frac{total\ number\ of\ colonies\ in\ the\ test\ group}{total\ number\ of\ seeded\ cells\ in\ the\ test\ group}\ x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE\ of\ the\ dose\ group}{CE\ of\ the\ vehicle\ control}\ x\ 100$$

Statistics:
Due to the negative findings, a statistical evaluation was not carried out.
Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies (MFcorr.) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.
A test substance is generally considered negative in this test system if:

- The corrected mutation frequency (MFcorr.) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

Results:

Analytical determinations:
The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor. The stability of the test substance at room temperature in the vehicle DMSO and in water for 4 hours was verified analytically.
Preliminary cytotoxicity assay:
In the preliminary experiment in the presence and absence of metabolic activation a significant test substance induced cytotoxicity leading to a reduction of relative cloning efficiency below 20 % was not observed. The relative cloning efficiency ($CE_{1\ relative}$) after treatment with the highest concentration (3500 µg/mL; approx. 10 mM) ranged between 88.9 to 106.5 % depending on the treatment interval and metabolic activation.
Precipitation of the test substance was not observed up to the highest tested concentration with and without metabolic activation. No marked effect on osmolarity was observed. The pH of the stock solution was adjusted prior to application to physiological levels using 2N NaOH.
Based on these data the highest concentration tested in the mutagenicity experiments was 3500 µg/mL without and with metabolic activation.
Mutagenicity assays:
A significant cytotoxic effect was not observed in both experiments up to the highest tested concentration irrespective of treatment interval and presence of metabolic activation. The obtained relative cloning efficiency did not drop below 86.3 % under any of the tested conditions.
A relevant increase in the number of mutant colonies was not observed in the original and confirmatory experiments with and without metabolic activation (see Table B.6.8-43, Table B.6.8-44).
The mutant frequencies obtained at any tested concentration with or without metabolic activation were within the range of the concurrent vehicle control as well as within the range of the historical negative control data.
The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. Test substance precipitation did not occur up the highest tested concentration of 3500 µg/mL.
Treatment with the positive controls EMS and MCA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Table B.6.8-43: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE1 (survival) (4 h after treatment; approx. 200 cells/flask seeded		CE2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	11	3.06	3.80	85.3	100.0	81.6	100.0
M656H031							
218.8 µg/mL	5	1.39	1.73	75.7	88.7	80.9	99.1
437.5 µg/mL	1	0.28	0.34	76.4	89.6	80.3	98.4
875.0 µg/mL	3	0.84	1.11	77.0	90.3	75.7	92.8
1750.0 µg/mL	1	0.28	0.35	80.2	94.0	78.9	96.7
3500.0 µg/mL	3	0.84	1.02	81.7	95.8	81.8	100.2
Positive control EMS							
300.0 µg/mL	325	90.28	137.00	77.7	91.1	65.7	80.5
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	6	1.67	1.98	85.1	100.0	84.3	100.0
M656H031							
218.8 µg/mL	4	1.11	1.37	83.3	97.9	82.2	97.5
437.5 µg/mL	3	0.84	1.03	83.3	97.9	78.0	92.5
875.0 µg/mL	5	1.39	1.84	81.9	96.2	77.8	92.3
1750.0 µg/mL	2	0.56	0.74	80.2	94.2	76.4	90.6
3500.0 µg/mL	1	0.28	0.36	84.0	98.7	78.8	93.5
Positive control MCA							
10.0 µg/mL	228	63.34	107.83	74.0	87.0	58.8	69.8

a number of colonies approx 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
b correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period

Table B.6.8-44: Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE1 (survival) (4 h after treatment; approx. 200 cells/flask seeded		CE2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 24-hour exposure period							
Vehicle (DMSO)	7	1.95	2.60	81.9	100.0	75.1	100.0
M656H031							
218.8 µg/mL	8	2.23	3.13	76.3	93.2	72.3	96.3
437.5 µg/mL	0	0.00	0.00	73.8	90.1	75.1	100.0
875.0 µg/mL	6	1.67	2.39	72.2	88.2	70.6	94.0
1750.0 µg/mL	5	1.39	1.99	73.4	89.6	73.5	97.9
3500.0 µg/mL	7	1.95	2.65	70.7	86.3	72.8	96.9
Positive control EMS							
300.0 µg/mL	529	146.95	245.20	59.5	72.6	60.1	80.0
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	7	1.95	2.50	83.4	100.0	75.5	100.0
M656H031							
250 µg/mL	0	0.00	0.00	75.3	90.3	83.3	110.3
500 µg/mL	2	0.56	0.78	79.7	95.6	72.3	95.8
1000 µg/mL	5	1.39	1.75	78.0	93.5	73.2	97.0
2000 µg/mL	1	0.28	0.34	76.7	92.0	75.2	99.6
3500 µg/mL	3	0.84	0.99	75.3	90.3	82.8	109.7
Positive control MCA							
10.0 µg/mL	283	78.61	118.42	62.9	75.4	67.9	89.9

a number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
b correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test, M656H031 did not induce forward mutations in the HPRT locus in CHO cells *in vitro*.

Data point: KCA 5.8

Report: [redacted] 2008c (ASB2008-7223)
Reg.No. 360 712 (metabolite M31 of BAS 656 H) - *In vitro* chromosome aberration assay in V79 cells
2008/1063692

Guideline(s):	OECD 473, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH, EPA 870.5375
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.8
Report:	[redacted] 2008a (ASB2014-8430) Amendment No. 1 to the report: Reg.No. 360 712 (metabolite M31 of BAS 656 H) - *In vitro* chromosome aberration assay in V79 cells 2008/1070759
Guideline(s):	OECD 473, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH, EPA 870.5375
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656H031 (Reg.No. 360 712) was tested *in vitro* for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in two independent experiments in the presence and absence of metabolic activation.

Test Material:	Reg.No. 360712 (Metabolite M31 of BAS 656 H)
Description:	Solid, white
Lot/Batch #:	L81-46
Purity:	98.7 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Jul 2018 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions. The stability of the test substance dissolved in the vehicle DMSO and in aqueous solution over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	A negative control was not employed in this study
Solvent control:DMSO	
Positive control, -S9:	Ethylmethanesulfonate 500 µg/mL
Positive control, +S9:	Cyclophosphamide 0.5 µg/mL
Activation:	S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the

so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: Chinese hamster V79 cells with high proliferation rate (doubling time of about 12 - 16 hours), a high plating efficiency (>90 %) and a stable karyotype (modal number of 22 chromosomes). Stocks of the V79 cell line were maintained at -196 °C in liquid nitrogen. Each batch used for the cytogenetic experiments was checked for mycoplasma contamination, karyotype stability and plating efficiency incl. vital staining.

Culture medium: MEM medium with glutamine supplemented with
- 10 % (v/v) foetal calf serum (FCS)
- 1 % (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1 % (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance (4-hour treatment), MEM medium was used without FCS supplementation. For exposure periods of more than 4 hours MEM medium with 10 % (v/v) FCS was used.

Test concentrations:

a) Preliminary toxicity assay: 218.8, 437.5, 875.0, 1750.0 and 3500.0 µg/mL with and without metabolic activation (4- and 18-hour exposure, (18 h preparation interval)

Mutation assay:

1st experiment: 218.8, 437.5, 875.0, 1750.0 and 3500.0 µg/mL with and without metabolic activation

2nd experiment: 218.8, 437.5, 875.0, 1750.0 and 3500.0 µg/mL without metabolic activation (18 h preparation interval) as well as with metabolic activation (28 h preparation interval)
875, 1750 and 3500 µg/mL without metabolic activation (28 h preparation interval)

Dates of experimental work: 21-Jul-2008 to 09-Sep-2008.

Preliminary cytotoxicity assay:

Cytotoxicity was assessed by mitotic index, cell attachment (morphology) and quality of the slides. The cells were prepared at a sampling time of 18 hours (about 1.5-fold cell cycle time) after 4 and 18 hours exposure time without S9 mix and after 4 hours exposure time with S9 mix. The pre-test was performed following the method described for the main experiment. In addition to the mitotic index, the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

Cytogenicity Assay:

Cell treatment: Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours or continuous treatment experiments for 18 hours. The preparation intervals were 18 h and 28 h post treatment begin. Duplicate cultures were run for each dose and condition. The cells were incubated in Quadriperm® dishes at 37 °C, 5 % CO_2 and ≥90 % humidity. Two chambers of a Quadriperm dish were used for each concentration.

For determination of cytotoxicity, additional cell cultures (using 25 cm² plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.

Spindle inhibition: 100 µL colcemid was added to each chamber 2-3 hours prior to harvesting.

Cell harvest: At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4 % KCl solution (37 °C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.

Slide preparation: The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.

Metaphase analysis: Slides were coded prior to analysis. As a rule, the first 100 consecutive well spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analysed for structural chromosome aberrations. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analysed is reduced from the planned 200 mitoses/test group.
A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

Statistics:
The proportion of metaphases with aberrations was calculated for each group.
A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.
Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- A statistically significant, dose-related, and reproducible increase in the number of structural chromosomal aberrations (excl. gaps).
- The proportion of aberrations (excl. gaps) exceeded both the concurrent negative control range and the historical negative control range.

A test substance is generally considered negative in this test system if:

- The number of cells with structural aberrations (excl. gaps) in the dose groups is not statistically significant increased above the concurrent negative/vehicle control value and is within the historical negative control data range.

Results:

Analytical determinations:
The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor. The stability of the test substance at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.
Preliminary cytotoxicity assay:
In the preliminary experiment in the presence and absence of metabolic activation a significant test substance induced cytotoxicity leading to a reduction of at least one of the toxicity parameters to more than 50 % of the respective control was not observed. The overall lowest mitotic index observed after treatment with the highest concentration (3500 µg/mL; approx. 10 mM) regardless any test condition was 78.8 %.
Precipitation of the test substance was not observed up to the highest tested concentration with and without metabolic activation. No marked effect on osmolarity was observed. The pH of the stock

solution was adjusted prior to application to physiological levels using 2N NaOH.
Based on these data the highest concentration tested in the mutagenicity experiments was 3500 µg/mL without and with metabolic activation.
Cytogenicity assays:
In this study treated cells could be evaluated up to the highest used test substance concentration (3500 µg/mL). Cytotoxicity (mitotic index of 36.3 %) as described by a drop in the relative mitotic index below 50 % was observed in the 2nd Experiment after 18 hours treatment at 28 hours preparation interval in the absence of S9 mix at 3500 µg/mL only.
A relevant increase in cells with structural chromosomal aberrations was not observed in any of the performed experiments neither with nor without metabolic activation. For all tested treatment or preparation intervals the number of aberrant cells was within the historical control range and close to the value obtained for the concurrent control group. In the 1st Experiment after 4 hours treatment in the absence of S9 mix a dose related increase of structural chromosome aberrations was observed (1 - 2.5 %). However, the values were clearly within the historical negative control data range (0.0 - 5.5 % aberrant metaphase cells, exclusive gaps) and, therefore, this observation was regarded as biologically irrelevant (see Table B.6.8-45, Table B.6.8-46). The frequency of cells containing numerical aberrations was not affected by treatment with the test substance.
The osmolarity of the incubations were not altered by the addition of the test substance. The pH was adjusted to physiological conditions prior to treatment. Precipitation of the test substance did not occur up to the highest tested concentration.
Vehicle and positive controls were all in a range to ensure the validity of the test.

Table B.6.8-45: Chromosome aberration test with M656H031 – without metabolic activation

Exp.	Exposure/ Preparation period	Test groups	S9 mix	P	Genotoxicity				Cytotoxicity*	
					Aberrant cells [%]				Cell number [%]	Mitotic index [%]
					incl. gaps#	excl. gaps#	with exchanges	Polyploid cells [%]		
1	4/18 h	Vehicle control[1]	-	-	2.5	1.0	0.5	0.0	100.0	100.0
		M656H031								
		218.8 µg/mL	-	-	-	-	-	-	103.2	-
		437.5 µg/mL	-	-	-	-	-	-	101.2	-
		875.0 µg/mL	-	-	3.0	1.0	1.0	0.0	105.2	76.7
		1750.0 µg/mL	-	-	2.5	1.5	1.5	1.5	96.0	81.9
		3500.0 µg/mL	-	-	3.0	2.5	2.0	0.0	91.6	91.7
		Positive control[2]	-	-	23.0S	21.0[S]	9.0[S]	0.0	n.t.	122.3
2	18/18 h	Vehicle control[1]	-	-	5.0	2.0	0.5	2.4	100.0	100.0
		M656H031								
		218.8 µg/mL	-	-	7.0	3.0	1.0	3.4	100.6	113.7
		437.5 µg/mL	-	-	5.0	2.0	0.0	3.8	100.0	101.6
		875.0 µg/mL	-	-	7.0	2.0	1.0	2.9	98.8	96.2
		1750.0 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	91.9	-
		3500.0 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	79.7	-
		Positive control[2]	-	-	37.0[S]	33.0[S]	19.0[S]	0.0	n.t.	96.7
2	18/28 h	Vehicle control[1]	-	-	3.0	2.0	0.5	1.0	100.0	100.0
		M656H031								
		875.0 µg/mL	-	-	-	-	-	-	90.8	-
		1750.0 µg/mL	-	-	-	-	-	-	101.8	79.3
		3500.0 µg/mL	-	-	3.5	2.0	1.5	0.0	73.8	36.3
		Positive control[2]	-	-	24.0[S]	24.0[S]	19.0[S]	0.0	n.t.	65.8

P: Precipitation determined at the end of exposure period
*: Relative values compared with the respective vehicle control
#: Inclusive cells carrying exchanges
n.t.: Not tested
n.s.: Not scorable due to low metaphase numbers and/or poor metaphase quality
-: Not scored
S: Aberration frequency statistically significant higher than corresponding control values
1: DMSO 1 % (v/v)
2: EMS 500 µg/mL

Table B.6.8-46: Chromosome aberration test with M656H031 – with metabolic activation

Exp.	Exposure/ Preparation period	Test groups	S9 mix	P	Genotoxicity				Cytotoxicity*	
					Aberrant cells [%]				Cell number [%]	Mitotic index [%]
					incl. gaps#	excl. gaps#	with exchanges	Polyploid cells [%]		
1	4/18 h	Vehicle control[1]	+	-	3.5	1.5	1.0	0.0	100.0	100.0
		M656H031								
		218.8 µg/mL	+	-	-	-	-	-	96.6	-
		437.5 µg/mL	+	-	-	-	-	-	97.7	-
		875.0 µg/mL	+	-	2.0	1.5	1.0	0.0	111.8	108.5
		1750.0 µg/mL	+	-	3.5	1.5	0.5	0.0	101.9	107.6
		3500.0 µg/mL	+	-	4.5	1.5	1.5	0.5	82.1	100.0
		Positive control[2]	+	-	20.0[S]	18.0[S]	11.0[S]	0.0	n.t.	89.6
2	18/18 h	Vehicle control[1]	+	-	3.5	2.5	2.0	1.0	100.0	100.0
		M656H031								
		218.8 µg/mL	+	-	-	-	-	-	102.5	-
		437.5 µg/mL	+	-	-	-	-	-	93.4	-
		875.0 µg/mL	+	-	4.0	2.5	1.0	0.5	91.0	142.5
		1750.0 µg/mL	+	-	5.0	3.5	2.5	0.0	91.5	101.4
		3500.0 µg/mL	+	-	3.0	2.0	2.0	0.0	99.2	128.8
		Positive control[2]	+	-	11.0[S]	11.0[S]	6.0[S]	1.0	n.t.	125.6

P: Precipitation determined at the end of exposure period
*: Relative values compared with the respective vehicle control
#: Inclusive cells carrying exchanges
n.t.: Not tested
-: Not scored
S: Aberration frequency statistically significant higher than corresponding control values
1: DMSO 1 % (v/v)
2: CPP 0.5 µg/mL

Conclusion:

The study is considered to be acceptable.
Based on the results of the study, M656H031 did not exhibit clastogenic potential *in vitro* in the presence or absence of metabolic activation.

Short-term toxicity of M656PH031:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point: KCA 5.8

Report: [redacted] 2013c (ASB2014-8417)
Reg.No. 360712 (metabolite of BAS 656 H) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet
2013/1042165

Guideline(s):	OECD 407, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Reg.No. 360712 (Metabolite of BAS 656 H)
Description:	Solid/white
Batch/purity #:	L81-132, 100.0 %
Stability of test compound:	Stable until 01 Jul 2019. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.
Vehicle and/or positive control:	Rodent diet
Test animals:	
Species:	Rat
Strain:	Wistar Crl:WI (Han), Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 166.5 ± 9.5 g, ♀ 132.4 ± 7.9 g
Source:	Charles River, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, *ad libitum*
Housing:	Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment Motor activity measurements were conducted in polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes per hour
Photo period:	12 h light/12 h dark (06:00-18:00/18:00-06:00)
Dates of experimental work:	28-Jun-2012 - 29-Jan-2013. (In life dates: 28-Jun-2012 (start of administration) to 27-Jul-2012 (necropsy)).

Animal assignment and treatment:
M656H031 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomisation list based on body weights.
Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. One diet preparation per dose was performed for this study.

Analyses performed prior to the start of the administration period revealed that the test substance was stable in the diet for at least 32 days.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analysed. The samples were also used for determination of the test article concentration. For the mid dose level single samples were analysed. No test article was determined in control diets.

Table B.6.8-47: Analysis of diet preparations for homogeneity and test item content

Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
1200 ppm	28.06.12	1157.3 ± 19.7[#]	96.4	1.7
4000 ppm	28.06.12	3967.5	99.2	n.a.
12000 ppm	28.06.12	11882.9 ± 468.5[#]	99.0	4.0

n.a.: not applicable;
[#] based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity samples in the range of 1.7 to 4 % indicate the homogenous distribution of M656H031 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 96.4 to 99.2 % of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H031 in the vehicle.
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-48: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians.

Table B.6.8-49: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except reticulocytes and differential blood count, urine pH, urine volume, urine specific gravity	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians. For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urinalysis, except volume, colour, turbidity and specific gravity	Pair wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Table B.6.8-50: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behaviour during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. faeces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Individual food consumption was determined once weekly and calculated as mean food consumption in grams per animal and day.
Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$Food\ efficiency\ at\ day\ x = \frac{BW_x - BW_y}{FC_{y\ to\ x}}\ x\,100$$

BW_x and BW_y body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).

Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted
Ophthalmoscopy:
Not performed in this study.
Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1.	behaviour when removed from cage	10.	respiration
2.	fur	11.	tremors
3.	skin	12.	convulsions
4.	salivation	13.	abnormal movements
5.	nose discharge	14.	impairment of gait
6.	lacrimation	15.	activity/arousal level
7.	eyes/pupil size	16.	faeces (number of faecal pellets/appearance/ consistency) within two minutes
8.	posture	17.	urine (appearance/quantity) within two minutes
9.	palpebral closure	18.	number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behaviour during "handling"
2.	touch response	9.	vocalisation
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

Motor activity measurement:
Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

Haematology and clinical chemistry:
Blood was withdrawn in the morning from fasted, isoflurane anesthetised animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomised sequence.
The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
	Red blood cells		White blood cells		Clotting Potential
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Haemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Haematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
Clinical chemistry:					
	Electrolytes		Metabolites and Proteins		Enzymes:
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Urinalysis:
For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analysed in a randomised order.
The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		Semi quantitative parameters		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

Sacrifice and pathology:
The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	#	kidneys	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)@
✓		#	bone marrow§	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands	✓		#	lymph nodes#	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides¥	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity‡	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetised animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles				

§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; ‡l histopathology at level III, ¥left epididymidis collected for histopathology

The organs or tissues were fixed in 4 % formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.
The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:
• Increased/decreased grade of cortico-medullar ratio (related only to area)
• Increase of starry sky cells
• Changes of cellular density in the cortex
• Changes of cellular density in the medulla

Spleen:
• Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
• Altered cellular composition of follicles
• Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):
• Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
• Altered cellular composition of paracortex
• Altered number of germinal centers
• Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):
• Changes of the cellularity of follicles (including mantle zone and germinal centers)
• Changes of the cellularity of interfollicular area
Bone marrow:
• Changes of the cellularity
• Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

Results:

Observations:
No clinical signs were observed throughout the study.
No mortality was observed throughout the study.
Ophthalmoscopy was not performed in this study.
FOB and Motor Activity
Neither home cage nor open field observations revealed any indication of treatment related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
Comparing the single intervals with the control groups, no significant deviations were measured with the exception of a decreased value at interval 1 in males of test group 3 (12000 ppm). As a consequence, the overall motor activity measurement in male animals of test group 3 (12000 ppm) was significantly decreased. As no FOB parameter was changed and no findings occurred during the detailed clinical observation, the change was assessed as being spontaneous and not related to treatment. No statistically significant differences of overall motor activity between control and treated animals were observed for males of the other test groups (1200 and 4000 ppm) as well as for the female animals of all test groups.
Body weight and body weight gain:
No statistically significant differences of absolute body weights or body weight gain were noted (see Table B.6.8-51 and Figure B.6.8-17).

Table B.6.8-51: Mean body weight of rats administered M656H031 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1200	4000	12000	0	1200	4000	12000
Body weight [g]								
- Day 0	168.4	171.4	167.9	167.2	131.7	133.6	131.6	130.0
- Day 28	297.4	194.0	281.5	283.6	188.2	192.8	189.8	195.4
Δ% (compared to control)#		-1.1	-5.3	-4.6		2.4	0.9	3.8
Overall body weight gain [g]	129	122.6	113.7	116.5	56.5	59.2	58.2	65.4
Δ% (compared to control)#		-4.9	-11.9	-9.7		4.8	3.1	15.8

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

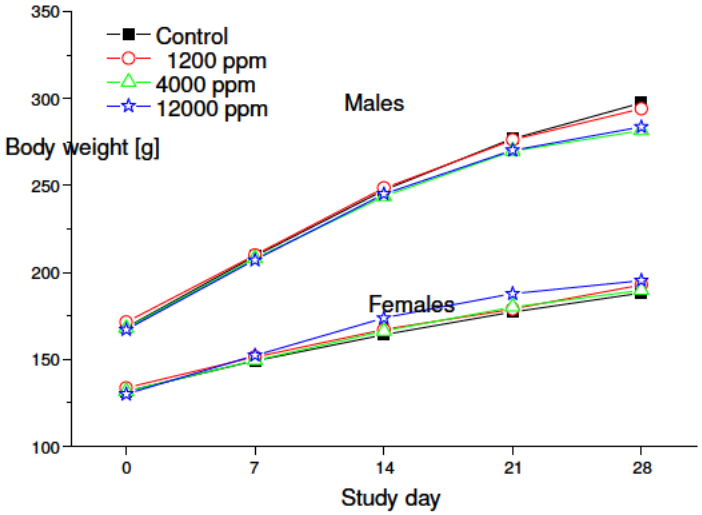


Figure B.6.8-17: Body weight development of rats administered M656H031 for at least 28 days

Food consumption and compound intake:
No test substance related, adverse findings were observed. All recorded values were within the biological range typical for this strain of rats.
The mean daily test substance intake over the entire study period was calculated and is shown in the following table:

Table B.6.8-52: Calculated intake of M656H031

Test group	Concentration in the vehicle (ppm)	Mean daily test substance intake (mg/kg bw/d)	
		Males	Females
1	1200	108	111
2	4000	342	352
3	12000	1068	1140

Water consumption:
No test substance related, adverse changes with regard to water consumption were observed.

Blood analysis:
Haematological findings:
No treatment related changes among haematological parameters were observed.
In female animals of test group 1 (1200 ppm), relative basophil counts were lower compared to controls (see Table B.6.8-53). However, the alteration was not dose dependent and, therefore, this change was regarded as incidental and not treatment related.

Table B.6.8-53: Selected haematology findings in rats administered M656H031 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		108	342	1068		111	352	1140
Basophilic leucocytes [giga/L]	0.02	0.03	0.03	0.03	0.02	0.01	0.02	0.02
[%]	0.4	0.4	0.5	0.4	0.5	0.2*	0.5	0.5

*p ≤ 0.05 (Kruskal-Wallis and Wilcoxon-test, two sided)

Clinical chemistry findings:
No treatment related changes among clinical chemistry parameters were observed.
Urinalysis:
No treatment related changes among urinalysis parameters were observed.
Necropsy:
Organ weight:
Terminal body weights of treated rats displayed no statistically significant differences to the controls (see Table B.6.8-54).
When compared to the control group 0, the mean absolute organ weights of treated animals showed no significant deviations. A non-significant decrease of -47 % was recorded for the mean uterus weight of females of test group 3 (12000 ppm).
In relation to terminal body weight the liver weights in male animals were significantly increased.

Table B.6.8-54: Selected mean absolute and relative organ weights of rats administered M656H031 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		108	342	1068		111	352	1140
Terminal body weight [g]	268.74	266.96	257.64	256.44	171.58	175.94	170.64	179.94
[% of control]	100	99	96	95	100	103	99	105
Uterus weight, absolute [g]					0.712	0.604	0.588	0.380
[% of control]					100	85	83	53
Uterus weight, relative [%]					0.414	0.344	0.346	0.212
[% of control]					100	83	84	51
Liver weight, absolute [g]	7.210	7.120	7.132	7.556	4.840	4.808	4.688	4.825
[% of control]	100	99	99	105	100	99	97	100
Liver weight, relative [%]	2.681	2.671	2.761	2.946*	2.822	2.735	2.746	2.680
[% of control]	100	100	103	110	100	97	97	95

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

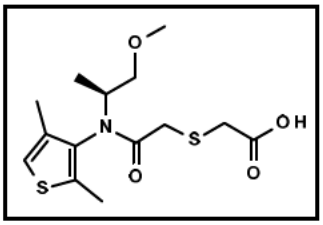
No other statistically significant changes of absolute or relative organ weights were observed.

Gross and histopathology
A single macroscopic finding (pelvic dilation of the kidney) recorded in males of test group 3 (12000 ppm) belongs to the spectrum of background lesion and was considered to be incidental in nature and not related to treatment.
The difference in uterus weights was consistent with the different sexual cycle state observed histologically in each test group. Thus the uterus weight decrease in females of test group 3 (12000 ppm) (see Table B.6.8-54) reflects a physiologic uterus state and not a treatment related effect.
No histopathological correlate was found for the liver weight increase. Furthermore, this increase was of low magnitude and weak statistical significance. Therefore, the relative liver weight increase in males of test group 3 (12000 ppm) was considered to be incidental and not related to treatment.
All other histopathological findings were either single observations, were equally distributed between control and treated groups or displayed no dose-response relationship. Therefore, these findings were considered to be incidental.

Conclusion:

The study is considered to be acceptable.
The administration of M656H031 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1068 mg/kg bw/d) and in female (1140 mg/kg bw/d) Wistar rats.

Metabolite M656PH032 formerly assigned M32:
M656PH032 is a metabolite of dimethenamid-P determined in hen and groundwater. In the hen metabolism study M656H032 was observed at 1.05 mg/kg in excreta. M656H032 was also observed in an *in vitro* metabolism study on liver and kidney.



Structural alerts for M656PH032:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times predicted M656PH032 (see molecule 7 of report ASB2014-8408) to be not mutagenic in the Ames test without or with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (see molecule 7 of report ASB2014-8410) the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure similar to transformation products of M656PH030 and M656PH031. For this alpha-beta unsaturated carbonyl was an alert given for induction of chromosomal aberration by interaction with topomerases/proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (13 in total) gave no alert for chromosomal aberration. Formation of M656PH032 as a metabolite of M656PH031 is predicted by OASIS times (ASB2014-8410).
In the DEREK analysis conducted structural alerts for M656PH032 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (Molecule 12) in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low. In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.
In conclusion in one of the structure activity evaluation tools employed there was a limited alert for

chromosomal aberration *in vitro* with metabolic activation considered of low relevance for the *in vivo* situation.

Genotoxicity studies of M656PH032:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Woitkowiak C., 2013a (ASB2014-8452) Reg.No. 395234 (metabolite of BAS 656 H, dimethenamid) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2013/1113379
Guideline(s):	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
Deviations:	No deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656H032 (Reg.No. 395234) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent plate incorporation assays and a third pre-incubation experiment.

Test Material	Reg.No. 395234 (Metabolite of BAS 656 H, dimethenamid)
Description:	Liquid; brown, clear
Lot/Batch#:	RS-TGA-100396
Purity:	92.1 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2014 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control:	The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each

experiment via the spontaneous rate.

Test concentrations:

Plate incorporation assay:

(1st and 2nd experiment): Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2800 and 5600 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above. In addition, a 2nd experiment with TA1537 was performed in the absence and presence of metabolic activation due to inconclusive bacteriotoxic values observed in the 1st approach.

Pre-incubation assay:

(3rd experiment): The test substance/vehicle/positive control substance, bacterial and S9-mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2800 and 5600 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 05-Mar-2013 to 22-Mar-2013.

Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates).

Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

Statistics:

No special statistical tests were performed.

Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

A dose related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

Results:

Analytical determinations:

The test substance was stable over the study period under the storage conditions.

The stability of the test substance in the vehicle dimethyl sulfoxide (DMSO) was verified analytically.

Toxicity:

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (reduced his- or trp-background growth, slight decrease in the number of his+ or trp+ revertants, slight reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 2800 µg/plate onward. In the pre-incubation assay bacteriotoxicity (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 333 µg/plate onward.
Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-55).
Precipitation was not observed up to the maximum concentration.

Table B.6.8-55: Bacterial gene mutation assay with M656H032 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		*E. coli*	
1st experiment: Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	28	15	51	39	11	9	11	9	93	85
M656H032										
33 µg/plate	26	18	60	42	17	7	8	5	88	77
100 µg/plate	30	19	56	43	8	8	10	6	81	87
333 µg/plate	30	17	57	45	12	8	8	4	93	80
1000 µg/plate	24	18	52	36	13	10	10	4	88	92
2800 µg/plate	22	15	41	44	9	10	5	5	87	88
5600 µg/plate	13	17	34	27	12	10	3	3	46	50
Pos. control§	1291	837	1464	923	411	1071	224	545	372	740
2nd experiment: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	-	-	-	-	-	-	8	7	-	-
M656H032										
33 µg/plate	-	-	-	-	-	-	9	5	-	-
100 µg/plate	-	-	-	-	-	-	8	6	-	-
333 µg/plate	-	-	-	-	-	-	8	8	-	-
1000 µg/plate	-	-	-	-	-	-	5	7	-	-
2800 µg/plate	-	-	-	-	-	-	8	3	-	-
5600 µg/plate	-	-	-	-	-	-	6	3	-	-
Pos. control§	-	-	-	-	-	-	514	627	-	-
3rd experiment: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	24	16	39	31	10	9	11	8	65	68
M656H032										
33 µg/plate	24	14	36	30	10	9	11	7	66	66
100 µg/plate	20	15	37	29	9	9	10	7	64	69

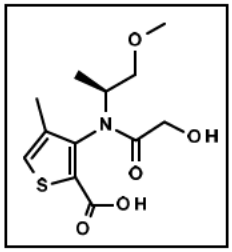
Strain	TA 98		TA 100		TA 1535		TA 1537		*E. coli*	
333 µg/plate	24	16	38	22	11	10	8	6	60	73
1000 µg/plate	17	10	26	21	10	6	5	7	61	65
2800 µg/plate	17	15	35	24	8	5	7	3	67	68
5600 µg/plate	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]
Pos. control[§]	983	1148	806	883	323	731	218	554	407	870
[§] = Compound and concentrations see Material and Methods (I.A.2.) above										

[B]= reduced background growth

Conclusion:

The study is considered to be acceptable.
According to the results of the present study, the test substance M656H032 was not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Metabolite M656PH043 former assigned M43/M44:
M656PH043 is a metabolite of dimethenamid-P predicted in groundwater.



Structural alerts for M656PH043:
In the OECD-toolbox no alerts for DNA-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. For protein-binding according to the OASIS module a nucleophilic addition to carbon-hetero double-bonds was predicted instead of nucleophilic substitution, while in the OECD module for both structures the prediction was to act via direct acylation to formation of actetates. No prediction on protein binding potency was possible for M656PH043, while dimethenamid-P was predicted to be moderately reactive. Thus these limited alerts are not considered of relevance.
OASIS-Times predicted M656PH043 (see ASB2014-8408 and ASB2014-8410) to be not mutagenic in the Ames test and not to induce chromosomal aberration *in vitro* with the limitation that the molecule was out of the prediction domain.
In the DEREK analysis conducted structural alerts for M656PH043 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P.
No alert for genotoxicity was identified in this model. The Vega prediction (Molecule 14) in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low.
In conclusion there was no alert for genotoxicity.

Genotoxicity studies of M656PH043:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point: KCA 5.8

Report: Woitkowiak C., 2014a (ASB2014-8453)

	Reg.No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhy murium/Escherichia coli, reverse mutation assay 2013/1361332
Guideline(s):	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13 No. L 142, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.14 No. L 142, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656PH043 (Reg. No. 5917262, former assigned M43) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and in a pre-incubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

Test Material	Reg.No. 5917262 (Metabolite of BAS 656-PH, dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-113
Purity:	94.6 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Sep 2015 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control:	The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

Test concentrations:

Plate incorporation assay: Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2650 and 5300 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: The test substance/vehicle/positive control substance, bacterial and S9-mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2650 and 5300 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 17-Sep-2013 to 10-Oct-2013

Plate incorporation assay:
To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates).
Pre-incubation assay:
100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9-mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.
Titer determination:
In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.
Statistics:
No special statistical tests were performed.
Evaluation criteria: The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

Results:

Analytical determinations:
The test substance was stable over the study period under the storage conditions.
The stability of the test substance in the vehicle dimethyl sulfoxide (DMSO) was verified analytically (BASF Project No. 01Y0077/13Y011).
Toxicity:
A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
A bacteriotoxic effect (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants, reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 2650 µg/plate onward. In the pre-incubation assay bacteriotoxicity (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants, reduction in the titer) was observed under all test conditions from about 2650 µg/plate onward.
Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-56).
Precipitation was not observed up to the maximum concentration.

Table B.6.8-56: Bacterial gene mutation assay with M656PH043 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	25	19	91	73	11	10	9	5	72	66
M656PH043										
33 µg/plate	26	20	88	70	12	8	9	6	72	65
100 µg/plate	28	18	84	72	11	10	8	5	69	65
333 µg/plate	26	17	84	70	9	9	8	5	71	64
1000 µg/plate	27	20	91	70	11	9	8	5	65	59
2650 µg/plate	17	18	64	74	12	10	7	3	50	59
5300 µg/plate	12	12	60	38	9	7	7	4	36	36
Pos. control[§]	1107	570	1432	1255	245	1417	259	567	510	857
Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	28	19	71	54	11	11	6	6	72	68
M656PH043										
33 µg/plate	28	19	70	55	12	11	5	6	67	71
100 µg/plate	27	20	69	57	11	10	6	6	66	68
333 µg/plate	27	20	73	55	12	10	5	8	73	68
1000 µg/plate	28	19	71	51	10	11	6	6	73	60
2650 µg/plate	11	6	54	0[B]	7	7	2	2	54	22
5300 µg/plate	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]
Pos. control[§]	1182	444	1240	1031	243	1138	363	416	238	1030

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
[B] = reduced background growth

Conclusion:

The study is considered to be acceptable.
According to the results of the present study, the test substance M656PH043 was not mutagenic in the *Salmonella typhimurium* / *Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point:	KCA 5.8
Report:	[redacted] 2013a (ASB2014-8459) Reg.No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P): *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1246089
Guideline(s):	OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656PH043 (Reg.No. 5917262) was tested *in vitro* for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the TK+/- locus. Two independent experiments were conducted in the presence and absence of metabolic activation.

Test Material	Reg. No. 5917262 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-113
Purity:	94.6 % (tolerance ± 1.0 %) for details see Certificate of Analysis ASAP13_121 (Dose selection adjusted to purity considering the preliminary information concerning the purity at the start of the experiment (97.37 %).)
Stability of test compound:	Stable in DMSO
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL
Activation:	S9 was produced from the livers of induced 8 - 12 weeks old male Wistar [Hsd Cpb: WU] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3) parts followed by centrifugation at 9000 g. An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

Test organism: The L5178Y cell line, which is characterised by a high proliferation rate (doubling time 10-12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50 %. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with hypoxanthine (5.0×10^{-3} M), aminopterin (2.0×10^{-5} M), thymidine (1.6×10^{-3} M) and glycin (5.0×10^{-3} M) followed by a recovery period of 2 days in RPMI 1640 medium containing hypoxanthine (1.0×10^{-4} M) and thymidine (1.6×10^{-3} M). After this incubation the cells were returned to complete culture medium (see below).

Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15 % horse serum (24 hour treatment, 3 % HS during 4 hour treatment), 1 % of 100 U/100 µg/mL penicillin/streptomycin, 220 µg/mL sodium-pyruvate, and 0.5 - 0.75 % amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, $Na_2HPO_4 \times 2\ H_2O$ 192 mg, KH_2PO_4 150 mg

Locus examined: Thymidine Kinase Locus (TK+/-)

Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 23.44 to 3000 µg/mL

b) Mutation assay:

1st and 2nd experiment: 93.8, 187.5, 375.0, 750.0, 1500.0, 3000.0 µg/mL with and without metabolic activation

Dates of experimental work: 24-Jul-2013 to 16-Sep-2013.

Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.

1×10^7 cells (3×10^6 cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

Mutation Assay:

Cell treatment and expression: In the mutation experiment 1×10^7 (3×10^6 during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3 % horse serum (15 % horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or

absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3 x 10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.
Selection:
After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4 x 10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5 % CO_2/95.5 % humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.
Size distribution of the colonies:
Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations:
Pre-test:

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control

Main test:

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) x (cell number at 72 h/if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control
relative total growth:	relative suspension growth x relative cloning efficiency/100
cloning efficiency (viability):	ln(mean number of empty wells per plate/96)/cells seeded per well
relative cloning efficiency:	cloning efficiency x 100/cloning efficiency of corresponding control
cells survived:	cloning efficiency x cell number seeded in TFT medium
mutant colonies/10^6 cells:	small mutant colonies + large mutant colonies

threshold:	number of mutant colonies per 10^6 cells of each solvent control plus 126
cloning efficiency (viability):	cloning efficiency determined after the expression period to measure viability of the cells without selective agent

Statistics:
A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

Evaluation criteria:
The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose dependent and correspondingly statistically significant.

A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10 % of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

Results:

Analytical determinations:
Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP13_121).
Preliminary cytotoxicity assay:
The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 23.44 µg/mL and 3000 µg/mL were chosen with regard to the molecular weight (287.3 g/mol) and the purity of the test item (according to the preliminary information concerning the purity of the test item (97.37 %)). No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.
Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. In the pre-experiment and in both main experiments the pH was adjusted at the two highest concentrations using 2N NaOH. There was no relevant shift of the osmolarity at the maximum concentration.

The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3000 µg/mL without and with metabolic activation.
Mutagenicity assays:
Relevant cytotoxic effects indicated by a relative total growth of less than 50 % of survival were observed in the first culture of experiment II at 375.0 µg/mL and above without metabolic activation.
No visible precipitation of the test item in the culture medium was observed.
No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The threshold of 126 above the corresponding solvent control was not reached (see Table B.6.8-57, Table B.6.8-58).
The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies.

Table B.6.8-57: Gene mutation in mammalian cells - 1st experiment

	Conc. µg/mL	**S9 mix**	**relative total growth**	**mutant colonies/10^6 cells**	**threshold**	**relative total growth**	**mutant colonies/10^6 cells**	**threshold**
Experiment I/4 h treatment			Culture I			Culture II		
Solv. Control DMSO		-	100.0	115	241	100.0	68	194
Pos. Control MMS	19.5	-	23.4	416	241	17.3	532	194
M656PH043								
	93.8	-	culture was not continued[#]			culture was not continued[#]		
	187.5	-	68.4	122	241	61.2	79	194
	375.0	-	63.4	146	241	87.8	89	194
	750.0	-	64.1	108	241	79.5	62	194
	1500.0	-	83.7	113	241	92.3	94	194
	3000.0	-	79.4	114	241	65.9	72	194
Solv. Control DMSO		+	100.0	132	258	100.0	71	197
Pos. Control CPA	3.0	+	37.3	550	258	56.9	449	197
Pos. Control CPA	4.5	+	36.1	478	258	23.4	281	197
M656PH043								
	93.8	+	culture was not continued[#]			culture was not continued[#]		
	187.5	+	87.1	135	258	129.2	58	197
	375.0	+	103.3	104	258	110.1	77	197
	750.0	+	113.7	105	258	143.0	57	197
	1500.0	+	89.4	139	258	156.6	59	197
	3000.0	+	117.1	78	258	111.9	51	197

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
[#] culture was not continued as a minimum of only four concentrations is required by the guidelines

Table B.6.8-58: Gene mutation in mammalian cells - 2nd experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II/24 h treatment			Culture I			Culture II		
Solv. Control DMSO		-	100.0	78	204	100.0	56	182
Pos. Control MMS	13.0	-	11.5	348	204	22.5	338	182
M656PH043								
	93.8	-	culture was not continued#			culture was not continued#		
	187.5	-	164.4	57	204	139.4	56	182
	375.0	-	42.8	86	204	128.1	48	182
	750.0	-	45.6	92	204	124.9	59	182
	1500.0	-	42.1	97	204	112.2	42	182
	3000.0	-	27.1	47	204	162.1	53	182
Experiment II/4 h treatment								
Solv. Control DMSO		+	100.0	60	186	100.0	50	176
Pos. Control CPA	3.0	+	49.8	197	186	54.7	215	176
Pos. Control CPA	4.5	+	24.4	354	186	29.8	324	176
M656PH043								
	93.8	+	culture was not continued#			culture was not continued#		
	187.5	+	114.1	55	186	91.7	64	176
	375.0	+	107.3	58	186	110.5	42	176
	750.0	+	91.4	34	186	87.0	93	176
	1500.0	+	86.3	57	186	93.7	63	176
	3000.0	+	88.2	72	186	107.4	55	176

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test, M656PH043 did not induce forward mutations in the TK+/- locus in L5178Y cells *in vitro*.

Data point: KCA 5.8

Report: [redacted] 2013a (ASB2014-8466)
Reg.No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* micronucleus test in chinese hamster V79 cells
2013/1246088

Guideline(s): OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July

2012 - B.49: *In vitro* Mammalian Cell Micronucleus Test

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

M656PH043 (Reg. No. 5917262, former assigned M43) was tested *in vitro* for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments.

Test Material: Reg. No. 5917262 (Metabolite of BAS 656-PH, Dimethenamid-p)
Description: Solid, beige
Lot/Batch #: L82-113
Purity: 94.6 % (tolerance ± 1.0 %)
Stability of test compound: Stable in DMSO (solvent)
Solvent used: Dimethylsulfoxide (DMSO)
Control Materials:
Negative control: A negative control was not employed in this study
Solvent control:DMSO
Positive controls, -S9: Mitomycin C
(MMC, 0.1 µg/mL, dissolved in deionised water)
Griseofulvin (8.0 µg/mL, dissolved in DMSO)
Positive control, +S9: Cyclophosphamide
(CPA, 10.0 µg/mL, dissolved in saline)
Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

Test organisms: Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥70 %).
Culture medium/conditions: About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal

essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) foetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).
Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 - 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

Test concentrations:
a) Preliminary toxicity assay: Experiment I:
5.9-3000.0 µg/mL with and without metabolic activation
Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I (see Table B.6.8-59).
b) Cytogenicity assay: Experiment II:
5.9-3000.0 µg/mL without metabolic activation
93.8-3000.0 µg/mL with metabolic activation

Table B.6.8-59: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with M656PH043

Prepara-tion interval	Expo-sure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 h	4 h	I	5.9	11.7	23.4	46.9	93.8	187.5	375.0	750.0	1500.0	3000.0
24 h	24 h	II	5.9	11.7	23.4	46.9	93.8	187.5	375.0	750.0	1500.0	3000.0
			With S9 mix									
24 h	4 h	I	5.9	11.7	23.4	46.9	93.8	187.5	375.0	750.0	1500.0	3000.0
24 h	4 h	II					93.8	187.5	375.0	750.0	1500.0	3000.0

Dates of experimental work: 15-Jul-2013 - 27-Aug-2013
Preliminary cytotoxicity assay: With respect to the molecular weight and the purity (97.37 %, preliminary information at the start of the experiment) of the test item, 3000.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 5.9 and 3000.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.
Cytogenicity Assay:
Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.
Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose x H_2O, 192 mg/L Na_2HPO_4 x 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 20 hours.
Exposure period 24 hours: The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10 % (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were

performed.
Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer generated random code to prevent scorer bias.
Analysis of micronuclei and cytotoxicity: Evaluation was performed manually using microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.
Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)
Statistics:
Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.
Evaluation criteria:
A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose related and reproducible increase in the number of cells containing micronuclei is observed.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

Results:

Analytical determinations:
Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP13_121).
Preliminary cytotoxicity assay:
In the pre-test no cytotoxicity was observed at the evaluated concentrations in the absence and presence of S9 mix.
Cytogenicity assays:
In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. In addition, no visible precipitation of the test item in the culture medium was observed.
No mutagenicity was observed in Experiment I in the absence and presence of S9 mix and in

Experiment II in the presence of S9 mix. The rates of micronucleated cells after treatment with the test item (0.35 - 1.35 %) were close to the rates of the solvent control values (0.55 - 1.25 %) and within the range of the laboratory historical solvent control data. In Experiment II in the absence of S9 mix statistically significant increases were observed after treatment with 1500.0 and 3000.0 µg/mL (1.25 and 1.95 % micronucleated cells). Dose dependency was observed and the highest value exceeded the range of the historical control data (0.05 - 1.50 % micronucleated cells) (see Table B.6.8-60).
Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table B.6.8-60: Summary of results of the micronucleus test with M656PH043

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 h without S9 mix				
I	24 h	Solvent control[1] Positive control[2] 750.0 1500.0 3000.0	2.87 2.60 2.85 2.80 2.74	0.80 4.95[S] 0.70 1.35 0.90
Exposure period 24 h without S9 mix				
II	24 h	Solvent control[1] Positive control[3] 750.0 1500.0 3000.0	2.92 2.47 2.82 2.87 2.65	0.55 14.45[S] 0.55 1.25[S] 1.95[S]**
Exposure period 4 h with S9 mix				
I	24 h	Solvent control[1] Positive control[4] 750.0 1500.0 3000.0	2.07 1.80 1.97 2.24 2.10	1.25 8.85[S] 1.25 1.10 1.35
II	24 h	Solvent control[1] Positive control[4] 750.0 1500.0 3000.0	1.82 1.57 1.94 1.79 1.97	1.05 12.95[S] 0.35 0.80 1.30

* The total number of micronucleated cells was determined in a sample of 2000 cells
** The total number of micronucleated cells was determined in a sample of 4000 cells
s Number of micronucleated cells statistically significantly higher than corresponding control values
1 DMSO 0.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 Griseofulvin 8.0 µg/mL
4 CPA 10.0 µg/mL

Conclusion:

Based on the results of the study, M656PH043 was mutagenic in this *in vitro* micronucleus test, when tested up to the highest required concentrations.

Data point: KCA 5.8

Report: [redacted]2013b (ASB2014-8471)
Reg.No. 5917262 (metabolite of BAS 656-PH dimethenamid-P) - Micronucleus assay in bone marrow cells of the mouse
2014/1001781

Guideline(s):	OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.8
Report:	Grauert M., Kamp H., 2014a (ASB2014-8483) Reg. No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in 30 % dimethyl sulphoxide + 70 % polyethylenglycol (v+v) 2014/1098005
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.8
Report:	Becker M., Landsiedel R., 2014a (ASB2014-8478) Reg. No. 5917262 (metabolite of BAS 656-PH, Dimethenamid-P) - Plasma analysis for external studies 2014/1092436
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656PH043 (Reg. No. 5917262, former assigned M43) was tested for the ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

Test Material:	Reg.No. 5917262 (Metabolite of BAS 656-PH, dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-113
Purity:	94.6 % (tolerance ± 1.0 %)
Stability of test compound:	Confirmed indirectly by dose formulation analytics (ASB2014-8483). Homogeneity of the preparations was ensured by mixing.
Solvent used:	30 % dimethyl sulphoxide (DMSO), 70 % polyethylene glycol (PEG)
Control Materials:	
Negative:	No negative control was employed in this study.
Solvent control:	DMSO/PEG 400 (3/7)
Positive control:	Cyclophosphamide (CPA) 40 mg/kg
Test animals:	
Species:	Mice

Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 9 weeks
Weight at dosing:	Males mean value 34.6 g (SD ± 1.7 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2 males and 2 females for each pre-test
Micronucleus assay:	7 males/dose; 5 males/control
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Single housing in Makrolon Type II (pre-test) / III (main study) cages, with wire mesh top
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	45 - 65 %
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00-18:00/18:00-06:00)
Test compound doses:	
Range finding test:	1000 and 2000 mg/kg (administered once orally)
Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.
Dates of experimental work:	15-Oct-2013 to 13-Nov-2013.
Preliminary range finding test:	Male and female NMRI mice were treated once by oral gavage with a test substance dose of 1000 (1st pre-test) and 2000 mg/kg bw (2nd pre-test).

Micronucleus test:
Treatment and sampling: Groups of male mice were treated once with either the vehicle or 500, 1000 or 2000 mg M656PH043/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were surveyed for evident clinical signs of toxicity throughout the study.
Twenty-four hours after the administration the mice were killed and the femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with foetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. The supernatant was discharged and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation: A small drop of the suspension was spread on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.
Slide evaluation: In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored.
To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.
Statistics:
The number of polychromatic erythrocytes with micronuclei was analysed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test.
Evaluation criteria:
A test item was considered as mutagenic if it induces either a dose related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

Results:

Analytical determinations:
The stability of the test substance in the solvent was confirmed indirectly by dose formulation analytics (ASB2014-8483).
Preliminary range finding test:
None of the male or female mice died after oral administration of 1000 and 2000 mg/kg bw. At 1000 mg /kg bw clinical signs comprised ruffled fur and eyelid closure in both sexes. At 2000 mg/kg bw ruffled fur only was observed in one mal animal. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.
Micronucleus assay:
Signs of systemic toxicity were not observed up to 2000 mg/kg bw test substance. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle. Plasma-analytics however (ASB2014-8478), demonstrated that M656PH043 was systemically available.
The mean number of polychromatic erythrocytes was not substantially decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that M656PH043 did not have any cytotoxic properties in the bone marrow.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item (see Table B.6.8-61). The mean values of micronuclei observed after treatment with the test substance were below or very near to the value of the vehicle control group. Moreover, microneucleus values obtained in all dose groups were within the historical negative control range.
The positive control cyclophosphamide showed a statistically significant increase of induced micronucleus frequency thereby ensuring the validity of the test system.

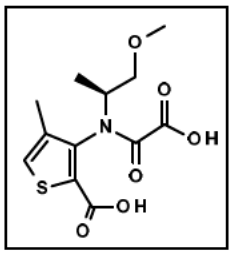
Table B.6.8-61: Micronucleus test in mice administered M656PH043 by oral gavage

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Vehicle	24	0.130	1-4	1280
M656PH043				
500 mg/kg bw	24	0.093	0-5	1243
1000 mg/kg bw	24	0.171	0-8	1225
2000 mg/kg bw	24	0.100	0-4	1241
Positive control				
Cyclophosphamide	24	1.950	24-56	1161
48 h sampling				
Sterile water	48	0.080	1-3	1191
M656PH043				
2000 mg/kg bw	48	0.064	1-2	1143

Conclusion:

The study is considered to be acceptable.
Based on the result of this study M656PH043 did not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Metabolite M656PH045 formerly assigned M45/M46:
M656PH045 is a metabolite of dimethenamid-P determined in groundwater.



Structural alerts for M656PH045:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times (see ASB2014-8408) predicted M656PH045 to be not mutagenic in the Ames test with and without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (see ASB2014-8410) the prediction was negative for the metabolite itself and the structural alerts identified for transformation products (alpha-beta polarised carbonyls) were not predicted to become active with the limitation that the molecules were out of the prediction domain.
In the DEREK analysis conducted structural alerts for M656PH045 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (Molecule 15) in both modules (CAESAR and SarPy) was non-mutagenic, however the reliability of this prediction was low.
In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

Genotoxicity studies of M656PH045 formerly assigned M45/M46:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Woitcowiak C., 2013a (ASB2014-8487) Reg.No. 5917261 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhymurium/Eschericia coli, reverse mutation assay 2013/1361403
Guideline(s):	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656PH045 (Reg.No. 5917261, former assigned M45) in the presence and absence of metabolic activation in a plate incorporation assay and a pre-incubation experiment.

Test Material	Reg.No. 5917261 (Metabolite of BAS 656-PH, Dimethenamid-p)
Description:	Solid, grey
Lot/Batch #:	L82-128
Purity:	98.4 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Sep 2015 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Ultrapure water
Control Materials:	
Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control:	The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

Test concentrations:
Plate incorporation assay:
(1st experiment): Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and with out S9) for all tester strains indicated above.

Pre-incubation assay:
(2nd experiment): The test substance/vehicle/positive control substance, bacterial and S9-mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 2500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 18-Sep-2013 to 10-Oct-2013.

Plate incorporation assay:
To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates).

Pre-incubation assay:
100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

Titer determination:
In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

Statistics: No special statistical tests were performed.

Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- A dose related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

Results:

Analytical determinations:
The test substance was stable over the study period under the storage conditions. The stability of the test substance in the vehicle ultrapure water was determined analytically. Purity of the test item was verified by HPLC analysis.
Toxicity:
A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
Bacteriotoxic effects (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants, reduction in the titer) were observed in the standard plate test and in the pre-incubation assay depending on the strain and test conditions from about 1000 µg/plate onward.
Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-62).
Precipitation was not observed up to the maximum concentration.

Table B.6.8-62: Bacterial gene mutation assay with M656PH045 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		*E. coli*	
1st experiment: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	21	16	61	58	11	11	8	6	61	58
M656PH045										
33 µg/plate	20	16	64	61	11	10	8	7	65	54
100 µg/plate	22	16	64	60	10	10	8	6	68	54
333 µg/plate	20	14	61	64	10	10	8	6	64	56
1000 µg/plate	11	14	55	64	8	12	5	6	68	54
2500 µg/plate	9	11	22	60	8	11	5	3	37	54
5000 µg/plate	9	12	20	54	5	9	0[B]	4	30	24
Pos. control[§]	1297	328	1353	1305	375	1236	263	699	447	752
2nd experiment: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	31	18	75	69	11	13	6	6	78	71
M656PH045										
10 µg/plate	26	18	86	67	10	12	7	5	74	70
33 µg/plate	28	22	81	70	11	13	6	6	73	66
100 µg/plate	28	21	81	75	11	12	7	6	77	70
333 µg/plate	27	18	80	67	10	10	6	6	73	66
1000 µg/plate	26	18	62	71	11	10	7	6	75	62
2500 µg/plate	0[B]	4	39	37	7	7	0[B]	3	16	16
Pos. control[§]	1182	444	1240	1031	243	1138	363	416	238	1030

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
[B] = Reduced Background Growth

Conclusion:

The study is considered to be acceptable.
According to the results of the present study, the test substance M656PH045 did not induce revertants in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point: KCA 5.8

Report: [redacted]., 2013b (ASB2014-8460)
Reg.No. 5917261 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells
2013/1246091

Guideline(s): OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

M656PH045 (Reg.No. 5917261) was tested *in vitro* for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the TK+/- locus. Two independent experiments were conducted in the presence and absence of metabolic activation.

Test Material	Reg. No. 5917261 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-122
Purity:	99.7 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in deionised water (The stability of a comparable batch (L82-128) was verified analytically.)
Solvent used:	deionised water
Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	deionised water
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL
Activation:	Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[*a*]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313) in the pre-experiment and in experiment I and II. An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM

$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

Test organism: The L5178Y cell line, which is characterised by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50 %. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with hypoxanthine (5.0×10^{-3} M), aminopterin (2.0×10^{-5} M), thymidine (1.6×10^{-3} M) and glycin (5.0×10^{-3} M) followed by a recovery period of 2 days in RPMI 1640 medium containing hypoxanthine (1.0×10^{-4} M) and thymidine (1.6×10^{-3} M). After this incubation the cells were returned to complete culture medium (see below).

Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15 % horse serum (24 hour treatment, 3 % HS during 4 hour treatment), 1 % of 100 U/100 µg/mL penicillin/streptomycin, 220 µg/mL sodium-pyruvate, and 0.5 - 0.75 % amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, $Na_2HPO_4 \times 2\ H_2O$ 290 mg, KH_2PO_4 150 mg

Locus examined: Thymidine Kinase Locus (TK+/-)

Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 39.06 to 5000 µg/mL

b) Mutation assay:

1st and 2nd experiment: 156.3, 312.5, 625.0, 1250.0, 2500.0, 5000.0 µg/mL with and without metabolic activation

Dates of experimental work: 29-Jul-2013 to 30-Sep-2013.

Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.

1×10^7 cells (3×10^6 cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

Mutation Assay:

Cell treatment and expression: In the mutation experiment 1×10^7 (3×10^6 during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3 % horse serum (15 % horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection:
After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4 x 10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5 % CO_2/95.5 % humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.
Size distribution of the colonies:
Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).
Calculations:
Pre-test

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control

Main test:

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) x (cell number at 72 h/if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control
relative total growth:	relative suspension growth x relative cloning efficiency/100
cloning efficiency (viability):	ln(mean number of empty wells per plate/96)/cells seeded per well
relative cloning efficiency:	cloning efficiency x 100/cloning efficiency of corresponding control
cells survived:	cloning efficiency x cell number seeded in TFT medium
mutant colonies/10^6 cells:	small mutant colonies + large mutant colonies
threshold:	number of mutant colonies per 10^6 cells of each solvent control plus 126
cloning efficiency (viability):	cloning efficiency determined after the expression period to measure viability of the cells without selective agent

Statistics:
A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C,

Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.
Evaluation criteria:
The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10 % of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

Results:

Analytical determinations:
Purity of the test item was verified by HPLC analysis.
Preliminary cytotoxicity assay:
The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 39.06 µg/mL and 5000 µg/mL were chosen). No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.
Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. In the pre-experiment and in both main experiments the pH was adjusted at the two highest concentrations using 2N NaOH. There was no relevant shift of the osmolarity at the maximum concentration.
The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 5000 µg/mL without and with metabolic activation.
Mutagenicity assays:
No relevant cytotoxic effect indicated by a relative total growth of less than 50 % of survival in both parallel cultures was observed in experiment I and II with and without metabolic activation. A single, moderately cytotoxic effect was noted in the second culture of experiment II at the maximum concentration of 5000 µg/mL in the presence of metabolic activation. However, this moderate reduction of the relative total growth was not judged as true cytotoxic effect as it was not reproduced in the parallel culture or in the first experiment with metabolic activation. No precipitation was observed by the unaided eye up to the maximum concentration.
No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The induction factor of 126 above the corresponding solvent control

was exceeded in the first experiment with metabolic activation at 2500 µg/mL in culture I. However, this isolated increase was judged as biologically irrelevant as it was neither dose dependent nor reproduced in the parallel culture under identical experimental conditions (see Table B.6.8-63, Table B.6.8-64).
The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies.

Table B.6.8-63: Gene mutation in mammalian cells - 1st experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I/4 h treatment			Culture I			Culture II		
Solv. Control with DMSO		-	100.0	60	186	100.0	71	197
Pos. Control with MMS	19.5	-	20.8	398	186	21.0	388	197
M656PH045								
	156.3	-	culture was not continued[#]			culture was not continued[#]		
	312.5	-	78.1	112	186	88.3	109	197
	625.0	-	95.4	92	186	92.6	121	197
	1250.0	-	96.5	80	186	106.3	65	197
	2500.0	-	75.7	98	186	62.2	136	197
	5000.0	-	83.8	104	186	68.5	128	197
Solv. Control with DMSO		+	100.0	95	221	100.0	95	221
Pos. Control with CPA	3.0	+	43.0	553	221	36.9	537	221
Pos. Control with CPA	4.5	+	39.4	642	221	21.9	788	221
M656PH045								
	156.3	+	culture was not continued[#]			culture was not continued[#]		
	312.5	+	116.4	120	221	68.0	119	221
	625.0	+	136.7	90	221	65.7	206	221
	1250.0	+	98.4	155	221	84.6	106	221
	2500.0	+	53.5	280	221	90.8	141	221
	5000.0	+	62.8	177	221	85.9	123	221

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
[#] culture was not continued as a minimum of only four concentrations is required by the guidelines

Table B.6.8-64: Gene mutation in mammalian cells - 2nd experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II/24 h treatment			Culture I			Culture II		
Solv. Control with DMSO		-	100.0	148	274	100.0	75	201
Pos. Control with MMS	13.0	-	19.6	402	274	25.7	271	201
M656PH045								
	156.3	-	culture was not continued#			culture was not continued#		
	312.5	-	78.8	137	274	78.0	56	201
	625.0	-	70.6	108	274	84.6	55	201
	1250.0	-	88.4	124	274	55.6	89	201
	2500.0	-	56.4	169	274	60.4	50	201
	5000.0	-	62.0	112	274	56.5	46	201
Experiment II/4 h treatment								
Solv. Control with DMSO		+	100.0	135	261	100.0	106	232
Pos. Control with CPA	3.0	+	37.3	234	261	30.5	331	232
Pos. Control with CPA	4.5	+	30.7	352	261	25.4	403	232
M656PH045								
	156.3	+	culture was not continued#			culture was not continued#		
	312.5	+	77.9	162	261	56.1	97	232
	625.0	+	59.0	117	261	55.4	130	232
	1250.0	+	115.6	107	261	59.1	115	232
	2500.0	+	121.7	83	261	62.3	145	232
	5000.0	+	88.1	81	261	42.1	156	232

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test, M656PH045 did not induce forward mutations in the TK+/- locus in L5178Y cells *in vitro*.

Data point: KCA 5.8

Report: [redacted] 2013b (ASB2014-8467)
Reg.No. 5917261 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* micronucleus test in Chinese hamster V79 cells
2013/1246090

Guideline(s): OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July

2012 - B.49: *In vitro* Mammalian Cell Micronucleus Test

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

M656PH045 (Reg.No. 5917261, former assigned M45) was tested *in vitro* for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments.

Test Material: Reg. No. 5917261 (Metabolite of BAS 656-PH, dimethenamid-P)
Description: Solid, beige
Lot/Batch #: L82-122
Purity: 99.7 % (tolerance ± 1.0 %)
Stability of test compound: Stable in deionised water (solvent) (The stability of a comparable batch (L82-128) was verified analytically.)
Solvent used: deionised water
Control Materials:
Negative control: A negative control was not employed in this study
Solvent control: deionised water
Positive controls, -S9: Mitomycin C
(MMC, 0.1 µg/mL, dissolved in deionised water)
Griseofulvin (9.0 µg/mL, dissolved in DMSO)
Positive control, +S9: Cyclophosphamide
(CPA, 10.0/15.0 µg/mL in Exp. I/II, dissolved in saline)
Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

Test organisms: Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥70 %).

Culture medium/conditions: About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) foetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).
Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5-1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

Test concentrations:

a) Preliminary toxicity assay: Experiment I:
9.8 - 5000.0 µg/mL with and without metabolic activation
Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I (see Table B.6.8-65).

b) Cytogenicity assay: Experiment II:
9.8 - 5000.0 µg/mL without metabolic activation
156.3 - 5000.0 µg/mL with metabolic activation

Table B.6.8-65: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg.No. 5917261 (Metabolite of BAS 656-PH, dimethenamid-P)

Preparation interval	Exposure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 h	4 h	I	9.8	19.5	39.1	78.1	156.3	312.5	625.0[P]	1250.0[P]	2500.0[P]	5000.0[P]
24 h	24 h	II*	9.8	19.5	39.1	78.1	156.3	312.5	625.0	1250.0	2500.0	5000.0[P]
24 h	24 h	II	9.8	19.5	39.1	78.1	156.3	312.5	625.0	1250.0	2500.0	5000.0
			With S9 mix									
24 h	4 h	I	9.8	19.5	39.1	78.1	156.3	312.5	625.0	1250.0	2500.0	5000.0
24 h	4 h	II*					156.3	312.5	625.0	1250.0	2500.0	5000.0
24 h	4 h	II					156.3	312.5	625.0	1250.0	2500.0	5000.0

*: Was repeated due to technical problems
[P]: Precipitation occurred at the end of treatment

Dates of experimental work: 17-Jul-2013 - 02-Dec-2013.

Preliminary cytotoxicity assay:
5000.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 9.8 and 5000.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

Cytogenicity Assay:
Exposure period 4 hours:
The culture medium of exponentially growing cell cultures was replaced with serum free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.
Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed

twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose x H_2O, 192 mg/L Na_2HPO_4 x 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 20 hours.
Exposure period 24 hours:
The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10 % (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.
Preparations of cultures:
For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer generated random code to prevent scorer bias.
Analysis of micronuclei and cytotoxicity:
Evaluation was performed manually using microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.
Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)
Statistics:
Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.
Evaluation criteria:
A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose related and reproducible increase in the number of cells containing micronuclei is observed

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

Results:

Analytical determinations:
Purity of the test item was verified by HPLC analysis.

Preliminary cytotoxicity assay:
In the pre-test no cytotoxicity was observed at the evaluated concentrations in the absence and presence of S9 mix. Precipitation of the test item was observed microscopically at the end of treatment at 625.0 µg/mL and above in the absence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.
Cytogenicity assays:
In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. Visible precipitation of the test item in the culture medium was observed microscopically at the end of treatment in the absence of S9 mix at 625.0 µg/mL and above in Experiment I.
The rates of micronucleated cells after treatment with the test item in Experiment I in the presence of S9 mix and in Experiment II in the absence and presence of S9 mix (0.70 - 1.40 %) were close to the rates of the solvent control values (1.00 - 1.60 %) and within the range of the laboratory historical solvent control data. However, in Experiment I in the absence of S9 mix after treatment with 156.3 µg/mL one single increase in micronucleated cells (1.70 %) above the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells) was observed. Since the value was not statistically significant, the finding was regarded as biologically irrelevant (see Table B.6.8-66).
Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table B.6.8-66: Summary of results of the micronucleus test with M656PH045

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 h without S9 mix				
I	24 h	Solvent control[1] Positive control[2] 156.3 312.5 625.0P 5000.0P	2.90 2.60 2.96 2.93 2.94 2.92	1.60 6.10S 1.70 0.85 1.15 n.e.
Exposure period 24 h without S9 mix				
II	24 h	Solvent control[1] Positive control[3] 1250.0 2500.0 5000.0	3.05 2.65 2.89 2.86 2.73	1.00 11.50S 1.25 1.00 1.25
Exposure period 4 h with S9 mix				
I	24 h	Solvent control[1] Positive control[4] 1250.0 2500.0 5000.0	2.19 1.74 2.27 2.41 2.28	1.30 9.65S 0.70 1.40 1.20
II	24 h	Solvent control[1] Positive control[5] 1250.0 2500.0 5000.0	2.25 1.73 2.28 2.16 2.13	1.35 12.55S 1.10 1.60 1.20

* The number of micronucleated cells was determined in a sample of 2000 cells
s Number of micronucleated cells statistically significantly higher than corresponding control values
P Precipitation occurred microscopically at the end of treatment
1 Deionsed water 10.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 Griseofulvin 9.0 µg/mL
4 CPA 10.0 µg/mL
5 CPA 15.0 µg/mL
n.e. not evaluated

Conclusion:

The study is considered to be acceptable.
Based on the results of the study, M656PH045 did not induce the formation of micronuclei in this *in vitro* test, when tested up to the highest required concentrations.

Short-term toxicity of M656PH045:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	[redacted]., 2014c (ASB2014-8418) Reg. No. 5917261 (metabolite of BAS 655-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018092
Guideline(s):	OECD 407, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Reg.No. 5917261, metabolite of dimethenamid-P
Description:	Solid/grey
Batch/purity #:	L82-128, 98.4 %
Stability of test compound:	Stable until 01 Sep 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.
Vehicle and/or positive control:	Rodent diet
Test animals:	
Species:	Rat
Strain:	Wistar Crl:WI (Han) Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 141.8 - 156.8 g, ♀ 119.9 - 136.4 g
Source:	Charles River, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, *ad libitum*
Housing:	Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment Motor activity measurements were conducted in polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about

	800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes per hour
Photo period:	12 h light/12 h dark (06:00 - 18:00/18:00 - 06:00)
Dates of experimental work:	05-Nov-2013 – 05-Mar-2014. (In life dates: 11-Nov-2013 (start of administration) to 10-Dec-2013 (necropsy))

Animal assignment and treatment:
M656H045 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomisation list based on body weights.
Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. One diet preparation per dose was performed for this study.
Analyses performed prior to the start of the administration period revealed that the test substance was stable in the diet for at least 34 days.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analysed. The samples were also used for determination of the test article concentration. For the mid dose level single samples were analysed. No test article was determined in control diets.

Table B.6.8-67: Analysis of diet preparations for homogeneity and test item content

Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
1200 ppm	8. Nov. 13	1220.1 ± 81.5	101.7	6.8
4000 ppm	8. Nov. 13	3675.600	91.9	
12000 ppm	8. Nov. 13	12014.2 ± 116.8	100.1	1.0

n.a.: not applicable;
based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures.

Relative standard deviations of the homogeneity samples in the range of 1.0 to 6.8 % indicate the homogenous distribution of M656H045 in the diet preparations. The actual (mean) average test substance concentrations were in the range of 91.9 % to 101.7 % of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H045 in the vehicle.
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-68: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Table B.6.8-69: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine color and turbidity	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table B.6.8-70: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. behaviour during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmos
6. respiration	15. faeces discharge during examination (appearance/consistency)
7. activity/arousal level	16. urine discharge during examination
8. tremors	17. pupil size
9. convulsions	

Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.
Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$Food\ efficiency\ at\ day\ x = \frac{BW_x - BW_y}{FC_{y\ to\ x}}\ x\ 100$$

BWx and BWy body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).
Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted.
Ophthalmoscopy:
Not performed in this study.
Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1.	behaviour when removed from cage	10.	respiration
2.	fur	11.	tremors
3.	skin	12.	convulsions
4.	salivation	13.	abnormal movements
5.	nose discharge	14.	impairment of gait
6.	lacrimation	15.	activity/arousal level
7.	eyes/pupil size	16.	faeces (number of faecal pellets/appearance/ consistency) within two minutes
8.	posture	17.	urine (appearance/quantity) within two minutes
9.	palpebral closure	18.	number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behaviour during "handling"
2.	touch response	9.	vocalisation
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

Motor activity measurement:
Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
Haematology and clinical chemistry:
Blood was withdrawn in the morning from fasted, isoflurane anesthetised animals from the retro-orbital plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomised sequence.
The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
	Red blood cells		White blood cells		Clotting Potential
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Haemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Haematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
Clinical chemistry:					
	Electrolytes		Metabolites and Proteins		Enzymes:
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Urinalysis:
For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analysed in a randomised order.
The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		Semi quantitative parameters		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Colour and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

Sacrifice and pathology:
The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	#	kidneys	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)@
✓		#	bone marrow§	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands£	✓		#	lymph nodes#	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides¥	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity‡	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetised animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles£				

§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; ‡l histopathology at level III, ¥left epididymidis collected for histopathology, £seminal vesicles and coagulation weight determined together

The organs or tissues were fixed in 4 % formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.
The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the oestrous cycle in ovaries, uterus, cervix, and vagina.

Results:

Observations:
No clinical signs were observed throughout the study. No mortality was observed throughout the study.
Ophthalmoscopy was not performed in this study.
FOB and Motor Activity:
Neither home cage nor open field observations revealed any indication of treatment related effects. The same holds true for the sensimotor tests and reflexes including quantitative parameters. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
Comparing the single intervals with the control groups, no significant deviations were measured.
Body weight and body weight gain:
No statistically significant differences of absolute body weights or body weight gain were noted (see Table B.6.8-71, Figure B.6.8-18).

Table B.6.8-71: Mean body weight of rats administered M656PH045 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1200	4000	12000	0	1200	4000	12000
Body weight [g]								
- Day 0	150.3	151.8	151.4	150.9	128	126.4	127.3	126.5
- Day 28	289.3	286.7	291.0	280.1	186.4	179	180.8	178.4
Δ% (compared to control)#		-0.9	0.6	-3.2		4.0	-3	-4.3
Overall body weight gain [g]	139.0	135.0	139.6	129.2	58.4	52.6	53.5	51.9
Δ% (compared to control)#		-2.9	0.5	-7.1		9.9	-8.4	-11.2
# Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)								

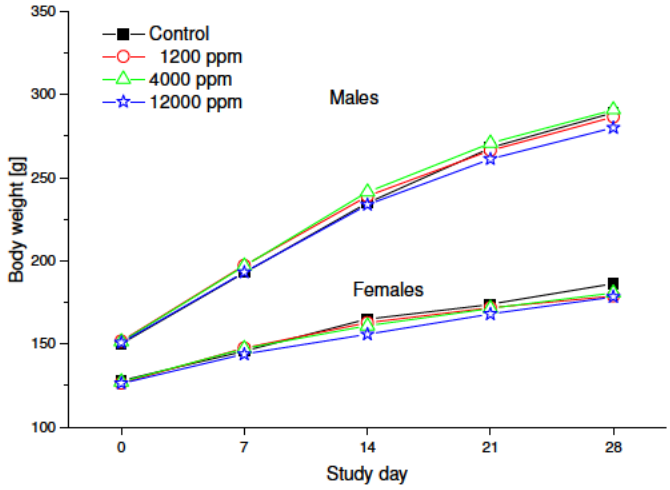


Figure B.6.8-18: Body weight development of rats administered M656PH045 for at least 28 days

Food consumption and compound intake:
No test substance related, adverse findings were observed. All recorded values were within the biological range typical for this strain of rats. Some values were not taken into account for groups summaries as these were declared as outliers. Increased food spilling was assumed for male animals the 4000 ppm group between study days 18 to 21 and 25 to 28 and for male animals of the 12000 ppm group between study days 25 to 28.
The mean daily test substance intake over the entire study period was calculated and is shown in the following (see Table B.6.8-72).

Table B.6.8-72: Calculated intake of M656PH045

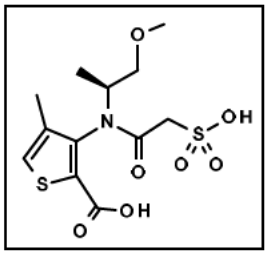
Test group	Concentration in the vehicle (ppm)	Mean daily test substance intake (mg/kg bw/d)	
		Males	Females
1	1200	102	100
2	4000	376	391
3	12000	1174	1298

Water consumption:
No test substance related, adverse changes with regard to water consumption were observed.
Blood analysis:
Haematological findings:
No treatment related changes among haematological parameters were observed. Prothrombin time could not be measured due to a technical failure of the instrument.
Clinical chemistry findings:
No treatment related changes among clinical chemistry parameters were observed.
Urinalysis:
No treatment related changes among urinalysis parameters were observed.
Necropsy:
Organ weight:
Terminal body weights of treated rats displayed no statistically significant differences to the controls.
When compared to the control group, all mean absolute and relative organ weights of treated animals did not show significant differences when compared to the control group.
Gross and histopathology:
Isolated macroscopic findings were recorded in one male control group animal (focus in epdidymidis) and one low dose animal (reduced testes size). A spermatogonic granuloma was identified as histopathological correlate for the focus in the epididymidis. Tubular degeneration of the testis was diagnosed for the animal showing the testis size reduction and also for a male control group animal. These lesions were considered to be incidental or spontaneous in nature and without any relation to treatment.
All other histopathological findings were either single observations, were equally distributed between control and treated groups. Therefore, these findings were considered to be incidental.

Conclusion:

The study is considered to be acceptable.
The administration of M656H045 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1174 mg/kg bw/d) and in female (1298 mg/kg bw/d) Wistar rats.

Metabolite M656PH047 formerly assigned M47/M48:
M656PH047 is a metabolite of dimethenamid-P determined in groundwater.



Structural alerts for M656PH047:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times (see ASB2014-8408) predicted M656PH047 to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration *in vitro* (see molecule 27 of report ASB2014-8410) the prediction was negative for the metabolite itself and negative for all presumed transformation products thereof (7 in total). The identified alert for a thiol was not predicted to become active. The overall prediction was therefore negative for *in vitro* chromosomal aberration, with the limitation that the structures were out of the prediction domain.

In the DEREK analysis conducted the structural alerts for M656PH047 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (see Molecule 16 of ASB2014-8412) in both modules CAESAR and SarPy was inconclusive. While the CAESAR module predicted M656PH047 to be Ames mutagenic the prediction of the SarPy module was non-mutagenic. However the reliability of these predictions was low as no similar compounds with known experimental data were found in the database and some similar molecules found in the database disagree with the prediction.
In conclusion no conclusive, relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

Genotoxicity studies of M656PH047:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Woitkowiak C., 2014b (ASB2014-8454) Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhymurium/Escherichia coli reverse mutation assay 2013/1397766
Guideline(s):	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656PH047 (Reg. No. 5917260, former assigned M47) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and in a pre-incubation experiment.

Test Material	Reg.No. 5917260 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-137
Purity:	90.7 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2015 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control:	The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Solvent/final concentration: 100 µL/plate
Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.
The optical density of the fresh bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 109 cells per mL).

Test concentrations:

Plate incorporation assay: Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2750 and 5500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: The test substance/vehicle/positive control substance, bacterial and S9-mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2750 and 5500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 23-Oct-2013 to 14-Nov-2013

Plate incorporation assay:
To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates).
Pre-incubation assay:
100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.
Statistics:
No special statistical tests were performed.
Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- A dose related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and *E.coli* WP2 uvrA) or tripled (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.

Results:

Analytical determinations:
The test substance was stable over the study period under the storage conditions.
The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (BASF Project No. 01Y0082/13Y015).
Toxicity:
A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
No bacteriotoxic effect (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants) was observed in the standard plate test up to the highest required concentration.
In the pre-incubation assay bacteriotoxicity (slight decrease in the number of his+ or trp+ revertants)

was occasionally observed depending on the strain and test conditions at 5500 µg/plate.
Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-73).
Test substance precipitation was found in the pre-incubation test at a concentration of 5500 µg/plate with and without S9 mix.

Table B.6.8-73: Bacterial gene mutation assay with M656PH047 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	24.3	24.3	54.0	42.3	14.0	7.0	10.7	6.7	62.7	59.7
M656PH047										
33 µg/plate	28.0	19.0	52.3	40.7	11.3	7.7	8.7	6.7	58.0	57.3
100 µg/plate	26.3	18.3	52.3	40.3	10.3	9.7	7.3	6.7	62.0	57.3
333 µg/plate	26.0	15.7	43.0	39.3	9.0	10.0	6.0	4.0	62.7	60.3
1000 µg/plate	29.7	18.7	49.0	41.3	9.0	7.3	8.7	5.0	59.0	59.7
2750 µg/plate	19.7	21.3	44.0	40.7	10.3	10.3	8.7	5.7	65.3	50.7
5500 µg/plate	20.0	16.3	46.0	37.0	10.3	8.3	7.3	5.7	57.7	52.7
Pos. control[§]	2110.0	343.3	2803.7	4304.7	208.0	5337.0	248.0	1691.0	149.0	829.7
Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	25.7	24.3	44.0	38.7	9.0	7.0	7.7	6.7	69.7	67.3
M656PH047										
33 µg/plate	30.7	14.7	47.7	28.3	12.3	11.0	7.7	6.7	70.7	67.7
100 µg/plate	28.7	19.7	67.0	38.3	11.7	13.0	7.0	5.3	74.3	60.7
333 µg/plate	28.0	18.3	57.0	35.7	10.7	10.0	7.3	10.3	73.3	70.0
1000 µg/plate	31.7	20.3	66.7	45.7	13.7	9.7	9.3	7.7	69.7	58.0
2750 µg/plate	21.7	19.0	64.7	37.0	9.3	5.7	6.3	9.7	64.7	59.7
5500 µg/plate	15.3[P]	13.3[P]	58.7[P]	30.3[P]	9.3[P]	6.0[P]	4.3[P]	3.0[P]	59.0[P]	26.0[P]
Pos. control[§]	2420.7	375.0	3005.7	3191.3	170.3	3234.7	225.0	1169.7	148.3	351.3

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
[P]= Precipitation

Conclusion:

The study is considered to be acceptable.
According to the results of the present study, the test substance M656PH047 was not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point:	KCA 5.8
Report:	[redacted] 2014a (ASB2014-8461) Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P): *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2014/1018055
Guideline(s):	OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656PH047 (Reg. No. 5917260, former assigned M47) was tested *in vitro* for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the TK+/- locus. Two independent experiments were conducted in the presence and absence of metabolic activation.

Test Material	Reg. No. 5917260 (Metabolite of BAS 656-PH, dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-137
Purity:	90.7 % (tolerance ± 1.0 %), dose calculation adjusted to purity
Stability of test compound:	Stable in DMSO over 4 hours
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL
Activation:	Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[*a*]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 29.8 mg/mL (Lot. No.: 050913) in the pre-experiment and in experiment I and II. An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

Test organism:
The L5178Y cell line, which is characterised by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50 %. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with hypoxanthine (5.0×10^{-3} M), aminopterin (2.0×10^{-5} M), thymidine (1.6×10^{-3} M) and glycin (5.0×10^{-3} M) followed by a recovery period of 2 days in RPMI 1640 medium containing hypoxanthine (1.0×10^{-4} M) and thymidine (1.6×10^{-3} M). After this incubation the cells were returned to complete culture medium (see below).
Culture media:
Complete culture medium:
RPMI 1640 medium supplemented with 15 % horse serum (24 hour treatment, 3 % HS during 4 hour treatment), 1 % of 100 U/100 µg/mL penicillin/streptomycin, 220 µg/mL sodium-pyruvate, and 0.5-0.75 % amphotericin used as antifungal agent.
Selection medium:
RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT.
Saline G solution:
Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, $Na_2HPO_4 \times 2H_2O$ 192 mg, KH_2PO_4 150 mg
Locus examined: Thymidine Kinase Locus (TK+/-)
Test concentrations:
a) Preliminary toxicity assay: Eight concentrations ranging from 15.1 to 1930 µg/mL
b) Mutation assay:
1st and 2nd experiment: 120.9, 241.9, 483.8, 967.5, 1935.0, 3870.0 µg/mL with and without metabolic activation
Dates of experimental work: 05-Nov-2013 to 02-Dec-2013.
Preliminary cytotoxicity assay:
A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.
1×10^7 cells (3×10^6 cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.
Mutation Assay:
Cell treatment and expression:
In the mutation experiment 1×10^7 (3×10^6 during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3 % horse serum (15 % horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution".

Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection:

After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5 % CO_2/95.5 % humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.

Size distribution of the colonies:

Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations:

Pre-test:

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control

Main test:

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) x (cell number at 72 h/if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control
relative total growth:	relative suspension growth x relative cloning efficiency/100
cloning efficiency (viability):	ln(mean number of empty wells per plate/96)/cells seeded per well
relative cloning efficiency:	cloning efficiency x 100/cloning efficiency of corresponding control
cells survived:	cloning efficiency x cell number seeded in TFT medium
mutant colonies / 106 cells:	small mutant colonies + large mutant colonies
threshold:	number of mutant colonies per 106 cells of each solvent control plus 126
cloning efficiency (viability):	cloning efficiency determined after the expression period to measure viability of the cells without selective agent

Statistics:

A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C,

Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.
Evaluation criteria:
The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose related increase in mutant frequencies using an appropriate statistical trend.

If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.
However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10 % of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

Results:

Analytical determinations:
Purity of the test item was verified by HPLC, Q-NMR analysis.
Preliminary cytotoxicity assay:
The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 15.1 µg/mL and 1930 µg/mL were chosen. No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment. A minor reduction of the relative suspension growth to approximately 50 % occurred at the maximum concentration following 24 hours treatment without metabolic activation.
Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. The pH was adjusted to neutral using 2M sodium hydroxide solution. There was no relevant shift of the osmolarity at the maximum concentration.
The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3870 µg/mL without and with metabolic activation.
Mutagenicity assays:
Relevant and reproducible cytotoxic effects indicated by a relative total growth (RTG) below 50 % in both parallel cultures solely occurred at the highest two concentrations of the second experiment without metabolic activation. RTG levels below 50 % were also noted in the second culture of the second experiment with metabolic activation at 483.8 and 1935 µg/mL. However, no comparable effect occurred in the parallel culture under identical experimental conditions. No visible precipitation of the test item in the culture medium was observed.
No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The threshold of 126 above the corresponding solvent control was

not reached (see Table B.6.8-74,

Table B.6.8-75).
The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies at acceptable levels of toxicity with at least one of the concentrations of the controls.

Table B.6.8-74: Gene mutation in mammalian cells - 1st experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I/4 h treatment			Culture I			Culture II		
Solv. Control DMSO		-	100.0	59	185	100.0	110	236
Pos. Control MMS	19.5	-	33.5	313	185	34.2	319	236
M656PH047								
	120.9	-	culture was not continued#			culture was not continued#		
	241.9	-	91.5	91	185	139.0	74	236
	483.8	-	95.6	65	185	138.7	70	236
	967.5	-	86.6	76	185	102.7	210	236
	1935.0	-	72.9	121	185	83.0	120	236
	3870.0	-	91.4	92	185	97.6	86	236
Solv. Control DMSO		+	100.0	47	173	100.0	91	217
Pos. Control CPA	3.0	+	80.8	123	173	106.9	218	217
Pos. Control CPA	4.5	+	39.8	261	173	63.1	391	217
M656PH047								
	120.9	+	culture was not continued#			culture was not continued#		
	241.9	+	72.6	119	173	161.4	67	217
	483.8	+	56.4	138	173	184.1	115	217
	967.5	+	67.6	162	173	196.2	55	217
	1935.0	+	83.7	88	173	127.6	69	217
	3870.0	+	62.9	137	173	194.6	58	217

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Table B.6.8-75: Gene mutation in mammalian cells - 2nd experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II/24 h treatment			Culture I			Culture II		
Solv. Control DMSO		-	100.0	65	191	100.0	53	179
Pos. Control MMS	13.0	-	11.1	411	191	8.2	441	179
M656PH047								
	241.9	-	culture was not continued#			culture was not continued#		
	483.8	-	87.3	67	191	113.8	49	179
	967.5	-	71.3	72	191	92.4	50	179
	1935.0	-	81.7	52	191	51.2	57	179
	2902.5	-	19.5	50	191	32.4	78	179
	3870.0	-	21.9	62	191	25.4	66	179
Experiment II/4 h treatment								
Solv. Control DMSO		+	100.0	147	273	100.0	53	179
Pos. Control CPA	3.0	+	47.8	285	273	49.9	220	179
Pos. Control CPA	4.5	+	43.8	372	273	14.8	746	179
M656PH047								
	120.9	+	culture was not continued#			culture was not continued#		
	241.9	+	107.9	60	273	60.9	93	179
	483.8	+	96.4	79	273	33.5	174	179
	967.5	+	77.0	156	273	50.4	147	179
	1935.0	+	77.5	123	273	41.9	118	179
	3870.0	+	78.0	149	273	70.4	77	179

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Conclusion:

The study is considered to be acceptable.
Based on the results of the study it is concluded that under the conditions of the test M656PH047 does not induce forward mutations in the TK+/- locus in L5178Y cells *in vitro*.

Data point: KCA 5.8

Report: [redacted] 2014a (ASB2014-8472)
Reg.No. 5917260 (metabolite of BAS 656, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1018032

Guideline(s): OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test

	methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.8
Report:	Grauert M., Kamp H., 2014b (ASB2014-8484) Reg. No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in sterile water 2014/1098002
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.8
Report:	Becker M., Landsiedel R., 2014b (ASB2014-8479) Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for external studies 2014/1092434
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656PH047 (Reg. No. 5917260, former assigned M47) was tested for the ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

Test Material:	Reg.No. 5917260 (Metabolite of BAS 656-PH, Dimethenamid-p)
Description:	Solid, beige
Lot/Batch #:	L82-137
Purity:	90.7 %
Stability of test compound:	Confirmed indirectly by dose formulation analytics (see separate report, BASF study code 04Y0082/13Y060).
Solvent used:	sterile water
Control Materials:	
Negative:	No negative control was employed in this study.
Solvent control:	sterile water
Positive control:	Cyclophosphamide (CPA) 40 mg/kg
Test animals:	
Species:	Mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 12 weeks

Weight at dosing:	Males mean value 35.8 g (SD ± 1.5 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2 males and 2 females for each pre-test
Micronucleus assay:	7 males/dose; 5 males/control
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Single housing in Makrolon Type II (pre-test) / III (main study) cages, with wire mesh top
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	45 - 65 %
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00/18:00 - 06:00)
Test compound doses:	
Range finding test:	1000 and 2000 mg/kg (administered once orally)
Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.
Dates of experimental work:	12-Nov-2013 to 09-Dec-2013

Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 1000 (1st pre-test) and 2000 mg/kg bw (2nd pre-test).

Micronucleus test:

Treatment and sampling:

Groups of male mice were treated once with either the vehicle or 500, 1000 or 2000 mg M656PH047/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose animals were treated for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were surveyed for evident clinical signs of toxicity throughout the study.

Twenty-four hours after the administration the mice were killed and the femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with foetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. The supernatant was discharged and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.

Slide preparation:

A small drop of the suspension was spread on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.

Slide evaluation:

In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored.

To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

Statistics:

The number of polychromatic erythrocytes with micronuclei was analysed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test.

Evaluation criteria:

A test item was considered mutagenic if it induces either a dose related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

Results:

Analytical determinations:
The stability of the test substance in the solvent was confirmed indirectly by dose formulation analytics (see ASB2014-8484).
Preliminary range finding test:
None of the male or female mice died after oral administration of 1000 and 2000 mg/kg bw. At 2000 mg /kg bw clinical signs comprised ruffled fur and eyelid closure in both sexes. At 1000 mg/kg bw ruffled fur only was observed. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.
Micronucleus assay:
The only clinical symptom in the main experiment was ruffled fur in four of 14 mice treated with the high dose of test item observed 1 hour post application. The animals treated with the mid dose level and low dose level did not exhibit any clinical symptoms. Analysis in blood plasma confirmed the systemic availability of M656PH047 (ASB2014-8479).
The mean number of polychromatic erythrocytes was not substantially decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that M656PH047 did not have any cytotoxic properties in the bone marrow.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item (see Table B.6.8-76). The mean values of micronuclei observed after treatment with the test substance were even slightly below the value of the vehicle control groups in all dose groups. Moreover, micronucleus values obtained in all dose groups were very well within the historical negative control range.
The positive control cyclophosphamide showed a statistically significant increase of induced micronucleus frequency thereby ensuring the validity of the test system.

Table B.6.8-76: Micronucleus test in mice administered M656PH047 by oral gavage

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Vehicle	24	0.120	1-4	1287
M656PH047				
500 mg/kg bw	24	0.107	0-4	1241
1000 mg/kg bw	24	0.114	2-3	1240
2000 mg/kg bw	24	0.086	0-5	1234
Positive control				
Cyclophosphamide	24	3.030	57-67	1109
48 h sampling				
Vehicle	48	0.140	2-5	1153
M656PH047				
2000 mg/kg bw	48	0.057	0-4	1208

Conclusion:

The study is considered to be acceptable.
Based on the result of this study M656PH047 did not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Short-term toxicity of M656PH047:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point: KCA 5.8

Report: [redacted] 2014d (ASB2014-8419)
Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet
2014/1018064

Guideline(s): OECD 407, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147

Deviations: No relevant deviations

GLP: Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material:	Reg.No. 5917260, metabolite of dimethenamid-P
Description:	Solid/brown
Batch/purity #:	L82-137, 90.7 %
Stability of test compound:	Stable until 01 Nov 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.
Vehicle and/or positive control:	Rodent diet
Test animals:	
Species:	Rat
Strain:	Wistar Crl:WI (Han) Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 155.9 - 169.8 g, ♀ 118.8 - 145.1 g
Source:	Charles River, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, *ad libitum*
Housing:	Group housing (5 animals per cage) in polysulfonate H cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria and play tunnel large supplied by PLEXX B.V., Elst Netherlands for environmental enrichment. Motor activity measurements were conducted in polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes per hour
Photo period:	12 h light/12 h dark

(06:00 - 18:00/18:00 - 06:00)
Dates of experimental work: 09-Dec-2013 – 05-Mar-2014.
(In life dates: 17-Dec-2013 (start of administration) to 15-Jan-2014 (necropsy)).

Animal assignment and treatment:
M656H047 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1320 (low dose), 4400 (intermediate dose) and 13200 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomisation list based on body weights.
Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Eight diet preparations per dose were performed for this study (preparation twice a week).
Analyses performed prior to the start of the administration period revealed that the test substance was stable in the diet for 4 days.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1320 ppm) and top dose level (13200 ppm) and subsequently analysed. The samples were also used for determination of the test article concentration. For the mid dose level single samples were analysed. No test article was determined in control diets.

Table B.6.8-77: Analysis of diet preparations for homogeneity and test item content

Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
1320 ppm	17. Dez. 13	1313.3 ± 57.8	99.5	4.4
4400 ppm	17. Dez. 13	4346.9	98.8	
13200 ppm	17. Dez. 13	13039.8 ± 332.4	98.8	2.5

n.a.: not applicable;
based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures.

Relative standard deviations of the homogeneity samples in the range of 2.5 to 4.4 % indicate the homogenous distribution of M656H047 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 98.8 to 99.5 % of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H047 in the vehicle.
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-78: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Table B.6.8-79: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine color and turbidity	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table B.6.8-80: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behaviour during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmos
6. respiration	15. faeces discharge during examination (appearance/consistency)
7. activity/arousal level	16. urine discharge during examination
8. tremors	17. pupil size
9. convulsions	

Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Substance intake for day } x = \frac{FC_x \times C}{BW_x}$$

BWx and BWy body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Substance intake for day } x = \frac{FC_x \times C}{BW_x}$$

FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).
Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted.
Ophthalmoscopy:
Not performed in this study.
Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1.	behaviour when removed from cage	10.	respiration
2.	fur	11.	tremors
3.	skin	12.	convulsions
4.	salivation	13.	abnormal movements
5.	nose discharge	14.	impairment of gait
6.	lacrimation	15.	activity/arousal level
7.	eyes/pupil size	16.	faeces (number of faecal pellets/appearance/ consistency) within two minutes
8.	posture	17.	urine (appearance/quantity) within two minutes
9.	palpebral closure	18.	number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behaviour during "handling"
2.	touch response	9.	vocalisation
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

Motor activity measurement:
Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
Haematology and clinical chemistry:
Blood was withdrawn in the morning from fasted, isoflurane anesthetised animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation, these samples were stored frozen but not analysed. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomised sequence.

The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
	Red blood cells		White blood cells		Clotting Potential
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Haemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Haematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
Clinical chemistry:					
	Electrolytes		Metabolites and Proteins		Enzymes:
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Urinalysis:
For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analysed in a randomised order.
The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		Semi quantitative parameters		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

Sacrifice and pathology:
The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:												
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).												
C	W	H		C	W	H		C	W	H		
✓	✓	#	adrenals	✓	✓	#	kidneys	✓			skin	
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)@	
✓		#	bone marrow§	✓		#	larynx	✓	✓	#	spleen	
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow	
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)	
✓		#	coagulating glands£	✓		#	lymph nodes#	✓	✓	#	testes	
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus	
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid	
✓	✓	#	epididymides¥	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea	
✓		#	esophagus	✓		#	nose/nasal cavity‡	✓		#	urinary bladder	
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix	
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina	
			gall bladder	✓			pharynx					
✓		✓	gross lesions	✓		#	pituitary					
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetised animals)	
✓	✓	#	heart	✓		#	rectum					
✓		#	ileum	✓			salivary glands*					
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles£					
§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; ‡l histopathology at level III, ¥left epididymidis collected for histopathology, £seminal vesicles and coagulation weight determined together												

The organs or tissues were fixed in 4 % formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.
The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers

Lymph nodes (mesenteric and axillar lymph nodes):
• Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
• Altered cellular composition of paracortex
• Altered number of germinal centers
• Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):
• Changes of the cellularity of follicles (including mantle zone and germinal centers)
• Changes of the cellularity of interfollicular area
Bone marrow:
• Changes of the cellularity
• Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

Results:

Observations:
No clinical signs were observed throughout the study. No mortality was observed throughout the study.
Ophthalmoscopy was not performed in this study.
FOB and Motor Activity:
Neither home cage nor open field observations revealed any indication of treatment related effects. The same holds true for the sensimotor tests and reflexes. Most deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental. The statistically significantly reduced forelimb grip strength in males of the low dose did not show a dose response relationship and thus was not considered to be treatment related.
With regard to the overall motor activity as well as single intervals no significant deviations were observed between treated animals and the control group.
Body weight and body weight gain:
No test substance related differences with regard to the mean body weights and body weight change values were noted for male and female animals of all dose groups (see Table B.6.8-81, Figure B.6.8-19). The significantly lower body weight change value in female animals of the 13200 ppm group in the interval from day 0 up to day 14 was assessed as being spontaneous in nature and not related to treatment, because no significant change occurred for the other intervals or the absolute mean body weight.

Table B.6.8-81: Mean body weight of rats administered M656H047 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1320	4400	13200	0	1320	4400	13200
Body weight [g]								
- Day 0	164.1	163.7	163.8	164.4	131.1	131.4	130.5	132.3
- Day 28	282.9	294.7	291.8	282.3	187.8	189.1	180.5	182.2
Δ% (compared to control)[#]		4.2	3.1	-0.2		0.7	-3.9	-3
Body weight change [g]								
- day 0-7	44.5	49.3	48.5	44.5	22.2	24.6	19.9	14.8
- day 0-14	80.3	87.7	87.6	81.0	41.2	42.6	35.5	27.9 *
- day 0-21	104.8	115.9	111.5	103.6	51.5	49.5	46.7	40.5
- day 0-28	118.8	131.1	128.0	117.9	56.6	57.7	50.1	49.8
Δ% (compared to control)[#]		10.3	7.7	-0.7		1.9	-11.6	-12

* $p \leq 0.05$ (Dunnett-test, two sided)
[#] Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means).

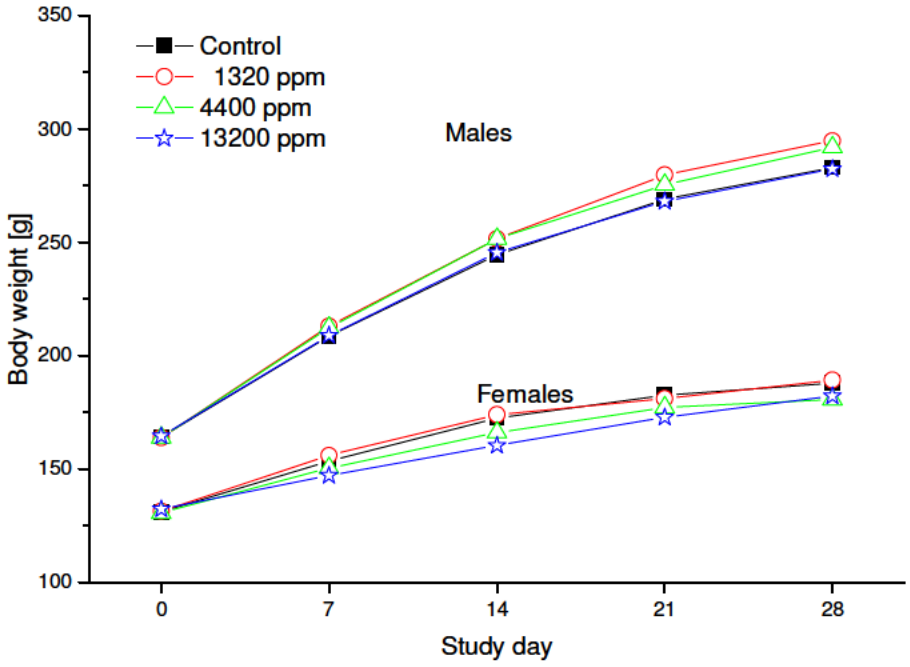


Figure B.6.8-19: Body weight development of rats administered M656H047 for at least 28 days

Food consumption and compound intake:
No test substance related, adverse findings were observed. A trend for an increase of food consumption during administration period was observed in male animals of the high dose group (see Table B.6.8-82). These changes were assessed as being spontaneous in nature and not related to treatment. All other recorded values were within the biological range typical for this strain of rats.

Table B.6.8-82: Mean food consumption of rats administered M656H047 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1320	4400	13200	0	1320	4400	13200
Food consumption [g]								
- Day 3-7	18.5	18.7	18.7	20.0	12.7	13.7	12.0	12.7
- Day 10-14	20.5	20.4	20.9	24.2	14.5	14.8	13.5	13.2
- Day 17-21	21.4	20.9	20.9	25.9	14.0	14.4	14.1	14.2
- Day 24-28	17.8	16.5	16.9	27.2	12.3	13.4	11.3	13.0
Total	78.2	76.5	77.4	97.3	53.5	56.3	50.9	53.1

The mean daily test substance intake over the entire study period was calculated and is shown in the Table B.6.8-83.

Table B.6.8-83: Calculated intake of M656H047

Test group	Concentration in the vehicle (ppm)	Mean daily test substance intake (mg/kg bw/d)	
		Males	Females
1	1320	99	107
2	4400	336	333
3	13200	1280	1066
Intake corrected for purity of M656PH047 of 90.7 %			
1	1200	90	97
2	4000	304	302
3	12000	1161	967

Water consumption:
No test substance related, adverse changes with regard to water consumption were observed.
Blood analysis:
Haematological findings:
No treatment related adverse findings among haematological parameters were observed.
In male animals of the 13200 ppm group, neutrophilic counts were significantly increased as compared to controls (see Table B.6.8-84). This was the only altered differential cell count fraction and even total white blood cell counts were not changed. The neutrophil cell counts in males of this test group were at the upper border of the historical control range. No alteration in haematology occurred in females. Therefore, the higher absolute neutrophil cell counts in males the 13200 ppm were regarded as incidental and not treatment related.

Table B.6.8-84: Selected haematology findings in rats administered M656H047 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		90	304	1161		97	302	967
Neutrophilic leucocytes [giga/L]	0.63	0.56	0.75	1.09*	0.45	0.65	0.63	0.42
[%]	11.7	13.1	15.1	15.7	11.8	14.8	17.6	13.7
	Historical control: 0.53-1.09 giga/L							

*p ≤ 0.05 (Kruskal-Wallis and Wilcoxon-test, two sided)

Clinical chemistry findings:
No treatment related changes among clinical chemistry parameters were observed.
At the end of the study in males of mid and high dose group total bile acid levels were increased (not statistically significant in test group 3). At least in males of 4400 ppm this was the only altered clinical chemistry parameter in these individuals. Therefore, the higher total bile acid levels in males of 4400 ppm were regarded as treatment related, but not adverse (ECETOC 2002a; ASB2014-8405). Regarding the 12000 ppm dose group higher serum total bile acid levels in males (see Table B.6.8-85) as well as increased urinary urobilinogen levels in rats of both sexes in the same test group (see Table B.6.8-86) indicated a higher rate of excretion of bilirubin and bile acids with the bile, followed by a higher intestinal resorption of bile acids and urobilinogen, the latter excreted by the kidneys. This effect might have been a consequence of an increased secondary metabolism induced by the compound. It was regarded as an adaptive and not an adverse effect.

Table B.6.8-85: Selected clinical chemistry findings in rats administered M656H047 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		90	304	1161		97	302	967
Total bile acids [µmol/L]	16.6	25.3	34.6**	44.7	14.5	31.2	17.6	29.4

*p ≤ 0.05 (Kruskal-Wallis and Wilcoxon-test, two sided)

Urinalysis:
In males and females of the 13200 ppm urinary urobilinogen levels were increased and additionally in females of this test group specific gravity of the urine was higher compared to controls. The higher urine specific gravity in females per se without any other finding in clinical pathology was regarded as treatment related, but not adverse. As discussed above the increased urobilinogen levels were attributed to an increased excretion of bilirubin and bile acids with subsequent intestinal resorption of bile acids and urobilinogen the latter being renal excreted. No other treatment related changes among urinalysis parameters were observed.

Table B.6.8-86: Selected urinary parameter findings in rats administered M656H047 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1320	4400	13200	0	1320	4400	13200
[mg/kg bw/day]		99	336	1280		107	333	1066
Urobilinoge^{K}	1	1	2	2*	1	1	1	3**
Specific gravity^{W} [g/L]	1.068	1.062	1.071	1.066	1.057	1.051	1.058	1.093*

*$p \leq 0.05$; ** $p \leq 0.01$
^{K} = Kruskal-Wallis + Wilcoxon test (two sided)
^{W} = Wilcoxon test (one sided)

Necropsy:
Organ weight:
Terminal body weights of treated rats displayed no statistically significant differences to the controls. When compared to the control group, the mean absolute organ weights of treated animals showed no significant deviations.
Gross and histopathology:
A single macroscopic finding (pelvic dilation of the kidney) recorded in males of test group 3 (12000 ppm) belongs to the spectrum of background lesion and was considered to be incidental in nature and not related to treatment.
There was no histopathological finding considered treatment related. All recorded histopathological findings were either single observations, were equally distributed between control and treated groups or displayed no dose response relationship. Therefore, these findings were considered to be incidental.

Conclusion:

The study is considered to be acceptable.
The administration of M656H047 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance related adverse signs of toxicity at concentrations of 1320, 4400 and 13200 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm corresponding to 1161 mg/kg bw/day in male and 967 mg/kg bw/day in female Wistar rats when corrected for purity of 90.7 %.

Metabolite M656PH049 former assigned M49:
M656PH049 is a groundwater metabolite.

Structural alerts for M656PH049:
In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential nucleophilic addition to the carbo-hetero double bond (keto-group) was identified in the OECD and the OASIS module. In contrast no DNA alert was identified for M656PH049 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox provides the alerts based on the functional groups identified only and does not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.
OASIS-Times (see ASB2014-8408) however predicted M656PH049 to be not mutagenic with regard to Ames test alerts without or with metabolic activation with the limitation that the molecule was out of the prediction domain. There was however an alert for *in vitro* chromosomal aberration (see ASB2014-8410) based on the alpha-beta-carbonyls polarised double bonds. Again the prediction

reliability was low as the structure was out of the prediction domain. Moreover the demethylated degradate (contained in the predicted metabolic pathway, molecule 23.1) was considered negative with the difference that this structure was covered by the prediction domain (see Figure B.6.8-20).

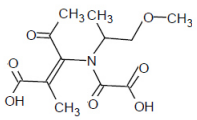
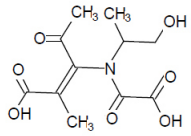
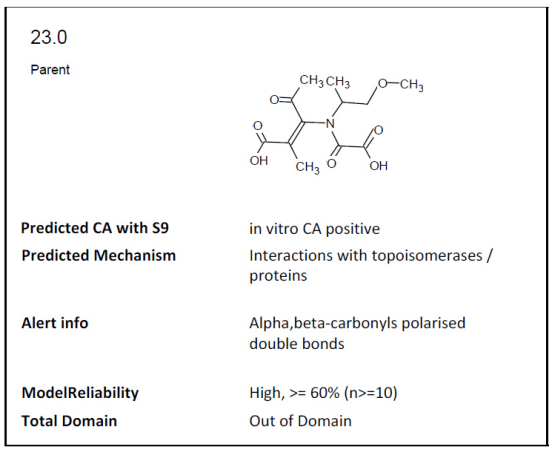
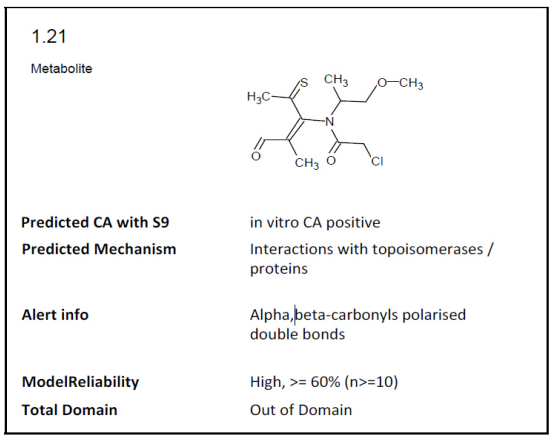
23.0 Parent		23.1 Metabolite	
			
Predicted CA with S9	in vitro CA positive	**Predicted CA with S9**	in vitro CA negative
Predicted Mechanism	Interactions with topoisomerases / proteins	**Predicted Mechanism**	
Alert info	Alpha,beta-carbonyls polarised double bonds	**Alert info**	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)	**ModelReliability**	
Total Domain	Out of Domain	**Total Domain**	In domain

Figure B.6.8-20: M656PH049 and its degradate OASIS times prediction for chromosomal aberration *in vitro*

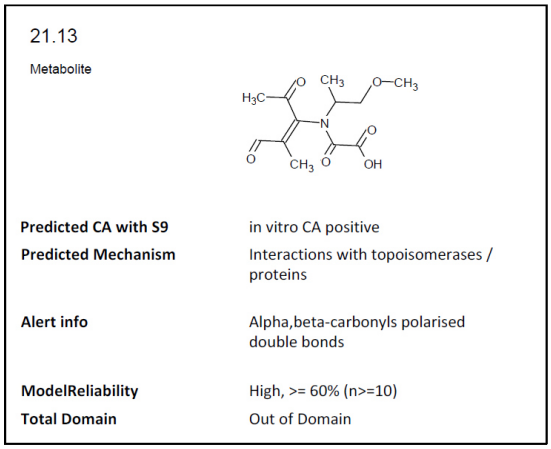
In addition the structural alert of concern was considered to be intrinsically covered by the toxicological testing of dimethenamid-P, and metabolites M656PH023 and M656PH054, as similar degradation products were presumed in their metabolic pathways.



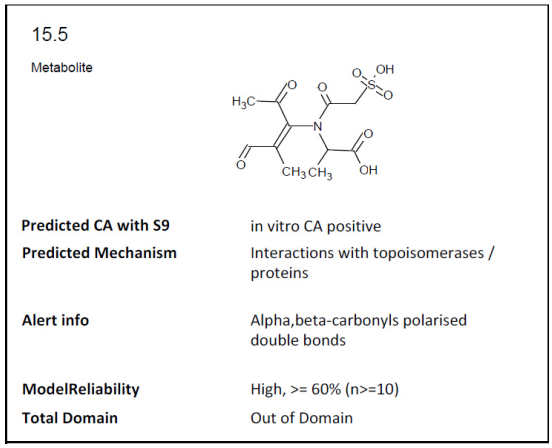
M656PH049



Degradate of dimethenamid-P



Degradate of M656PH023

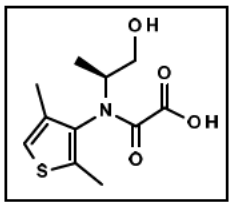


Degradate of M656PH054

Figure B.6.8-21: Coverage of M656PH049 by presumed degradates of tested compounds

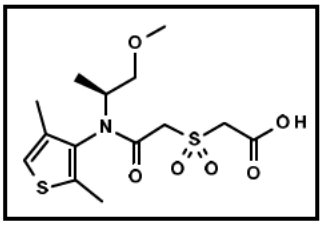
In the DEREK analysis conducted, structural alerts for M656PH049 (DocID 2014/10884549 here called M49a) were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. Moreover, there was also the alert for alpha-beta unsaturated ketone substructure indicating chromosome damage *in vitro*. As this alert is specific for an *in vitro* effect it was not considered relevant by the evaluator in adding an additional risk.
The Vega prediction (ASB2014-8412) in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low. Only moderately similar compounds with known experimental values have been found in the database and some similar molecules found in the database have experimental data that disagree with the prediction. Note: although the structure presented in the input mask of Vega showed the correct ring-open structure, in the output mask it was transferred to a ring-closed structure. Thus there remains some inclarity whether the calculations made are applicable to M656PH049.
The structural alert identified in several of the structure activity relationship models applied was that of unsaturated carbonyl compounds. They are known to present toxicological concerns in biological systems. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl and is mostly characterised for small, low molecular weight molecules (e.g. cyclohexen-1-one and methyl vinyl ketone). This alert is known to become active in *in vitro* systems but in particular for greater complex molecules like M656PH049 the evidence to become effective *in vivo* is lacking.
In conclusion a structural alert for chromosomal aberration *in vitro* was identified in several of the QSAR models employed that is considered of low relevance for mammalian systems *in vivo*. Moreover, the structural alert identified was considered to be covered by the toxicological testing conducted with dimethenamid-P, M656PH023 and M656PH054.

Metabolite M656PH050 formerly assigned M50:
M656PH050 is a ground-water and soybean metabolite, but was not determined in edible commodities.



Structural alerts for M656PH050:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times (see ASB2014-8408) predicted M656PH050 to be not mutagenic in the Ames test without or with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration *in vitro* (see ASB2014-8410) the prediction was negative for the metabolite itself and negative for all presumed transformation products thereof (12 in total). The identified alerts for alpha-beta unsaturated aldehydes, alpha-beta polarised carbonyls were not predicted to become active. The overall prediction was therefore negative for *in vitro* chromosomal aberration, with the limitation that the structures were out of the prediction domain.
In the DEREK analysis conducted structural alerts for M656PH050 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (ASB2014-8412) in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low. No similar compounds with known experimental values have been found in the database, some similar molecules found in the database have experimental data that disagree with the prediction and the accuracy of prediction for similar molecules in the training set is not optimal.
In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

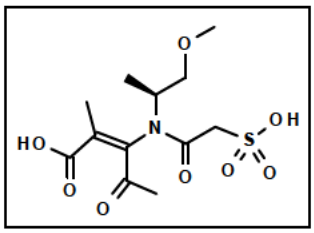
Metabolite M656PH051 former assigned M51:
M656PH051 is a metabolite observed in soybean and rotational crop metabolism but was not observed in any edible commodity. It is also a groundwater metabolite.



Structural alerts for M656PH051:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times predicted M656PH051 (see ASB2014-8408) to be not mutagenic in the Ames test without or with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (see ASB2014-8410) the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into ring-open structures similar to transformation products of M656PH030 and M656PH031. For this alpha-beta unsaturated carbonyls was an alert given for induction of chromosomal aberration by interaction with topomerases/proteins. Again the prediction was out of the total domain for this model.
In the DEREK analysis conducted structural alerts for M656PH051 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction (ASB2014-8413) was inconclusive. The CAESAR module predicted M656PH051 to be mutagenic. The reliability was low as no similar compounds with known experimental data were in the database and as similar molecules found in the database had experimental values that disagree with the prediction. Instead the SarPy module predicted M656PH051 to be not mutagenic. Again the reliability of this prediction was low as no similar compounds with known experimental value were found in the database and as the accuracy of prediction for similar molecules found in the database is not optimal. In conclusion no conclusive relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

Metabolite M656PH052 former assigned M52:
M656PH052 is a groundwater metabolite.



Structural alerts for M656PH052:
In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential nucleophilic addition to the carbo-hetero double bond (keto-group) was identified in the OECD and the OASIS module. In contrast no DNA alert was identified for M656PH052 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox only provides the alerts based on the functional groups identified only and does not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.
OASIS-Times (see ASB2014-8408) predicted M656PH052 to be not mutagenic in the Ames test without and with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (ASB2014-8410) the prediction was positive for the metabolite itself and for the demethylated transformation product thereof based on the structural alert for alpha-beta carbonyls with polarised double bonds. The overall prediction was therefore positive for *in vitro* chromosomal aberration, with the limitation that the structures were out of the prediction domain. As indicated by the TIMES prediction on S9-induced metabolisation formation of such ring opening structures is a plausible metabolic pathway in mammals and thus several similar ring-open thiols and alpha-beta unsaturated carbonyl degradates have been predicted for e.g. dimethenamid-P, M656PH023, M656PH027, M656PH047, M656PH030, M656PH031, M656PH032, M656PH043, M656PH054 (ASB2014-8410).
The identity of considered closest related transformation products is shown in Figure B.6.8-23 below.

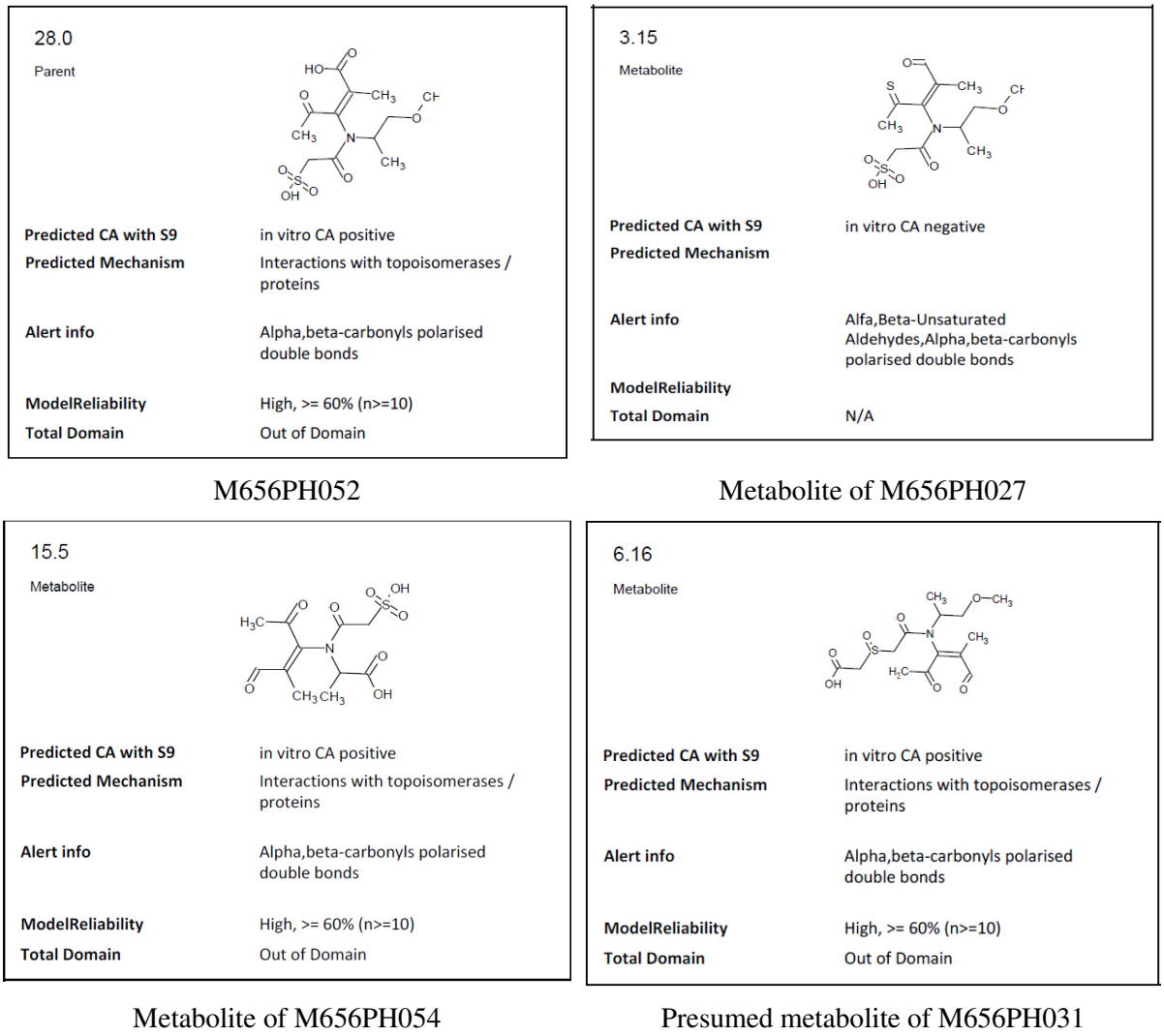


Figure B.6.8-22: Coverage of M656PH052 by presumed degradates of tested compounds

Thus it is reasonable to assume that the toxicological testing conducted with M656PH027, M656PH054 and M656PH031 covers intrinsically potentially structural alerts of M656PH052.
In the DEREK analysis conducted structural alerts for M656PH052 were the alpha-beta-unsaturated ketone alert for chromosome damage *in vitro*. This alert is considered specifically for an *in vitro* effect, not observed *in vivo*, hence this metabolite is not considered to add to any risk of *in vivo* effects of the parent molecule.
The Vega prediction (see ASB2014-8413) was inconclusive. In the CAESAR module the prediction was mutagenic. The reliability of this prediction was low as no similar compounds with known experimental data have been found in the database and similar compounds within the database have experimental values that disagree with the prediction. In particular the molecules identified in the database that gave evidence for mutagenicity contained other structural alerts not identified in M656PH052 that were considered potentially responsible for the mutagenic activity.

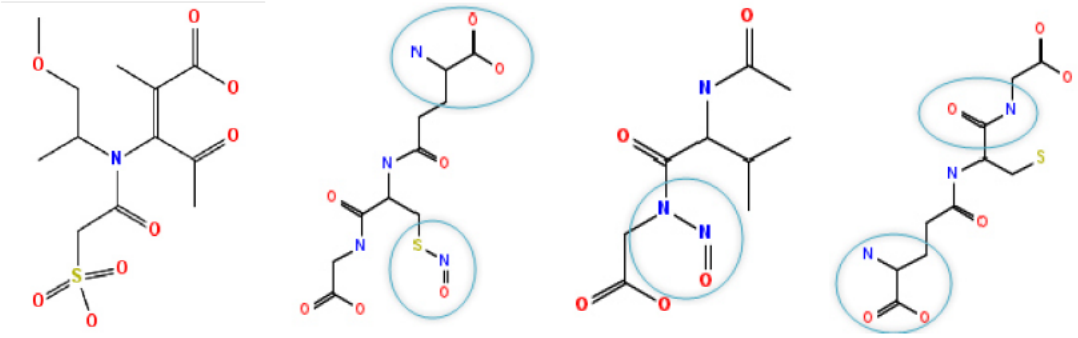
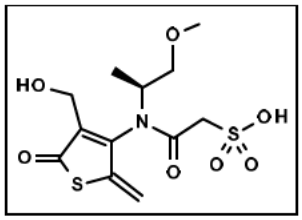


Figure B.6.8-23: Comparison of structural alerts of M656PH052 with similar structures identified by Vega CAESAR as mutagenic

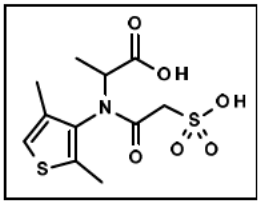
The SarPy module prediction was non-mutagenic, however the reliability of this prediction was also low. No similar molecules with known experimental data were in the database. Some similar molecules with experimental data disagree with the prediction and the accuracy of the prediction for similar molecules is not optimal.

Metabolite M656PH053 former assigned M53/M57:
M656PH053 is a groundwater metabolite.



Structural alerts for M656PH053:
In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential direct acylation was identified in the OECD and the OASIS module. In contrast no alert was identified for M656PH053 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox profiles provide the alerts based on the functional groups identified only and do not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.
OASIS-Times predicted M656PH053 (ASB2014-8408) to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (ASB2014-8410) the prediction was negative for the metabolite itself but positive for a structural alert contained in presumed transformation products thereof. This prediction based on the structural alert for alpha-beta carbonyls with polarised double bonds was not considered to become active as all presumed transformation products were predicted to be negative for chromosomal aberration *in vitro* due to the entire considered structural properties of the molecule (12 in total). The overall prediction was therefore negative for *in vitro* chromosomal aberration, with the limitation that the structures were out of the prediction domain.
The Vega prediction (ASB2014-8413) was negative. The CAESAR and SarPy module predictions were both non-mutagenic, however the reliability of this prediction was also low. No similar molecules with known experimental data were in the database. The accuracy of the prediction for similar molecules is not optimal and a prominent number of atom centred fragments of the molecule have not been found or are rare in the database.

Metabolite M656PH054 former assigned M54/M58:
M656PH054 is a groundwater metabolite.



Structural alerts for M656PH054:
In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule dimethenamid-P. Thus these limited alerts are not considered of relevance.
OASIS-Times predicted M656PH054 (ASB2014-8408) to be not Ames mutagenic with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (ASB2014-8410) the prediction was negative for the metabolite itself but positive for a structural alert contained in presumed transformation products thereof. This prediction based on the structural alert for a ring-opened alpha-beta carbonyl with polarised double (one out of 5 presumed transformation products). The overall prediction was therefore positive for *in vitro* chromosomal aberration, with the limitation that the structures were out of the prediction domain. For such a complex unsaturated system as determined for the transformation product of M656PH054, the relevance of the alert given is however of doubt for the *in vivo* situation.
In the DEREK analysis conducted the structural alerts for M656PH054 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (Molecule 19) in both modules CAESAR and SarPy was not mutagenic. The reliability of these predictions was reasonable as no similar compound with experimental data were in the training set.
Overall there was no alert for Ames mutagenicity in the QSAR models applied. There was however a limited alert for chromosomal aberration *in vitro* in the OASIS times module for a presumed transformation product. The structural alert was that of unsaturated carbonyl compounds. They are known to present toxicological concerns in biological systems *in vitro*. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl building a reactive centre for nucleophilic addition and is mostly characterised for small, low molecular weight molecules. This alert is known to become active in *in vitro* systems but in particular for greater complex molecules like M656PH054 the evidence to become effective *in vivo* is lacking.

Genotoxicity studies of M656PH054:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Woitkowiak C., 2014c (ASB2014-8455) Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhimurium / Escherichia Coli reverse mutation assay 2013/1363556
Guideline(s):	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656PH054 (Reg. No. 5920718, former assigned M54) in the presence and absence of metabolic activation in a plate incorporation assay and in a pre-incubation experiment.

Test Material: Reg.No. 5920718 (Metabolite of BAS 656-PH, dimethenamid-P)
Description: Solid, beige
Lot/Batch #: L82-121
Purity: 85.1 % (tolerance +/- 1.0 %)
Stability of test compound: The stability of the test substance under storage conditions over the test period was guaranteed until 01 Aug 2014 as indicated by the sponsor, and the sponsor holds this responsibility.
The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used: Ethanol
Control Materials:
Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed

with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate. The optical density of the fresh thawed bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 109 cells per mL).

Test concentrations:

Plate incorporation assay: Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 3000 and 6000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: The test substance/vehicle/positive control substance, bacterial and S9-mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 3000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 15-Oct-2013 to 24-Oct-2013

Plate incorporation assay:
To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates).

Pre-incubation assay:
100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9-mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

Statistics:
No special statistical tests were performed.

Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- A dose related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and *E.coli* WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.

Results:

Analytical determinations:
The test substance was stable over the study period under the storage conditions. The stability of the test substance in the vehicle ethanol was verified analytically (BASF Project No.: 01Y0083/13Y010).
Toxicity:
A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
A bacteriotoxic effect (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants) was observed in the standard plate test depending on the test conditions at 6000 µg/plate. In the pre-incubation assay, bacteriotoxicity (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants) was observed depending on the strain and test conditions from about 1000 µg/plate onward.
Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-87).
Test substance precipitation was found in the pre-incubation assay with S9 mix from about 1000 µg/plate onward.

Table B.6.8-87: Bacterial gene mutation assay with M656PH054 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27.3	21.3	65.0	50.0	11.7	11.0	7.7	7.0	73.7	53.3
M656PH054										
33 µg/plate	27.3	19.0	63.0	44.7	8.3	14.3	9.3	7.3	59.7	48.7
100 µg/plate	27.7	17.7	54.3	48.3	9.3	11.3	9.7	9.7	53.0	61.3
333 µg/plate	29.3	16.3	67.0	59.3	9.3	11.3	12.3	8.0	60.0	60.7
1000 µg/plate	25.7	22.7	60.0	52.7	12.0	6.0	9.7	7.0	54.0	56.3
3000 µg/plate	22.7	21.3	44.3[B]	49.3	11.0	8.7	8.0	4.7	58.3	54.0
6000 µg/plate	0.0[B]	15.0[B]	0.0[B]	53.3[B]	7.3[B]	7.3[B]	5.0[B]	3.3[B]	50.0[B]	32.0[B]
Pos. control[§]	2240.0	483.3	2275.0	4092.0	214.7	5197.3	244.0	2277.7	282.0	867.0
Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	32.7	23.0	67.3	37.3	11.0	13.0	11.0	8.0	72.0	64.3
M656PH054										
10 µg/plate	27.3	18.7	64.0	36.0	14.0	10.0	10.3	7.0	75.3	64.7
33 µg/plate	29.3	21.0	58.0	49.3	8.0	8.0	9.0	6.7	79.3	63.0
100 µg/plate	30.0	18.3	65.7	49.3	12.7	11.7	7.7	8.0	70.0	71.3
333 µg/plate	32.7	19.7	65.0	49.7	10.3	10.0	13.3	9.7	67.7	57.7
1000 µg/plate	33.0[BP]	21.0[B]	58.7[BP]	43.3[B]	9.7[BP]	6.3[B]	6.7[BP]	4.3[B]	79.3[BP]	63.7[B]
3000 µg/plate	24.0[BP]	6.3[B]	60.3[BP]	38.3[B]	6.3[BP]	4.3[B]	5.0[BP]	1.7[B]	47.3[BP]	32.3[B]
Pos. control[§]	2087.3	387.7	2382.3	2246.3	215.3	1812.0	199.3	954.7	157.3	327.3

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
[P]= Precipitation
[B] = Reduced Background Growth

Conclusion:

The study is considered to be acceptable.
According to the results of the present study, the test substance M656PH054 was not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point: KCA 5.8

Report: [redacted] 2013a (ASB2014-8462)
Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells
2013/1246093

Guideline(s): OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300, EPA 712-C-98-221

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Data point: KCA 5.8

Report: [redacted]., 2013d (ASB2014-8463)
Amendment No. 1 - Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells
2013/1404743

Guideline(s): OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300, EPA 712-C-98-221

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

M656PH054 (Reg.No. 5920718) was tested *in vitro* for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the TK+/- locus.

Test Material	Reg. No. 5920718 (Metabolite of BAS 656-PH, dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-121
Purity:	85.1 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in ethanol
Solvent used:	Ethanol
Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	Ethanol
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I and III); 13.0 µg/mL (experiment III)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL
Activation:	Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[*a*]pyrene and 2-aminoanthracene

in the Ames test.
The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313) in the pre-experiment and in experiment I and II, and 29.8 mg/mL (Lot. No.: 050913) in experiment III.
An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

Test organism: The L5178Y cell line, which is characterised by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50 %. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with hypoxanthine (5.0×10^{-3} M), aminopterin (2.0×10^{-5} M), thymidine (1.6×10^{-3} M) and glycin (5.0×10^{-3} M) followed by a recovery period of 2 days in RPMI 1640 medium containing hypoxanthine (1.0×10^{-4} M) and thymidine (1.6×10^{-3} M). After this incubation the cells were returned to complete culture medium (see below).

Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15 % horse serum (24 hour treatment, 3 % HS during 4 hour treatment), 1 % of 100 U/100 µg/mL penicillin/streptomycin, 220 µg/mL sodium-pyruvate, and 0.5 - 0.75 % amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, $Na_2HPO_4 \times 2\ H_2O$ 290 mg, KH_2PO_4 150 mg

Locus examined: Thymidine Kinase Locus (TK+/-)

Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 25.1 to 3214 µg/mL

b) Mutation assay:

1st, 2nd and 3rd experiment: 100.4, 200.9, 401.8, 803.5, 1607.0, 3214.0 µg/mL without and with metabolic activation (1st and 2nd experiment)
3800.0 µg/mL without and with metabolic activation (3rd experiment; this experiment was performed after data on the purity of the test item were available to test an additional concentration of 3800 µg/mL. This additional concentration was identical to a molar concentration of approximately 10 mM taking into account the purity of the test item (85.1 %) as provided by the sponsor)

Dates of experimental work: 02-Sep-2013 to 04-Nov-2013

Preliminary cytotoxicity assay:
A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.
1 x 10^7 cells (3 x 10^6 cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3 x 10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

Mutation Assay:
Cell treatment and expression:
In the mutation experiment 1×10^7 (3 x 10^6 during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3 % horse serum (15 % horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3 x 10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.
Selection:
After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4 x 10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5 % CO_2/95.5 % humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.
Size distribution of the colonies:
Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).
Calculations:
Pre-test:

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control

Main test:

total suspension growth (4 h treatment):	(cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)
total suspension growth (24 h treatment):	(cell number at 24 h/ cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at

	24 h < 300000 then cell number at 24 h) x (cell number at 72 h/if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)
relative suspension growth:	total suspension growth x 100/total suspension growth of corresponding control
relative total growth:	relative suspension growth x relative cloning efficiency/100
cloning efficiency (viability):	-ln(mean number of empty wells per plate/96)/cells seeded per well
relative cloning efficiency:	cloning efficiency x 100/cloning efficiency of corresponding control
cells survived:	cloning efficiency x cell number seeded in TFT medium
mutant colonies/106 cells:	small mutant colonies + large mutant colonies
threshold:	number of mutant colonies per 10^6 cells of each solvent control plus 126
cloning efficiency (viability):	cloning efficiency determined after the expression period to measure viability of the cells without selective agent

Statistics:
A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

Evaluation criteria:
The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose related increase in mutant frequencies using an appropriate statistical trend.

If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.
However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10 % of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

Results:

Analytical determinations:
Purity of the test item was verified by HPLC analysis.
Preliminary cytotoxicity assay:
The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h

treatment) of metabolic activation. Test item concentrations between 25.1 µg/mL and 3214 µg/mL were chosen. No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.
Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. In the pre-experiment and in the main experiments the pH was adjusted at the two highest concentrations using 2N NaOH. There was no relevant shift of the osmolarity at the maximum concentration.
The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3214 µg/mL without and with metabolic activation.
Mutagenicity assays:
Relevant cytotoxic effects indicated by a relative total growth of less than 50 % of survival were observed in the second culture of the first experiment at 803.5 µg/mL and above with metabolic activation. In the second experiment cytotoxic effects were noted in both cultures at 803.5 µg/mL and above without metabolic activation, and in the second culture with metabolic activation at 1607.0 µg/mL and above. In the additional third experiment relevant cytoxic effects of less than 50 % were noted in both cultures after 24 hours treatment performed without metabolic activation.
No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. Isolated increases exceeding the threshold of 126 above the corresponding solvent control were noted at 803.5 and 1607.0 µg/mL of the second culture of the first experiment with metabolic activation. These increases however, were not reproduced in the parallel culture at identical experimental conditions or in the second experiment with metabolic activation (see Table B.6.8-88, Table B.6.8-89). A significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was solely detected in the second culture of the second experiment without metabolic activation. This trend however, was judged as irrelevant since it actually was reciprocal, going down versus increasing concentrations.
The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies with at least one of the concentrations of the controls.

Table B.6.8-88: Gene mutation in mammalian cells - 1st experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I/4 h treatment			Culture I			Culture II		
Solv. control with ethanol		-	100.0	137	263	100.0	98	224
Pos. control with MMS	19.5	-	29.2	360	263	29.7	373	224
M656PH054								
	100.4	-	culture was not continued[#]			culture was not continued[#]		
	200.9	-	103.9	105	263	99.4	134	224
	401.8	-	103.3	119	263	57.2	153	224
	803.5	-	114.0	152	263	123.4	96	224
	1607.0	-	93.1	139	263	116.7	98	224
	3214.0	-	96.1	126	263	165.0	46	224
Solv. control with ethanol		+	100.0	96	222	100.0	90	216
Pos. control with CPA	3.0	+	54.4	326	222	45.8	312	216
Pos. control with CPA	4.5	+	22.0	831	222	51.3	532	216
M656PH054								
	100.4	+	culture was not continued[#]			culture was not continued[#]		
	200.9	+	103.0	81	222	101.6	101	216
	401.8	+	128.3	54	222	80.2	151	216
	803.5	+	118.7	62	222	49.0	238	216
	1607.0	+	124.4	63	222	44.2	276	216
	3214.0	+	155.9	54	222	49.3	185	216

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
[#] culture was not continued as a minimum of only four concentrations is required by the guidelines

Table B.6.8-89: Gene mutation in mammalian cells - 2nd and 3rd experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II/24 h treatment			Culture I			Culture II		
Solv. control with ethanol		-	100.0	64	190	100.0	98	224
Pos. control with MMS	13.0	-	10.0	682	190	12.5	1098	224
M656PH054								
	100.4	-	culture was not continued#			culture was not continued#		
	200.9	-	94.7	62	190	102.9	113	224
	401.8	-	164.2	56	190	97.3	125	224
	803.5	-	27.3	46	190	20.1	107	224
	1607.0	-	27.5	64	190	31.0	85	224
	3214.0	-	21.0	83	190	27.2	71	224
Experiment II/4 h treatment								
Solv. control with ethanol		+	100.0	94	220	100.0	101	227
Pos. control with CPA	3.0	+	41.4	277	220	25.9	339	227
Pos. control with CPA	4.5	+	46.8	338	220	18.0	458	227
M656PH054								
	100.4	+	culture was not continued#			culture was not continued#		
	200.9	+	127.1	88	220	93.6	63	227
	401.8	+	123.6	55	220	74.5	108	227
	803.5	+	182.7	81	220	72.3	135	227
	1607.0	+	125.3	71	220	49.0	106	227
	3214.0	+	112.0	104	220	41.9	137	227
Experiment III/4 h treatment			Culture I			Culture II		
Solv. control with ethanol		-	100.0	197	323	100.0	146	272
Pos. control with MMS	19.5	-	18.6	749	323	17.4	604	272
M656PH054	3800.0	-	87.1	195	323	45.2	201	272
Solv. control with ethanol		+	100.0	99	225	100.0	72	198
Pos. control with CPA	3.0	+	53.9	266	225	60.4	204	198
Pos. control with CPA	4.5	+	34.1	449	225	38.6	374	198
M656PH054	3800.0	+	69.5	152	225	56.5	122	198

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment III/24 h treatment								
Solv. control with ethanol		-	100.0	104	230	100.0	93	219
Pos. control with MMS	13.0	-	10.3	392	230	13.9	356	219
M656PH054	3800.0	-	25.5	106	230	27.1	149	219

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test, M656PH054 did not induce forward mutations in the TK+/- locus in L5178Y cells *in vitro*.

Data point: KCA 5.8

Report: [redacted] 2013c (ASB2014-8468)
Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* micronucleus test in Chinese hamster V79 cells
2013/1246092

Guideline(s): OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: *In vitro* Mammalian Cell Micronucleus Test

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

M656PH054 (Reg. No. 5920718) was tested *in vitro* for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in one experiment.

Test Material:	Reg. No. 5920718 (Metabolite of BAS 656-PH, dimethenamid-p)
Description:	Solid, beige
Lot/Batch #:	L82-121
Purity:	85.1 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in ethanol
Solvent used:	Ethanol
Control Materials:	
Negative control:	A negative control was not employed in this study
Solvent control:	Ethanol
Positive controls, -S9:	Mitomycin C (MMC, 0.1 µg/mL, dissolved in deionised water)
Positive control, +S9:	Cyclophosphamide (CPA, 10.0 µg/mL, dissolved in saline)
Activation:	S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three

consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at -80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

Test organisms: Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥70 %).
Culture medium/conditions:
About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) foetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).
Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5-1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.
Test concentrations:
Preliminary toxicity assay:
Cytogenicity assay:
6.3, 12.6, 25.1, 50.2, 100.4, 200.9, 401.8, 803.5, 1607.0, 3214.0 µg/mL with and without metabolic activation
Since the cultures fulfilled the requirements for cytogenetic evaluation, the preliminary test was designated main experiment (see Table B.6.8-90).

Table B.6.8-90: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg.No. 5920718 (Metabolite of BAS 656-PH, Dimethenamid-P)

Preparation interval	Expo-sure period	Concentration in µg/mL									
		Without S9 mix									
24 h	4 h	6.3	12.6	25.1	50.2	100.4	200.9	401.8	803.5	1607.0	3214.0
		With S9 mix									
24 h	4 h	6.3	12.6	25.1	50.2	100.4	200.9	401.8	803.5	1607.0[P]	3214.0[P]

[P]: Precipitation occurred at the end of treatment

Dates of experimental work: 25-Jul-2013 - 06-Aug-2013.

Preliminary cytotoxicity assay: With respect to the molecular weight of the test item, 3214.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 6.3 and 3214.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.
Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose x H_2O, 192 mg/L Na_2HPO_4 x 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 20 hours.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity:
Evaluation was performed manually using microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.
Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

Statistics:
Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.
Evaluation criteria:
A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose related and reproducible increase in the number of cells containing micronuclei is observed

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

Results:

Analytical determinations:
Purity of the test item was verified by Q-NMR analysis.
Cytogenicity assays:
No cytotoxicity was observed up to the highest applied concentration in the presence and absence of metabolic activation. Visible precipitation of the test item in the culture medium was observed microscopically at 1607.0 µg/mL and above in the presence of S9 mix at the end of treatment.
In the absence of S9 mix statistically significant increases in micronucleated cells were observed after treatment with 803.5 and 3214.0 µg/mL (1.05 and 0.85 %). These values were clearly in the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells) and therefore considered as being biologically irrelevant. In the presence of S9 mix statistically significant increases in micronucleated cells clearly exceeding the range of the laboratory historical control data (0.05 - 1.70 % micronucleated cells) were observed after treatment with 200.9, 401.8 and 803.5 µg/mL (2.75, 8.55 and 7.15 %) (see Table B.6.8-91). Since the test item was considered to be mutagenic after the first experiment, only one experiment was performed.
Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table B.6.8-91: Summary of results of the micronucleus test with M656PH054

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 h without S9 mix				
I	24 h	Solvent control[1] Positive control[2] 803.5 1607.0 3214.0	2.82 2.33 2.97 2.94 2.90	0.25 7.85[S] 1.05[S] 0.60 0.85[S]
Exposure period 4 h with S9 mix				
I	24 h	Solvent control[1] Positive control[3] 50.2 100.4 200.9 401.8 803.5 3214.0[P]	2.03 1.63 2.06 2.06 2.00 1.83 1.69 2.12	1.20 10.35[S] 1.30 1.80 2.75[S] 8.55[S] 7.15[S] n.e.

* The total number of micronucleated cells was determined in a sample of 2000 cells
S Number of micronucleated cells statistically significantly higher than corresponding control values
P Precipitation occurred microscopically at the end of treatment
n.e. not evaluated
1 Ethanol 0.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 CPA 10.0 µg/mL

Conclusion:

The study is considered to be acceptable.
Based on the results of the study, M656PH054 induced micronuclei under experimental conditions with metabolic activation in this *in vitro* test.

Data point: KCA 5.8

Report: [redacted] 2014b (ASB2014-8473)
Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1005221

Guideline(s): OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Data point: KCA 5.8

Report: Grauert E., Kamp H., 2014a (ASB2014-8485)
Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in sterile water
2014/1098011

Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Data point:	KCA 5.8
Report:	Becker M., Landsiedel R., 2014e (ASB2014-8480) Analytical report - Reg. No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for external studies 2014/1092437
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656PH054 (Reg. No. 5920718, Metabolite of BAS 656-PH, dimethenamid-P) was tested its ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

Test Material	Reg. No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P)
Description:	Solid, beige to brownish
Lot/Batch #:	L82-136
Purity:	86.5 %
Stability of test compound:	Stable in solvent (Confirmed indirectly by dose formulation analytics)
Solvent used:	Sterile water
Control Materials:	
Negative:	No negative control was employed in this study.
Solvent control:	Sterile water
Positive control:	Cyclophosphamide (CPA) 40 mg/kg bw
Test animals:	
Species:	Mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 11 weeks
Weight at dosing:	35.7 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males/dose/test group, 5 males per vehicle and control group, respectively
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	The animals were housed in groups in Makrolon Type II (pre-test)/III (main study), with wire mesh top.
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	45 - 65 %
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00/18:00 - 06:00)

Test compound concentration:
Range finding test: 1000 and 2000 mg/kg bw (administered once orally)
Micronucleus assay: 500, 1000 and 2000 mg/kg bw
The test substance was administered once orally using an application volume of 10 mL/kg.
Dates of experimental work: 22-Oct-2013 to 21-Nov-2013.

Preliminary cytotoxicity assay:
Male and female NMRI mice were administered the test substance once by oral gavage at a dose of 1000 mg/kg bw (1st pre-experiment) and 2000 mg/kg bw (2nd experiment).

Micronucleus test:
Treatment and sampling:
Groups of male mice were treated once orally with either the vehicle, positive control substance or 500, 1000 and 2000 mg M656PH054/kg bw. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period (48 hours). The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The animals were surveyed for evident clinical signs of toxicity throughout the study.
Twenty-four/48 hours after the administration the mice were killed and the femora were removed. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with foetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet resuspended.
Slide preparation:
A small drop of the resuspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide evaluation:
In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.
Statistics:
Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.
Evaluation criteria:
A test item was considered as mutagenic if it induces either a dose related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods to determine the significance of effects were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results. A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

Results:

Analytical determinations:
The stability of the test substance in the vehicle (water) was verified in a separate study under the responsibility of the sponsor and the results are reported in a separate report.
Preliminary range finding test:
None of the male or female mice died after single oral dosing of 1000 and 2000 mg/kg bw. However, clinical signs including reduction of spontaneous activity, eyelid closure, ruffled fur, abdominal posture, sunken flanks and salivation in the 2000 mg/kg bw dose group. No clinical signs were observed in the 1000 mg/kg bw dose group. On the basis of these data 2000 mg/kg bw were estimated to be suitable as highest dose. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.

Micronucleus assay:
Clinical signs observed in the animals treated with the high dose of 2000 mg/kg bw comprised reduction of spontaneous activity, eyelid closure, ruffled fur, hunchback, tiptoe walk, sunken flanks, moribund condition, discharge from the eyes and swollen abdomen. At necropsy 48 h after application, the stomach of all animals treated with the high dose of the test item was filled with fluid and enlarged. In the mid dose of 1000 mg/kg bw reduction of spontaneous activity and ruffled fur was observed. No clinical signs were observed in any of the animals treated with low of the test substance, the positive control or the vehicle. Concurrent analysis for M656PH054 in plasma (ASB2014-8480) confirmed that M656PH054 was systemically available.
After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control in most mice. Two mice dosed with 2000 mg/kg showed lower PCE numbers 48 hours after treatment.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were near to the value of the vehicle control group and within the historical vehicle control data range (see Table B.6.8-92).
The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (2.97 %), thereby demonstrating the sensitivity of the test system.

Table B.6.8-92: Micronucleus test in mice orally administered M656PH054

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Sterile water	24	0.090	1-3	1184
M656PH054				
500 mg/kg bw	24	0.107	0-3	1178
1000 mg/kg bw	24	0.129	0-7	1167
2000 mg/kg bw	24	0.143	1-6	1062
Positive control				
Cyclophosphamide	24	2.970	33-81	1089
48 h sampling				
Sterile water	48	0.100	1-5	1225
M656PH054				
2000 mg/kg bw	48	0.114	0-5	929

Conclusion:

The study is considered to be acceptable.
Based on the results of this study, M656PH054 did not induce the formation of micronuclei in mouse polychromatic erythrocytes under the selected conditions.

Short-term toxicity of M656PH054:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point: KCA 5.8

Report: [redacted] 2014e (ASB2014-8420)
Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) -

	Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018065
Guideline(s):	OECD 407, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Test Material:	Reg.No. 5920718, metabolite of dimethenamid-P
Description:	solid/beige to brownish
Batch/purity #:	L82-136/86.5 %
Stability of test compound:	Stable until 01 Nov 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.
Vehicle and/or positive control:	Rodent diet
Test animals:	
Species:	Rat
Strain:	Wistar Crl:WI (Han) Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 167.1 ± 7 g, ♀ 127.1 ± 4.6 g
Source:	Charles River, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, *ad libitum*
Housing:	Group housing (5 animals per cage) in polysulfonate H cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria and play tunnel large supplied by PLEXX B.V., Elst Netherlands for environmental enrichment Motor activity measurements were conducted in polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes per hour
Photo period:	12 h light/12 h dark (06:00 - 18:00/18:00 - 06:00)
Dates of experimental work:	09-Dec-2013 – 05-Mar-2014. (In life dates: 19-Dec-2013 (start of administration) to 17-Jan-2014 (necropsy)).

Animal assignment and treatment:
M656H054 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1390 (low dose), 4630 (intermediate dose) and 13900 ppm (top dose). As body weight loss was determined in all males of the high dose group between study day 0 and study day 7, the diet concentration was reduced to 9250 ppm from study day 7 onwards. For all female dose groups as well

as for the other male dose groups the concentrations remained throughout the 28-day study period. The animals were assigned to the treatment groups by means of a computer generated randomisation list based on body weights.
Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Three diet preparations per dose were performed for this study.
Analyses performed prior to the start of the administration period revealed that the test substance was stable in the diet for at least 10 days.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1390 ppm) and top dose level (13900 ppm) and subsequently analysed. The samples were also used for determination of the test article concentration. For the mid dose level and for the reduced high dose for males single samples were analysed. No test article was determined in control diets.

Table B.6.8-93: Analysis of diet preparations for homogeneity and test item content

Dose level	Sampling	Concentration Mean ± SD [ppm]	% of nominal concentration	Relative standard deviation [%]
1390 ppm	18. Dec. 13	1256.9 ± 51[#]	90.4	4.1
4630 ppm	18. Dec. 13	4957.7	107.1	n.a.
9250 ppm	26. Dec. 13	8965.6	96.9	n.a.
13900 ppm	18. Dec. 13	12836.7 ± 101[#]	92.4	0.8

n.a.: not applicable;
[#] based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures.

Relative standard deviations of the homogeneity samples in the range of 0.8 to 4.1 % indicate the homogenous distribution of M656H054 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 90.4 to 107.1 % of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H054 in the vehicle.
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-94: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means.
faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians.

Table B.6.8-95: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine colour and turbidity	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians. For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table B.6.8-96: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behaviour during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmos
6. respiration	15. faeces discharge during examination (appearance/consistency)
7. activity/arousal level	16. urine discharge during examination
8. tremors	17. pupil size
9. convulsions	

Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.
Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BWx and BWy body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).
Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted.
Ophthalmoscopy:
Not performed in this study.
Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1.	behaviour when removed from cage	10.	respiration
2.	fur	11.	tremors
3.	skin	12.	convulsions
4.	salivation	13.	abnormal movements
5.	nose discharge	14.	impairment of gait
6.	lacrimation	15.	activity/arousal level
7.	eyes/pupil size	16.	faeces (number of faecal pellets/appearance/ consistency) within two minutes
8.	posture	17.	urine (appearance/quantity) within two minutes
9.	palpebral closure	18.	number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behaviour during "handling"
2.	touch response	9.	vocalisation
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

Motor activity measurement:
Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
Haematology and clinical chemistry:
Blood was withdrawn in the morning from fasted, isoflurane anesthetised animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation, these samples were stored frozen but not analysed. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomised sequence.

The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
	Red blood cells		White blood cells		Clotting Potential
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Haemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Haematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
Clinical chemistry:					
	Electrolytes		Metabolites and Proteins		Enzymes:
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Urinalysis:
For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analysed in a randomised order.
The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		Semi quantitative parameters		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Colour and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

Sacrifice and pathology:
The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	✓	kidneys£	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)@
✓		#	bone marrow§	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands£	✓		#	lymph nodes#	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides¥	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity‡	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetised animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles£				

§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; £ additional stained with CAB for hyaline droplets, * mandibular and sublingual; ** oviduct not weighed; ‡ l histopathology at level III; ¥ left epididymidis collected for histopathology; £ seminal vesicles and coagulation weight determined together.

The organs or tissues were fixed in 4 % formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:
• Increased/decreased grade of cortico-medullar ratio (related only to area)
• Increase of starry sky cells
• Changes of cellular density in the cortex
• Changes of cellular density in the medulla
Spleen:
• Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
• Altered cellular composition of follicles
• Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):
• Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
• Altered cellular composition of paracortex
• Altered number of germinal centers
• Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):
• Changes of the cellularity of follicles (including mantle zone and germinal centers)
• Changes of the cellularity of interfollicular area
Bone marrow:
• Changes of the cellularity
• Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

Results:

Observations:
No clinical signs were observed throughout the study. No mortality was observed throughout the study.
Ophthalmoscopy was not performed in this study.
FOB and Motor Activity:
Neither home cage nor open field observations revealed any indication of treatment related effects. The same holds true for the sensimotor tests and reflexes including quantitative parameters. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
With regard to the overall motor activity as well as single intervals no significant deviations were observed between treated animals and the control group.
Body weight and body weight gain:
In the first week of treatment males of the high dose group showed significant body weight loss in combination with a severe reduced food consumption (Table B.6.8-97, Table B.6.8-102, Figure B.6.8-24). Although not significantly altered, a tendency to impaired body weight development was also observed in female animals after 7 days of treatment. Thus it was decided to reduce the diet concentration of that dose group from day 7 onwards from 13900 towards 9250 ppm. After the dose reduction the male animals showed an increased body weight gain from day 7 to 14, but body weight was still significantly reduced on day 14. The final body weight (94.8 % of control) and the overall body weight gain (86.4 % of control) still showed a trend of being lower than control. In the female

high dose group (13900/9250 ppm) a slight, not significantly reduced body weight development was noticed throughout the study, leading to a final significantly reduced body weight of 92.7 % of control and a not statistically significant reduction in body weight gain of 82.6 % of control, respectively (Table B.6.8-97).

Table B.6.8-97: Mean body weight of rats administered M656H054 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1390	4630	13900/9250[1]	0	1390	4630	13900 / 9250[1]
Body weight [g]								
- Day 0	166.8	166.5	166.4	168.5	128.1	129.9	125.4	124.9
- Day 7	211.4	212.1	211.7	153.2**	149.1	147.3	147	140.9
- Day 14	248.7	248.6	251.5	228.1*	165.6	170.2	158.1	157
- Day 21	267.6	278.2	281.4	260.3	183.8	181	176.2	170.2
- Day 28	291.8	297.4	295.0	276.5	189.4	183.9	179.9	175.5*
Δ% (compared to control)[#]		1.9	1.1	-5.2		2.9	-5	-7.3
Body weight gain [g]								
d 0 -> 7	44.6	45.5	45.3	-15.4**	21	17.4	21.6	16
d 0 -> 14	81.9	82.1	85.1	59.5**	37.5	40.3	32.8	32.2
d 0 -> 21	100.8	111.7	115.0	91.8	55.7	51.1	50.9	45.4
d 0 -> 28	125.0	130.9	128.5	108.0	61.3	54.0	54.5	50.6
Δ% (compared to control)[#]		4.7	2.9	-13.6		11.9	-11.1	-17.4

1 Due to significant weight loss from day 0 to day 7 the diet concentration of the high dose group was reduced to 9250 ppm from study day 7 onwards.
* $p \leq 0.05$; $p \leq 0.01$; Dunnett test (two-sided)
# Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means).

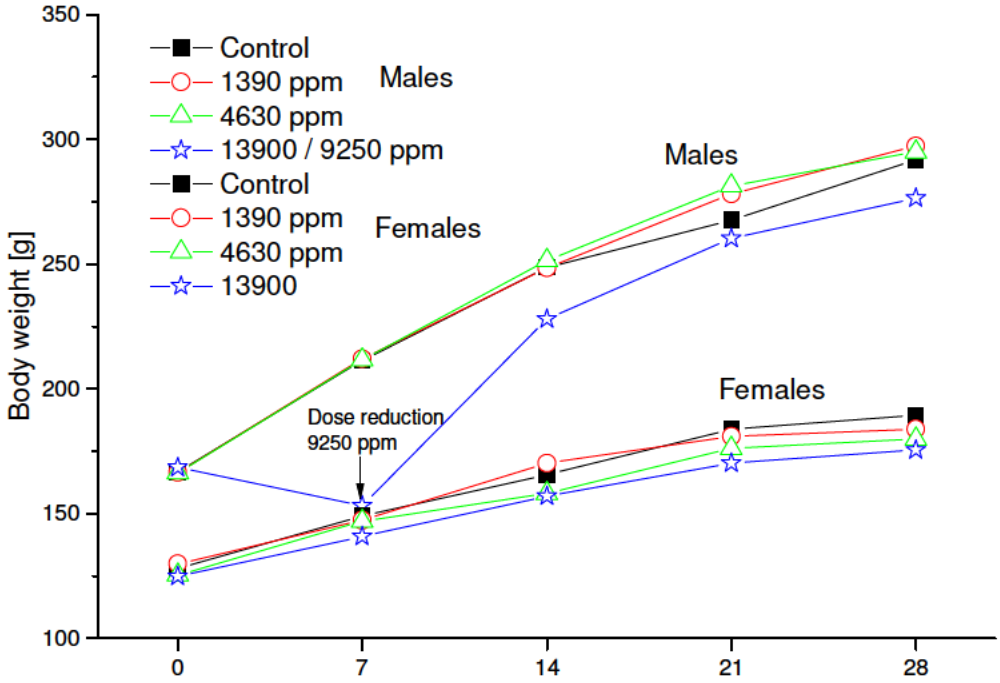


Figure B.6.8-24: Body weight development of rats administered M656H054 for at least 28 days

Food consumption and compound intake:
In the high dose males a severe decrease in food consumption was observed for males between days 4-7. After the reduction of the diet concentration on study day 7 the food consumption was comparable to control group values (Table B.6.8-98). All other recorded values were within the biological range typical for this strain of rats. Increased food spilling was observed for male animals of the 4630 ppm between study days 11 to 14 and 18 to 21, thus the group mean values were declared as outliers and not given.

Table B.6.8-98: Mean food consumption of rats administered M656H054 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1390	4630	13900/9250[1]	0	1390	4630	13900/9250[1]
Food consumption [g]								
- Day 4-7	19.4	20.9	20.5	7.1	14.1	14.3	14.7	13.4
- Day 10-14	21.1	24.1	21.5	22.5	15.0	15.9	-	14.1
- Day 17-21	19.8	32.0	21.8	21.1	15.6	21.1	-	15.3
- Day 24-28	23.9	29.2	25.2	24.3	16.9	16.8	24.4	16.8
Total	84.2	106.2	89.0	75.0	61.6	68.1	39.1	59.6

1 Due to significant weight loss from day 0 to day 7 the diet concentration of the high dose group was reduced to 9250 ppm from study day 7 onwards.

The mean daily test substance intake over the study period calculated on the values for week 2 to 4 is shown in the following (see Table B.6.8-99).

Table B.6.8-99: Calculated intake of M656H054

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1390	142	140
2	4630	400	546
3	9250 (from day 7 onwards)	826	850
	13900 (until day 7)	645	1319
Intake corrected for purity of M656PH054 of 86.5 %			
1	1200	123	120
2	4000	346	472
3	8000 (from day 7 onwards)	714	736
	12000 (until day 7)	558	1141

Water consumption:
No test substance related, adverse changes with regard to water consumption were observed.
Blood analysis:
Haematological findings:
No treatment related changes among haematological parameters were observed.
Clinical chemistry findings:
No treatment related changes among clinical chemistry parameters were observed. In males of the low 1390 ppm dose group triglyceride levels were higher compared to controls, but the increase was not dose dependent. Therefore, this alteration was regarded as incidental and not treatment related.

Table B.6.8-100: Selected clinical chemistry findings in rats administered M656H054 for at least 28 days (group means)

Sex		Males				Females			
Dose	[ppm]	0	1200	4000	12000/ 8000	0	1200	4000	12000/ 8000
	[mg/kg bw/day]		123	346	558/ 714		120	472	1141/ 736
Triglycerides	[mmol/L]	0.90	1.28*	0.76	0.85	0.56	0.51	0.49	0.54

*$p \leq 0.05$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Urinalysis:
No treatment related changes among urinalysis parameters were observed.
Necropsy:
Organ weight:
Terminal body weights of treated rats displayed no statistically significant differences to the controls (Table B.6.8-101).
When compared to the control group, the only significant deviation was the decrease in absolute testes weights of the high dose group (13900/9250 ppm). The decreased absolute weight of testes in male animals of test group 3 (13900 ppm) could be explained by the decreased terminal body weight in these animals. Additionally, there was no histopathological finding correlating to the decreased weight. Therefore, the decreased absolute weight of testes was not regarded as a treatment related effect. All other mean absolute organ weights of either male or female animals treated with M656PH054 showed no significant deviations.
In relation to terminal body weight no significant deviations from control were determined.

Table B.6.8-101: Selected mean absolute and relative organ weights of rats administered M656H054 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000/ 8000	0	1200	4000	12000/ 8000
[mg/kg bw/day]		123	346	558/ 714		120	472	1141/ 736
Terminal body weight [g]	265.96	269.5	267.3	253.34	172.34	168.9	165.38	161.36
[% of control]	100	101	101	95	100	98	96	94
Testes weight, absolute [g]	3.53	3.568	3.296	3.176**				
[% of control]	100	101	93	90				
Testes weight, relative [%]	1.33	1.326	1.238	1.253				
[% of control]	100	100	93	94				

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Gross and histopathology:
A single macroscopic finding (pelvic dilation of the kidney) recorded in one male of the low dose group (1390 ppm) belongs to the spectrum of background lesion and was considered to be incidental in nature and not related to treatment.
All histopathological findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.
The kidneys of male animals all dose groups (1390, 4630 as well as 13900 and 9250 ppm) showed a marginally increased severity of eosinophilic droplets in proximal tubular epithelial cells (Table B.6.8-102). Since there was no clear dose response relationship and since the grading and severity of eosinophilic droplets in treated animals were within the range of historical control data, the finding was regarded not to be treatment related.

Table B.6.8-102: Selected histopathological findings of rats administered M656H054 for at least 28 days

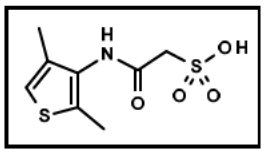
Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000/ 8000	0	1200	4000	12000/ 8000
[mg/kg bw/day		123	346	558/ 714		120	472	1141/ 736
Animals in group	5	5	5	5	5	5	5	5
Kidneys, eosinophilic droplets	5	5	5	5	0	0	0	0
[%]	100	100	100	100				
minimal	5	2	4	2				
moderate	0	3	1	3				
	Historical control: 0-100 %; grading minimal up to moderate							

Conclusion:

The administration of M656H054 via the diet to male and female Wistar rats for 4 weeks test substance related adverse signs of toxicity taking into account reduced food consumption in male

animals as well as impaired body weight development in male and female animals at concentrations of 13900 ppm in males leading to a concentration reduction to 9250 ppm from day 7 of treatment onwards. No adverse signs of toxicity were noticed at the low dose (1390 ppm) or mid dose (4360 ppm).
Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) in Wistar rats was 4000 ppm corresponding to 346 mg/kg bw/day in males and 472 mg/kg bw/day in females when corrected for purity of 86.5 %.

Metabolite M656H055 former assigned M55:
M656H055 is a groundwater metabolite.



Structural alerts for M656H055:
In the OECD-toolbox no alerts for DNA-binding were identified. The only alert for genotoxicity (H-acceptor path) was also identified for the parent molecule dimethenamid-P. There was a deviating alert for protein-binding in the OASIS-module by amide formation via ester-aminolysis, instead of nucleophilic substitution at the sp3 carbon. The relevance of this alert is however questionable.
OASIS-Times (ASB2014-8408) predicted M656H055 to be not mutagenic in the Ames test with and without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (ASB2014-8410) the prediction was negative for the metabolite itself but positive for structural alerts contained in presumed transformation products thereof. This prediction based on the one side on the structural alert for alpha-beta carbonyl with polarised double bond. The presumed transformation products were partly in the predictivity domain. Moreover there was an alert for cleavage into primary aromatic amine structures whose predicted chromosomal aberration was again in the prediciticitivity domain. The overall prediction was therefore positive for *in vitro* chromosomal aberration and considered reasonable.
In the DEREK analysis conducted the structural alerts for M656H055 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (Molecule 16) in both modules CAESAR and SarPy was not mutagenic. The reliability of these predictions was reasonable as no similar compound with experimental data were in the training set.
In conclusion, there was an alert for chromosomal aberration *in vitro*.

Genotoxicity studies of M656H055:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Woitkowiak C., 2012a (ASB2014-8456) Reg.No. 5749263 (metabolite of BAS 656 H, dimethenamid) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2012/1220415
Guideline(s):	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656H055 (Reg.No. 5749263, metabolite of dimethenamid) using ultrapure water as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and a pre-incubation experiment.

Test Material: Reg.No. 5749263 (Metabolite of BAS 656 H, Dimethenamid)
Description: Solid, beige
Lot/Batch #: L80-154
Purity: 69.8 % (tolerance +/- 1.0 %)
Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Nov 2013 as indicated by the sponsor, and the sponsor holds this responsibility.
The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used: Ultrapure water
Control Materials:
Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control: The vehicle control with and without S9-mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed

with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

Test concentrations:

Plate incorporation assay:
(1st experiment): Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 3600 and 7200 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay:
(2nd experiment): The test substance/vehicle/positive control substance, bacterial and S9-mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 3600 and 7200 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 16-May-2012 to 25-May-2012

Plate incorporation assay: To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with *E. coli* the Vogel Bonner agar plates were replaced by plates containing a SA1 selective agar according to Green and Muriel.

Pre-incubation assay: 100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9-mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

Titer determination: In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle

control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

Statistics:
No special statistical tests were performed.
Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- A dose related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

Results:

Analytical determinations:
The test substance was stable over the study period under the storage conditions.
Purity of the test item was verified by Q-NMR analysis.
Toxicity:
A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
No bacteriotoxic effect (reduced his- or trp-background growth, decrease in the number of his+ or trp+ revertants, reduction in the titer) was observed in the standard plate test and in the pre-incubation assay up to the highest required concentration.
Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-103).
Precipitation was not observed up to the maximum concentration.

Table B.6.8-103: Bacterial gene mutation assay with M656H055 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		*E. coli*	
1st experiment: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	31	19	88	72	15	10	8	6	82	73
M656H055										
33 µg/plate	27	20	90	73	13	12	9	7	89	79
100 µg/plate	28	15	93	73	11	10	10	6	91	72
333 µg/plate	22	17	88	68	16	13	8	7	88	82
1000 µg/plate	31	20	91	73	16	9	8	6	88	83
3600 µg/plate	29	18	82	71	14	12	5	6	81	80
7200 µg/plate	29	17	87	72	15	11	10	5	95	88
Pos. control§	762	632	817	608	308	768	180	423	248	1142
2nd experiment: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	28	21	90	79	13	11	8	6	44	36
M656H055										
33 µg/plate	26	20	91	79	12	13	9	7	43	41
100 µg/plate	26	21	92	81	12	10	8	6	46	41
333 µg/plate	26	19	82	87	12	11	7	7	42	37
1000 µg/plate	33	20	100	86	12	11	8	7	45	40
2800 µg/plate	27	18	87	82	13	11	6	6	41	37
5600 µg/plate	23	18	98	81	10	12	7	6	41	40
Pos. control§	715	627	874	731	139	720	141	448	265	749

§ = Compound and concentrations see Material and Methods (I.A.2.) above

Conclusion:

According to the results of the present study, the test substance M656H055 is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point: KCA 5.8

Report: [redacted] 2013a (ASB2014-8488)
Reg.No. 5749263 (metabolite of BAS 656 H, dimethenamid) - *In vitro* gene mutation test in CHO cells (HPRT locus assay)

2013/1282610

Guideline(s): OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300

Deviations: No relevant deviations

GLP: Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Acceptability: The study is considered to be acceptable.

Materials and methods:

M656H055 (Reg. No. 5749263, metabolite of dimethenamid) was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells.

Test Material: Reg. No. 5749263 (Metabolite of BAS 656 H, Dimethenamid)
Description: Solid, beige
Lot/Batch #: L80-154
Purity: 69.8 % (tolerance +/- 1.0 %)
Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Nov 2013 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used: Culture medium
Control Materials:
Negative control: A negative control was not employed in this study
Solvent control: Culture medium
Positive control -S9: Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9: 7,12-Dimethylbenz[a]anthracene (DMBA) 1.25 µg/mL
Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organism: Chinese hamster CHO cells with a high proliferation rate (doubling time of about 12 – 16 h), high plating efficiency (about 90 %) and a karyotype with a modal number of 20 chromosomes. Stocks of the CHO cell line were maintained at -196 °C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-

deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

Culture media:

Culture medium: Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10 % (v/v) foetal calf serum (FCS).

Pretreatment medium: ("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.

Selection medium: ("TG" medium): L-glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL and 1 % (v/v) stable glutamine (200 mM)

All media were supplemented with:

- 1 % (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1 % (v/v) amphotericin B (250 µg/mL)

During pulse exposure (4 hours) to the test substance, Ham‘s F12 medium was used without FCS supplementation. In the case of continuous treatment (24 hours) Ham‘s F12 medium with FCS supplementation was used.

Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 14.1 to 3600 µg/mL

b) Mutation assay:

1st experiment: 450, 900, 1800 and 3600 µg/mL with and without metabolic activation

2nd experiment: 450, 900, 1800 and 3600 µg/mL without metabolic activation
625, 1250, 2500 and 3600 µg/mL with metabolic activation

Dates of experimental work: 22-Apr-2013 to 06-Aug-2013.

Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with 9 test substance concentrations in serum free Ham's F12 medium for about 4 hours (with and without metabolic activation) or 24 hours (only without metabolic activation) after an attachment period of 20 - 24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 - 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

Mutation Assay:

Pretreatment of Cells:

Cells with a passage number ≥2 after thawing from the frozen cells stock were seeded into 75-cm^2 flasks and incubated with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3 - 4 days.

Cell treatment:

For each test group, about 1x106 cells per flask were seeded into 175-cm^2 flasks containing about 20 mL Ham's F12 medium supplemented with 10 % FCS and incubated for about 20 - 24 hours with 5 % CO_2 at 37 °C and >90 % humidity for cell attachment. 2 flasks were used for each test group.

After the cell attachment period the medium was replaced by the treatment medium. In case of experiments without metabolic activation the treatment medium consisted of 18 mL Ham's F12 medium without FCS plus 2 mL positive/vehicle control or test substance. In case of metabolic activation the treatment medium consisted of 14 mL Ham's F12 medium without FCS, 2 mL vehicle control or test substance preparation and 4 mL S9-mix. Analogously, for the positive control group 16 mL of medium was supplemented with 0.2 mL vehicle and 4 mL S9-mix. For the exposure period of more than 4 hours Hams´s F12 medium with 10 % FCS was used.

Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5 % CO_2, 37 °C and ≥90 % humidity.

Expression:
After incubation for 4 or 24 hours, respectively, the treatment medium was replaced by at least 20 mL Ham's F12 medium with 10 % FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of about 3 days following the 4 hour exposure or 2 days following the 24 hour exposure period. After an entire expression period of 7 - 9 days the cells were transferred into the selection medium (2nd passage).
Selection:
For the mutant selection, six 75-cm^2 flasks each were seeded with 3 x 10^5 cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.
Determination of Cytotoxicity:
Cloning efficiency 1 (survival):
The survival (cloning efficiency 1; CE1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25-cm^2 flasks using 5 mL Ham`s F12 medium with 10 % FCS. After a 20 - 24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 or 24 hours as described above. At the end of the treatment period the cells were washed with HBSS and the treatment medium was replaced by Ham's F12 medium with 10 % FCS. After a further incubation for about 5 to 8 days the colonies were fixed, stained and counted.
Cloning efficiency 2 (viability):
The viability (cloning efficiency 2; CE2) was determined in parallel to the selection of mutants after the expression period under the same conditions as described for cloning efficiency 1.
Calculations:
Mutant frequency:
Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\,absolute}} \times 100$$

Cloning efficiency (CE, %):
absolute:

$$CE_{absolute} = \frac{\text{total number of colonies in the test group}}{\text{total number of seeded cells in the test group}} \times 100$$

relative, in comparison to control:

$$CE_{relative} = \frac{\text{CE of the dose group}}{\text{CE of the vehicle control}} \times 100$$

Statistics:
Due to the negative findings, a statistical evaluation was not carried out.
Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies (MFcorr.) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency (MFcorr.) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

Results:

Analytical determinations:
The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor.
Preliminary cytotoxicity assay:
In the preliminary experiment in the presence and absence of metabolic activation a significant test substance induced cytotoxicity leading to a reduction of relative cloning efficiency below 20 % was not observed. The relative cloning efficiency (CE1 relative) after treatment with the highest concentration (3600 µg/mL) ranged between 72.6 to 111.7 % depending on the treatment interval and metabolic activation.
Precipitation of the test substance was not observed up to the highest tested concentration with and without metabolic activation. In the pre-test the parameters pH value and osmolarity were not influenced by the addition of the test substance preparation to the culture medium at the concentrations measured.
Based on these data the highest concentration tested in the mutagenicity experiments was 3600 µg/mL without and with metabolic activation.
Mutagenicity assays:
A significant cytotoxic effect was not observed in both experiments up to the highest tested concentration irrespective of treatment interval and presence of metabolic activation. The obtained relative cloning efficiency did not drop below 85.9 % under any of the tested conditions.
A relevant increase in the number of mutant colonies was not observed in both experiments with and without metabolic activation (see Table B.6.8-104).
The mutant frequencies obtained at any tested concentration with or without metabolic activation were close to the range of the concurrent vehicle control and within the range of the historical negative control data.
The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. Test substance precipitation did not occur up the highest tested concentration of 3600 µg/mL.
Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Table B.6.8-104: Gene mutation in mammalian cells

Exp.	Exposure period [h]	Test groups [µg/mL]	S9 mix	Prec.*	Genotoxicity ** MF corr. [per 10^6 cells]	Cytotoxicity ***	
						CE_1 [%]	CE_2 [%]
1	4	Negative control	-	-	9.16	100.0	100.0
		M656H055					
		450.0	-	-	5.26	96.0	105.8
		900.0	-	-	5.44	108.2	115.1
		1800.0	-	-	1.88	105.7	117.8
		3600.0	-	-	2.23	102.3	112.1
		Positive control[1]	-	-	202.16	116.4	85.6
2	24	Negative control	-	-	4.61	100.0	100.0
		M656H055					
		450.0	-	-	0.38	101.8	110.4
		900.0	-	-	3.33	98.0	116.3
		1800.0	-	-	0.71	95.0	109.2
		3600.0	-	-	2.64	85.9	102.4
		Positive control[1]	-	-	227.43	80.4	95.3
1	4	Negative control	+	-	5.50	100.0	100.0
		M656H055					
		450.0	+	-	3.57	112.5	102.8
		900.0	+	-	1.99	97.9	108.5
		1800.0	+	-	3.99	100.2	112.7
		3600.0	+	-	7.40	103.6	116.0
		Positive control[2]	+	-	438.70	90.9	87.8
2	4	Negative control	+	-	1.11	100.0	100.0
		M656H055					
		625.0	+	-	3.39	93.4	95.8
		1250.0	+	-	3.21	88.6	98.4
		2500.0	+	-	0.36	92.9	96.9
		3600.0	+	-	2.05	94.9	94.3
		Positive control[2]	+	-	416.09	70.0	79.9

* Precipitation in culture medium at the end of exposure period
** Mutant frequency MFcorr: mutant colonies per 10^6 cells corrected with the CE_2 value
*** Cloning efficiency related to the respective vehicle control
1 EMS 300 µg/mL
2 DMBA 1.25 µg/mL

Conclusion:

The study is considered to be acceptable.
Based on the results of the study and under the conditions of the test M656H055 did not induce forward mutations in the HPRT locus in CHO cells *in vitro*.

Data point:	KCA 5.8
Report:	[redacted] 2013a (ASB2014-8474) Reg. No. 5749263 (metabolite of BAS 656 H, dimethenamid) - Micronucleus assay in bone marrow cells of the mouse intraperitoneally administration 2012/1205857
Guideline(s):	OECD 474 (1997), EPA 870.5395, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12
Deviations:	No relevant deviations
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

M656H055 (Reg.No. 5749263, metabolite of dimethenamid) was tested for its ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

Test Material	Reg. No. 5749263 (metabolite of BAS 656 H, dimethenamid)
Description:	Solid, beige
Lot/Batch #:	L80-154
Purity:	69.8 %
Stability of test compound:	stable in solvent (Confirmed indirectly by dose formulation analytics
Solvent used:	sterile water
Control Materials:	
Negative:	No negative control was employed in this study.
Solvent control:	sterile water
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw
Test animals:	
Species:	Albino mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	10 - 11 weeks
Weight at dosing:	37.1 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males/dose/test group, 5 males per vehicle and control group, respectively
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (Harlan Laboratories B.V.; Horst; The Netherlands), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	The animals were housed in groups in Makrolon Type II/III, with wire mesh top.
Environmental conditions:	
Temperature:	20 - 24 °C

Humidity:	45 - 85 %
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00/18:00 - 06:00)
Test compound concentration:	
Range finding test:	2068 (per os) and 2000 (i.p.) mg/kg bw
Micronucleus assay:	525, 1000 and 2000 mg/kg bw The test substance was administered once intraperitoneally using an application volume of 20 mL/kg.
Dates of experimental work:	23-Jul-2012 to 05-Sep-2012.

Preliminary cytotoxicity assay:
Male and female NMRI mice were administered the test substance once by oral gavage at a dose of 2068 mg/kg bw (1st pre-experiment) and by intraperitoneal injection with 2000 mg/kg bw (2nd experiment).
Micronucleus test:

Treatment and sampling:	Groups of male mice were treated once with either the vehicle, positive control substance or 525, 1000 and 1500 mg Reg.No. 5749263/kg bw by intraperitoneal injection. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period (48 hours). The application volume was 20 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The application volume for the positive control groups was 10 mL/kg b.w. The animals were surveyed for evident clinical signs of toxicity throughout the study. Twenty-four/48 hours after the administration the mice were killed and the two femora were prepared free of all soft tissue. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with foetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet resuspended.
Slide preparation:	A small drop of the resuspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.
Statistics:	Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

Evaluation criteria:
A test item was classified as mutagenic if it induced either a dose related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods to determine the significance of effects were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results. A test item that failed to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

Results:

Analytical determinations:
The stability of the test substance in the vehicle (water) was verified in a separate study under the

responsibility of the sponsor and the results are reported in a separate report.
Preliminary range finding test:
None of the male or female mice died after single oral dosing of 2068 mg/kg bw or single i.p. injection of 2000 mg/kg bw. However, clinical signs including reduction of spontaneous activity, eyelid closure, tumbling, straub phenomena, hunchback, ruffled fur, stiff-legged walking, dark eyes, sunken flanks and pharyngeal reflex were observed within 6 hours after administration. On the basis of these data 2000 mg/kg bw were estimated to be suitable as highest dose. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.
Micronucleus assay:
Clinical signs were restricted to the animals treated with high dose of 2000 mg/kg bw and comprised reduction of spontaneous activity, eyelid closure, ruffled fur and hunchback. No clinical signs were observed in any of the animals treated with low and mid dose of the test substance, the positive control or the vehicle.
After treatment with the test item at 24 h and 48 h preparation interval the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that the test substance did not induce cytotoxic effects in the bone marrow.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were below or near to the value of the vehicle control group and all values in all dose groups were very well within the historical vehicle control data range (see Table B.6.8-105).
The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (2.91 %), thereby demonstrating the sensitivity of the test system.

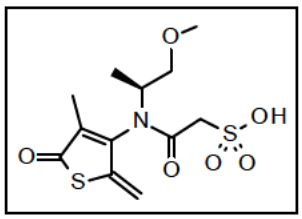
Table B.6.8-105: Micronucleus test in mice administered M656H055 by i.p. injection

Treatment	Sampling time	PCEs with micronuclei (5 %)	Range	PCE per 2000 erythrocytes
24 h sampling				
Sterile water	24	0.090	0-4	1384
M656H055				
525 mg/kg bw	24	0.079	0-4	1270
1000 mg/kg bw	24	0.100	0-4	1242
2000 mg/kg bw	24	0.050	0-2	1252
Positive control				
Cyclophosphamide	24	2.910	36-93	1200
48 h sampling				
Sterile water	48	0.080	0-6	1244
M656H055				
2000 mg/kg bw	48	0.057	0-4	1255

Conclusion:

The study is considered to be acceptable.
Based on the results of this study, M656H055 did not induce the formation of micronuclei in mouse polychromatic erythrocytes under *in vivo* conditions.

Metabolite M656PH059 former assigned M59/M60/M61:
M656PH059 is a groundwater metabolite.



Structural alerts for M656PH059:
In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential direct acylation was identified in the OECD and the OASIS module. In contrast no alert was identified for M656PH059 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox profiles provide the alerts based on the functional groups identified only and do not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.
OASIS-Times predicted M656PH059 (ASB2014-8408) to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (ASB2014-8410) the prediction was negative for the metabolite itself but positive for a structural alert contained in presumed transformation products thereof. This prediction based on the structural alert for alpha-beta carbonyls with polarised double bonds was not considered to become active as all presumed transformation products were predicted to be negative for chromosomal aberration *in vitro* due to the entire considered structural properties of the molecule (12 in total). The overall prediction was therefore negative for *in vitro* chromosomal aberration, with the limitation that the structures were out of the prediction domain.
The Vega prediction (ASB2014-8412) was inconclusive. In the CAESAR module the prediction was mutagenic. The reliability of this prediction was low as no similar compounds with known experimental data have been found in the database and similar compound within the database have experimental values that disagree with the prediction. Moreover, a prominent number of atom centred fragments of the compound have not been found or are rare in compounds found in the database. Vice versa compounds of the database that gave evidence for mutagenicity contained significantly other atom centred fragments not identified in M656PH059 that were considered potentially responsible for the mutagenic activity (see Figure B.6.8-25).

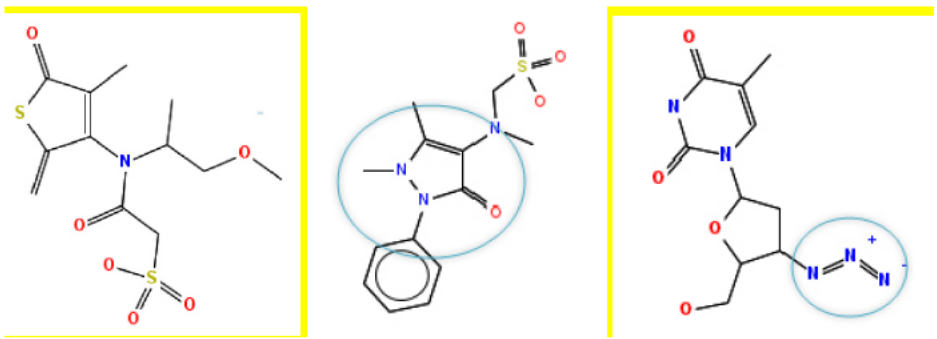
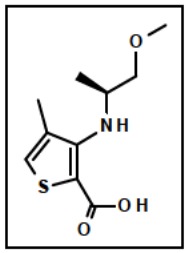


Figure B.6.8-25: Comparison of structural alerts of M656PH059 with similar structures identified by Vega CAESAR as mutagenic

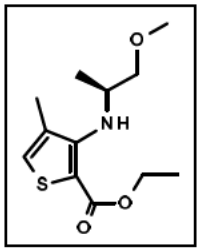
The SarPy module prediction was not mutagenic, however the reliability of this prediction was also low. No similar molecules with known experimental data were in the database. Some similar

molecules with experimental data disagree with the prediction. Moreover, a prominent number of atom centred fragments of the compound have not been found or are rare in compounds found in the database. Overall the structural similarity is considered insufficient.
There were contradictory predictions for Ames mutagenicity. While OECD toolbox ISS module considered M656PH059 Ames positive for the alert of an alpha, beta unsaturated carbonyl, this alert was not considered in the OASIS module and there was also no alert for Ames mutagenicity in the OASIS TIMES prediction neither for the molecule per se nor for presumed transformation products. The Vega CAESAR prediction for being mutagenic is not considered reliable as other structural alerts are considered responsible for the mutagenicity of the identified similar but not close enough related compounds. Thus there was no conclusive Ames alert identified for M656PH059. Overall there was some inconsistency in the prediction of an alert for chromosomal aberration *in vitro* predicted for either the molecule per se or presumed transformation products. The structural alert was that of unsaturated carbonyl compounds. They are known to present toxicological concerns in biological systems *in vitro*. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl building a reactive centre for nucleophilic addition and is mostly characterised for small, low molecular weight molecules. This alert is known to become active in *in vitro* systems but in particular for greater complex molecules like M656PH059 the evidence to become effective *in vivo* is lacking.

M656PH062 former assigned M62:
M656PH062 is a groundwater metabolite.



It was not possible to obtain M656PH062 as stable test item for toxicological testing as the compound was rapidly decarboxylated at the thiophene ring. Thus, decision was taken to test the ethylesterderivate of this metabolite as a surrogate taking into account that ester-bonds are easily cleaved in metabolic capable test systems. The structure for the surrogate test item is presented below.



Structural alerts for M656PH062:
In the OECD-toolbox the alert for DNA-binding of the OECD module differed from what was identified for the parent compound dimethenamid-P. For dimethenamid-P the alert was for iminium ion formation of the tertiary amine. Instead for M656PH062 it was the alert of nitrenium ion formation of the secondary heterocyclic amine. There was however no alert for DNA binding in the OASIS v. 1.2 module for both compounds. The only alert for genotoxicity (H-acceptor path) was also identified for the parent molecule dimethenamid-P.
OASIS-Times (ASB2014-8408) predicted M656PH062 to be not mutagenic in the Ames neither without nor with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration (ASB2014-8410) the prediction was negative for the metabolite itself and the structural alerts identified for transformation products (thiols) were not predicted to become active with the limitation that the molecules were out of the prediction domain.
In the DEREK analysis conducted the structural alerts for M656PH062 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (Molecule 22) in both modules CAESAR and SarPy was not mutagenic. The reliability of these predictions was low as no similar compound with experimental data were in the

training set and as similar molecules found in the database have experimental values that disagree with the prediction.
In conclusion, no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

Genotoxicity studies of M656PH062:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	Woitkowiak C., 2013b (ASB2014-8457) Reg.No. 5936274 (derivate of metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhymurium / Escherichia coli reverse mutation assay 2013/1373303
Guideline(s):	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

S. typhimurium and *E. coli* were exposed to M656PH062 (Reg. No. 5936274, former assigned M62, metabolite of dimethenamid-P) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and in a pre-incubation experiment.

Test Material	Reg.No. 5936274 (Derivate of metabolite of BAS 656-PH, dimethenamid-P)
Description:	Liquid, yellow/clear
Lot/Batch #:	L82-129
Purity:	90.1 % (tolerance +/- 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2015 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)
Control Materials:	
Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)
Vehicle control:	The vehicle control with and without S9-mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

Test organisms: *S. typhimurium* strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

The *Salmonella* strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.
The optical density of the fresh bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 109 cells per mL).

Test concentrations:

Plate incorporation assay: Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2650 and 5300 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: The test substance/vehicle/positive control substance, bacterial and S9-mix or phosphate buffer were incubated at 37 °C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 2650 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

Dates of experimental work: 15-Oct-2013 to 25-Oct-2013.

Plate incorporation assay: To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates).

Pre-incubation assay: 100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9-mix or phosphate buffer were incubated at 37 °C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

Statistics: No special statistical tests were performed.

Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and *E. coli* WP2 uvrA) or tripled (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolising system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.

Results:

Analytical determinations:
The test substance was stable over the study period under the storage conditions.
The stability of the test substance in the vehicle DMSO was verified analytically.

Toxicity:
A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
A bacteriotoxic effect (reduced his- and trp-background growth, decrease in the number of his+ revertants) was occasionally observed in the standard plate test depending on the strain and test conditions from about 2650 µg/plate onward.

In the pre-incubation assay bacteriotoxicity (reduced his- and trp-background growth, decrease in the number of his+ revertants) was occasionally observed depending on the strain and test conditions from about 333 µg/plate onward.
Mutation assays:
Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table B.6.8-106).
In the standard plate test, test substance precipitation was found from about 100 µg/plate onward with and without S9-mix.

Table B.6.8-106: Bacterial gene mutation assay with M656PH062 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		*E. coli*	
Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27.0	17.7	56.0	52.0	12.0	15.7	11.3	10.0	58.0	53.3
M656PH062										
33 µg/plate	26.7	16.3	66.3	50.3	12.0	15.7	9.3	10.3	51.0	52.0[P]
100 µg/plate	23.7[P]	17.3[P]	67.0[P]	52.7[P]	11.7[P]	14.3[P]	16.0[P]	7.0[P]	55.3[P]	61.0[P]
333 µg/plate	22.0[P]	15.7[P]	54.0[P]	65.3[P]	11.7[P]	13.0[P]	8.0[P]	8.7[P]	59.3[P]	52.0[P]
1000 µg/plate	21.3[P]	14.3[P]	58.3[P]	52.3[P]	8.7[P]	8.7[P]	10.7[P]	8.7[P]	56.3[P]	50.7[P]
2650 µg/plate	17.0[P]	11.7[P]	46.0[P]	44.0[P]	10.0[P]	10.7[P]	5.0[P]	5.7[P]	61.3[P]	50.0[P]
5300 µg/plate	22.7[P/B]	15.3[P/B]	41.0[P/B]	48.0[P/B]	7.0[P/B]	12.0[P/B]	3.0[P/B]	0.7[P/B]	51.7[P/B]	52.3[P/B]
Pos. control[§]	2240.0	483.3	2275.0	4092.0	214.7	5197.3	244.0	2277.7	282.0	867.0
Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27.0	15.7	51.7	35.7	12.7	9.3	8.0	9.3	43.3	48.0
M656PH062										
10 µg/plate	26.7	15.0	43.7	33.0	14.7	9.7	7.0	7.0	59.7	42.7
33 µg/plate	23.3	16.7	50.0	39.0	8.3	9.7	5.7	7.0	51.3	59.0
100 µg/plate	23.0	13.7	46.0	43.0	9.3	8.3	6.3	6.7	53.3	57.3
333 µg/plate	25.3	14.0	30.7	38.0	9.3	7.3	6.7	5.7	51.3	56.0
1000 µg/plate	25.0	11.0	42.7	33.7	9.3	11.0	6.3	4.0	63.0	37.7
2650 µg/plate	18.7	12.0[B]	31.7	25.3[B]	11.0	6.0[B]	7.7	2.7[B]	53.3	37.7[B]
Pos. control[§]	1900.0	379.3	684.7	316.7	194.7	1242.7	191.3	1670.7	120.7	406.0

[P] = Precipitation
[§] = Compound and concentrations see Material and Methods above
[B] = Reduced Background Growth

Conclusion:

According to the results of the present study, the test substance M656PH062 was not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen here.

Data point: KCA 5.8

Report: [redacted] 2013c (ASB2014-8464)
Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the Thymidine Kinase Locus (TK+/-) in mouse lymphoma L5178Y cells
2013/1307624

Guideline(s): OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300, EPA 712-C-98-221

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

The ethylester derivate of M656PH062 (Reg. No. 5936274, ethylester derivate of metabolite of dimethenamid-P) was tested *in vitro* for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the TK+/- locus.

Test Material	Reg. No. 5936274 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Liquid; yellow, clear
Lot/Batch #:	L82-129
Purity:	90.1 % (at the start of the experiment preliminary information on the purity of the test item indicated a purity ≥ 94.859 area-%)
Stability of test compound:	Stable in Ethanol
Solvent used:	Ethanol
Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	Ethanol
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL
Activation:	Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[*a*]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313) in the pre-experiment and in experiment I, and 29.8 mg/mL (Lot. No.: 050913) in experiment II. An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

Test organism: The L5178Y cell line, which is characterised by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50 %. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with hypoxanthine (5.0×10^{-3} M), aminopterin (2.0×10^{-5} M), thymidine (1.6×10^{-3} M) and glycin (5.0×10^{-3} M) followed by a recovery period of 2 days in RPMI 1640 medium containing hypoxanthine (1.0×10^{-4} M) and thymidine (1.6×10^{-3} M). After this incubation the cells were returned to complete culture medium (see below).

Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15 % horse serum (24 hour treatment, 3 % HS during 4 hour treatment), 1 % of 100 U/100 µg/mL penicillin/streptomycin, 220 µg/mL sodium-pyruvate, and 0.5 - 0.75 % amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, glucose 1100 mg, $Na_2HPO_4 \times 2\ H_2O$ 192 mg, KH_2PO_4 150 mg

Locus examined: Thymidine Kinase Locus (TK+/-)

Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 21.2 to 2713.0 µg/mL

b) Mutation assay:

1st experiment: 10.6, 21.3, 42.5, 85.0, 170.0, 255.0 µg/mL without metabolic activation
2.7, 5.3, 10.6, 21.3, 42.5, 63.8 µg/mL with metabolic activation

2nd experiment: 2.6, 5.3, 10.5, 21.0, 42.0, 63.0 µg/mL without metabolic activation
5.3, 10.5, 21.0, 31.5, 42.0, 63.0 µg/mL with metabolic activation

Dates of experimental work: 23-Sep-2013 to 04-Nov-2013.

Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.
1×10^7 cells (3×10^6 cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3×10^5 cells/mL, if necessary. The

relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

Mutation Assay:

Cell treatment and expression: In the mutation experiment 1 x 10^7 (3 x 10^6 during 24 h exposure) cells/flask (80-cm^2 flasks) suspended in 10 mL RPMI medium with 3 % horse serum (15 % horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3 x 10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection: After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4 x 10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5 % CO_2/95.5 % humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.

Size distribution of the colonies:

Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations:

Pre-test:

total suspension growth (4 h treatment): (cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment): (cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

relative suspension growth: total suspension growth x 100/total suspension growth of corresponding control

Main test:

total suspension growth (4 h treatment): (cell number at 24 h/cell number at 4 h) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment): (cell number at 24 h/cell number of seeded cells per mL (100000)) x (cell number at 48 h/if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) x (cell number at 72 h/if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth: total suspension growth x 100/total suspension growth

	of corresponding control
relative total growth:	relative suspension growth x relative cloning efficiency/100
cloning efficiency (viability):	ln(mean number of empty wells per plate/96)/cells seeded per well
relative cloning efficiency:	cloning efficiency x 100/cloning efficiency of corresponding control
cells survived:	cloning efficiency x cell number seeded in TFT medium
mutant colonies/106 cells:	small mutant colonies + large mutant colonies
threshold:	number of mutant colonies per 10^6 cells of each solvent control plus 126
cloning efficiency (viability):	cloning efficiency determined after the expression period to measure viability of the cells without selective agent

Statistics:
A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.
Evaluation criteria:
The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent solvent control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10 % of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

Results:

Analytical determinations:
Purity of the test item was verified by HPLC analysis.
Preliminary cytotoxicity assay:
The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 21.2 µg/mL and 2713.0 µg/mL were chosen with regard to the molecular weight (257.4 g/mol) corresponding to a molar

concentration of about 10 mM and considering the preliminary information concerning the purity of the test item (94.859 area-%) at the start of the experiment. No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.
Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. There was no relevant shift of the osmolarity and pH value even at the maximum concentration of the test item. Precipitation was observed by the unaided eye at 678.3 µg/mL and above with and without metabolic activation following 4 and 24 hours treatment.
The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 255 µg/mL without and 68.3 µg/mL with metabolic activation.
Mutagenicity assays:
Relevant cytotoxic effects indicated by a relative total growth of less than 50 % of survival were observed in both cultures of the first experiment at 170 µg/mL without metabolic activation and at 42.5 µg/mL and above with metabolic activation. In the second experiment without metabolic activation cytotoxic effects as described above were noted at 63.0 µg/mL without metabolic activation and at 31.5 µg/mL and above with metabolic activation. Precipitation of the test substance was not observed.
No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. Isolated increases exceeding the threshold of 126 above the corresponding solvent control were noted. As these increases were not reproducible and occurred at cytotoxic concentrations, they were considered as biologically not relevant (see Table B.6.8-107 and Table B.6.8-108).
The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies.

Table B.6.8-107: Gene mutation in mammalian cells - 1st experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I/4 h treatment			Culture I			Culture II		
Solv. control with ethanol		-	100.0	107	233	100.0	127	253
Pos. control with MMS	19.5	-	13.0	504	233	14.1	475	253
Ethylester derivative of M656PH062								
	10.6	-	106.9	71	233	135.1	136	253
	21.3	-	93.0	126	233	122.8	130	253
	42.5	-	169.5	80	233	83.5	113	253
	85.0	-	116.4	77	233	75.2	105	253
	1700.0	-	42.8	88	233	37.8	88	253
	255.0	-	culture was not continued[#]			culture was not continued[#]		
Solv. control with ethanol		+	100.0	52	178	100.0	69	195
Pos. control with CPA	3.0	+	17.8	241	178	23.3	369	195
Pos. control with CPA	4.5	+	14.4	599	178	15.1	649	195
Ethylester derivative of M656PH062								
	2.7	+	culture was not continued[##]			culture was not continued[##]		
	5.3	+	84.7	56	178	142.0	74	195
	10.6	+	80.5	65	178	106.6	70	195
	21.3	+	60.5	85	178	68.1	151	195
	42.5	+	10.6	172	178	46.0	201	195
	63.8	+	2.1	207	178	4.0	172	195

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
[#] culture was not continued due to exceedingly severe cytotoxic effects
[##] culture was not continued as a minimum of only four concentrations is required by the guidelines

Table B.6.8-108: Gene mutation in mammalian cells - 2nd experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II/24 h treatment			Culture I			Culture II		
Solv. control with ethanol		-	100.0	58	184	100.0	63	189
Pos. control with MMS	13.0	-	9.9	348	184	10.4	330	189
Ethylester derivative of M656PH062								
	2.6	-	culture was not continued##			culture was not continued##		
	5.3	-	96.0	50	184	124.4	56	189
	10.5	-	74.6	79	184	110.7	67	189
	21.0	-	80.4	71	184	110.7	62	189
	42.0	-	81.9	68	184	76.9	68	189
	63.0	-	29.3	49	184	46.7	46	189
Experiment II/4 h treatment								
Solv. control with ethanol		+	100.0	52	178	100.0	66	192
Pos. control with CPA	3.0	+	95.7	184	178	47.7	137	192
Pos. control with CPA	4.5	+	24.9	341	178	25.2	332	192
Ethylester derivative of M656PH062								
	5.3	+	culture was not continued##			culture was not continued##		
	10.5	+	117.3	83	178	151.9	33	192
	21.0	+	54.0	69	178	126.1	32	192
	31.5	+	43.1	109	178	46.2	48	192
	42.0	+	20.5	94	178	47.6	54	192
	63.0	+	9.7	187	178	11.7	75	192

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
culture was not continued as a minimum of only four concentrations is required by the guidelines

Conclusion:

Based on the results of the study and under the conditions of the test, the ethylester derivative of M656PH062 did not induce forward mutations in the TK+/- locus in L5178Y cells *in vitro*.

Data point: CA 5.8.1/47

Report: [redacted] 2014a (ASB2014-8469)
Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P): *In vitro* micronucleus test in chinese hamster V79 cells 2013/1307623

Guideline(s): OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: *In vitro* Mammalian Cell Micronucleus Test

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Materials and methods:

The ethylester derivate of M656PH062 (Reg. No. 5936274, ethylester derivate of metabolite of dimethenamid-P) was tested *in vitro* for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation.

Test Material: Reg. No. 5936274 (Metabolite of BAS 656-PH, dimethenamid-P)
Description: Liquid; yellow, clear
Lot/Batch #: L82-129
Purity: 90.1 %
Stability of test compound: Stable in ethanol (solvent)
Solvent used: Ethanol
Control Materials:
Negative control: A negative control was not employed in this study
Solvent control: Ethanol
Positive controls, -S9: Mitomycin C
(MMC, 0.1 µg/mL, dissolved in deionised water)
Positive control, +S9: Cyclophosphamide
(CPA, 15.0 µg/mL, dissolved in saline)
Activation: Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[*a*]pyrene and 2-aminoanthracene in the Ames test.
The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313).
An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

Test organisms: Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥70 %).
Culture medium/conditions: About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) foetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5-1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

Test concentrations:

a) Preliminary toxicity and cytogenicity assay:

5.3-2713.0 µg/mL with and without metabolic activation.
Since the cultures fulfilled the requirements for cytogenetic evaluation and the test item was considered to be mutagenic, this preliminary test was designated Main Experiment (see Table B.6.8-109).

Table B.6.8-109: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg. No. 5936274 (Metabolite of BAS 656-PH, dimethenamid-P)

Preparation interval	Exposure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 h	4 h	I	5.3	10.6	21.2	42.4	84.8	169.6[PS]	339.1[PS]	678.3[PS]	1356.5[PS]	2713.0[PS]
			With S9 mix									
24 h	4 h	I	5.3	10.6	21.2	42.4	84.8	169.6	339.1[PS]	678.3[PS]	1356.5[PS]	2713.0[PS]

[PS] Phase separation was observed at the end of treatment

Dates of experimental work: 18-Sep-2013 - 25-Sep-2013.

Preliminary cytotoxicity assay:

With regard to the molecular weight and the preliminary information on purity (94.859 %) of the test item, 2713.0 µg/mL of Reg. No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) (approx. 10 mM) were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 5.3 and 2713.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium were added.
Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose x H_2O, 192 mg/L Na_2HPO_4 x 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 20 hours.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-

generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity:

Evaluation was performed manually using microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

Evaluation criteria:

A test item was considered as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose related and reproducible increase in the number of cells containing micronuclei is observed

A test item can be considered as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

Results:

Analytical determinations:

Purity of the test item was verified by HPLC analysis.

Preliminary cytotoxicity assay:

Since the cultures fulfilled the requirements for cytogenetic evaluation and the test item was considered to be mutagenic, the preliminary test was designated Main Experiment (see below).

Cytogenicity assays:

In the absence and presence of S9-mix, concentrations showing clear cytotoxic effects were not evaluable for cytogenetic damage.

In the absence of S9-mix statistically significant increases in micronucleated cells, clearly exceeding the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells) were observed

after treatment with 10.6 and 21.2 µg/mL (3.05 and 6.30 %). In the presence of S9-mix one single statistically significant increase in micronucleated cells, clearly exceeding the range of the laboratory historical control data (0.05 - 1.70 % micronucleated cells) was observed after treatment with 21.2 µg/mL (8.55 %) (see Table B.6.8-110).
Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table B.6.8-110: Summary of results of the micronucleus test with ethylester derivative of M656PH062

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 h without S9 mix				
I	24 h	Solvent control[1] Positive control[2] 5.3 10.6 21.2	2.91 2.74 2.87 2.68 2.05	1.10 3.70[S] 1.10 3.05[S] 6.30[S]
Exposure period 4 h with S9 mix				
I	24 h	Solvent control[1] Positive control[3] 5.3 10.6 21.2	2.24 1.72 2.16 2.08 1.94	1.45 9.55[S] 1.40 1.35 8.55[S]

* The number of micronucleated cells was determined in a sample of 2000 cells.
S Number of micronucleated cells statistically significantly higher than corresponding control values
1 Ethanol 0.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 CPA 15.0 µg/mL

Conclusion:

Based on the results of the study, ethylester derivative of M656PH062 is considered as mutagenic in this *in vitro* micronucleus test, when tested up to the highest required concentrations.

Data point: KCA 5.8

Report: [redacted] 2014c (ASB2014-8475)
Reg.No. 5936274 (derivate of metabolite of BAS 656-PH, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1028628

Guideline(s): OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395

Deviations: No relevant deviations

GLP: Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Acceptability: The study is considered to be acceptable.

Data point: KCA 5.8

Report: Becker M., Landsiedel R., 2014c (ASB2014-8481)
Analytical report - Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for external studies

	2014/1092433
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.
Data point:	KCA 5.8
Report:	Grauert E., Landsiedel R., 2014a (ASB2014-8486) Analytical report - Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in corn oil 2014/1101991
Guideline(s):	No
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

The ethylester derivative of M656PH062 (Reg. No. 5936274, ethylester derivative of metabolite of dimethenamid-P) was tested for the ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

Test Material:	Reg.No. 5936274 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-129
Purity:	90.1 % (tolerance ± 1.0 %)
Stability of test compound:	Confirmed indirectly by dose formulation analytics.
Solvent used:	corn oil
Control Materials:	
Negative:	No negative control was employed in this study.
Solvent control:	corn oil
Positive control:	Cyclophosphamide (CPA) 40 mg/kg
Test animals:	
Species:	Mice
Strain:	Crl:NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8-11 weeks
Weight at dosing:	Males mean value 35.9 g (SD ± 1.7 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2 males and 2 females for each pre-test
Micronucleus assay:	7 males/dose; 5 males/control
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Single housing in Makrolon Type II (pre-test)/III (main study) cages, with wire mesh top
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	45 - 65 %

Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00/18:00 - 06:00)
Test compound doses:	
Range finding test:	1000 and 2000 mg/kg (administered once orally)
Micronucleus assay:	250, 500 and 1000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.
Dates of experimental work:	30-Oct-2013 to 26-Nov-2013.
Preliminary range finding test:	Male and female NMRI mice were treated once by oral gavage with a test substance dose of 1000 (1st pre-test) and 2000 mg/kg bw (2nd pre-test).
Micronucleus test:	
Treatment and sampling:	Groups of male mice were treated once with either vehicle or 250, 500 or 1000 mg M656PH062/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were surveyed for evident clinical signs of toxicity throughout the study. Twenty-four hours after the administration the mice were killed and the femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with foetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. The supernatant was discharged and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation:	A small drop of the suspension was spread on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.
Statistics:	The number of polychromatic erythrocytes with micronuclei was analysed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test.
Evaluation criteria:	A test item was considered as mutagenic if it induces either a dose related increase or a clear increase in the number of micro-nucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results. A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

Results:

Analytical determinations:
The stability of the test substance in the solvent was confirmed indirectly by dose formulation analytics (ASB2014-8486).
Preliminary range finding test:
One male animal of the 2000 mg/kg bw dose group died. No further mortalities were observed. At 1000 mg /kg bw clinical signs comprised reduction of spontaneous activity, abdominal position, hunchback, sunken flanks, ruffled fur and eyelid closure in both sexes. At 2000 mg/kg bw reduction of spontaneous activity, abdominal position, hunchback, sunken flanks, ruffled fur, eyelid closure, tumbling, apathy and tiptoe walk in both sexes was observed in animals of both sexes. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.
Micronucleus assay:
Clinical symptoms in the main experiment included ruffled fur, abdominal position, reduced spontaneous activity and eyelid closure in the animals treated with the high dose of the test item. The animals treated with the mid dose level exhibited reduced spontaneous activity and ruffled fur. The animals treated with the low dose exhibited ruffled fur only. Most signs occurred transiently after test item administration, only ruffled fur was observed in some mice up to the end of the observation period. Plasma analytics confirmed that the ethylester derivative of M656PH062 is systemically available (ASB2014-8481).
The mean number of polychromatic erythrocytes was not substantially decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that the test substance did not have any cytotoxic properties in the bone marrow.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item (Table B.6.8-111). The mean values of micronuclei observed after treatment with the test substance were even below to the value of the vehicle control group. Moreover, micronucleus values obtained in all dose groups were within the historical negative control range.
The positive control cyclophosphamide showed a statistically significant increase of induced micronucleus frequency thereby ensuring the validity of the test system.

Table B.6.8-111: Micronucleus test in mice administered M656PH062 by oral gavage

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Vehicle	24	0.110	0-7	1154
M656PH062				
250 mg/kg bw	24	0.086	0-4	1149
500 mg/kg bw	24	0.050	0-2	1186
1000 mg/kg bw	24	0.100	0-4	1195
Positive control				
Cyclophosphamide	24	2.430	36-61	1144
48 h sampling				
Sterile water	48	0.110	0-4	1222
M656PH062				
1000 mg/kg bw	48	0.071	0-2	1075

Conclusion:

Based on the result of this study M656PH062 did not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Short-term toxicity of M656PH062:

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.8
Report:	[redacted]., 2014f (ASB2014-8421) Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet, 2014/1018066
Guideline(s):	OECD 407, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

Ethylester derivative of M656PH062 (Reg.No. 5926274, derivative of metabolite of dimethenamid-P) was initially administered to Wistar rats at dietary dose levels of 0, 1200, 4000 and 12000 ppm. Due to severely impaired body weight development in males the high dose level of 12000 ppm was reduced to 8000 ppm form day 18 onwards.

Test material:	Reg.No. 5936274, derivate of metabolite of dimethenamid-P.
Description:	liquid / yellow, clear
Batch/purity #:	L82-129/90.1 %
Stability of test compound:	Stable until 01 Nov 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.
Vehicle and/or positive control:	Rodent diet
Test animals:	
Species:	Rat
Strain:	Wistar Crl:WI (Han) Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 160.1 ± 6.3 g, ♀ 130.7 ± 7.7 g
Source:	Charles River, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, *ad libitum*
Housing:	Group housing (5 animals per cage) in polysulfonate H cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria and play tunnel large supplied by PLEXX B.V., Elst Netherlands for environmental enrichment Motor activity measurements were conducted in polycarbonate cages

with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material

Environmental conditions:
Temperature: 20 - 24 °C
Humidity: 30 - 70 %
Air changes: 15 air changes per hour
Photo period: 12 h light/12 h dark (06:00 - 18:00/18:00 - 06:00)
Dates of experimental work: 05-Nov-2013 – 26-Feb-2014.
(In life dates: 14-Nov-2013 (start of administration) to 13-Dec-2013 (necropsy)).

Animal assignment and treatment:
The ethylester derivative of M656H062 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose). As body weight development was significantly impaired in all males of the high dose group and was also impaired in females between study day 0 and study day 14, the diet concentration was reduced to 8000 ppm from study day 18 onwards in males as well as in females. For the other dose groups the concentrations remained throughout the 28-day study period. The animals were assigned to the treatment groups by means of a computer generated randomisation list based on body weights.
Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. One diet preparation per dose was performed for this study.
Analyses performed prior to the start of the administration period revealed that the test substance was stable in the diet for at least 32 days.
Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analysed. The samples were also used for determination of the test article concentration. For the mid dose level (4000 ppm) and for the reduced high dose level (8000 ppm) single samples were analysed. No test article was determined in control diets.

Table B.6.8-112: Analysis of diet preparations for homogeneity and test item content

Dose level	Sampling	Concentration	% of nominal concentration	Relative standard deviation
		Mean ± SD		
		[ppm]		[%]
1200 ppm	12. Nov. 13	1143.7 ± 13[#]	95.3	1.1
4000 ppm	12. Nov. 13	3876.4	96.9	n.a.
8000 ppm	1. Dez. 13	7473.7	93.4	n.a.
12000 ppm	12. Nov. 13	11660.4 ± 147[#]	97.2	1.2

n.a.: not applicable;
[#] based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures.

Relative standard deviations of the homogeneity samples in the range of 1.1 to 1.2 % indicate the homogenous distribution of the ethylester derivative of M656H062 in the diet preparations. The actual (mean) average test substance concentrations were in the range of 93.4 to 97.2 % of the nominal concentrations. These results demonstrated the correctness of the concentrations of ethylester derivative of M656H062 in the vehicle.
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-113: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means.
faeces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians.

Table B.6.8-114: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine color and turbidity	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians. For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table B.6.8-115: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behaviour during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmos
6. respiration	15. faeces discharge during examination (appear ance/consistency)
7. activity/arousal level	16. urine discharge during examination
8. tremors	17. pupil size
9. convulsions	

Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.
Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day } x = \frac{BW_x - BW_y}{FC_{y \text{ to } x}} \times 100$$

BWx and BWy body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Substance intake for day } x = \frac{FC_x \times C}{BW_x}$$

FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).
Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted.
Ophthalmoscopy: Not performed in this study.
Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.
During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.
For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1.	behaviour when removed from cage	10.	respiration
2.	fur	11.	tremors
3.	skin	12.	convulsions
4.	salivation	13.	abnormal movements
5.	nose discharge	14.	impairment of gait
6.	lacrimation	15.	activity/arousal level
7.	eyes/pupil size	16.	faeces (number of faecal pellets/appearance/ consistency) within two minutes
8.	posture	17.	urine (appearance/quantity) within two minutes
9.	palpebral closure	18.	number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behaviour during "handling"
2.	touch response	9.	vocalisation
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

Motor activity measurement:
Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.
Haematology and clinical chemistry:
Blood was withdrawn in the morning from fasted, isoflurane anesthetised animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation, these samples were stored frozen but not analysed. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomised sequence.

The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
	Red blood cells		White blood cells		Clotting Potential
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Haemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Haematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
Clinical chemistry:					
	Electrolytes		Metabolites and Proteins		Enzymes:
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Urinalysis:
For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analysed in a randomised order.
The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		Semi quantitative parameters		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Colour and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

Sacrifice and pathology:
The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:												
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).												
C	W	H		C	W	H		C	W	H		
✓	✓	#	adrenals	✓	✓	#	kidneys	✓			skin	
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)@	
✓		#	bone marrow§	✓		#	larynx	✓	✓	#	spleen	
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow	
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)	
✓		#	coagulating glands£	✓		#	lymph nodes#	✓	✓	#	testes	
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	✓	thymus	
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid	
✓	✓	#	epididymides¥	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea	
✓		#	esophagus	✓		#	nose/nasal cavity‡	✓		#	urinary bladder	
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix	
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina	
			gall bladder	✓			pharynx					
✓		✓	gross lesions	✓		#	pituitary					
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetised animals)	
✓	✓	#	heart	✓		#	rectum					
✓		#	ileum	✓			salivary glands*					
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles£					

§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; ‡ l histopathology at level III; ¥left epididymidis collected for histopathology; £ seminal vesicles and coagulation weight determined together.

The organs or tissues were fixed in 4 % formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.
After the completion of the histopathological assessment by the study pathologist an internal peer review was performed by a second senior pathologist (Dr. Karin Küttler, Ludwigshafen, Germany) on liver and thyroid glands of all animals. Results presented in the study report reflect the consensus opinion of both the study pathologist and the reviewing pathologist.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:
• Increased/decreased grade of cortico-medullar ratio (related only to area)
• Increase of starry sky cells
• Changes of cellular density in the cortex
• Changes of cellular density in the medulla
Spleen:
• Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
• Altered cellular composition of follicles
• Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):
• Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
• Altered cellular composition of paracortex
• Altered number of germinal centers
• Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):
• Changes of the cellularity of follicles (including mantle zone and germinal centers)
• Changes of the cellularity of interfollicular area
Bone marrow:
• Changes of the cellularity
• Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

Results:

No clinical signs were observed throughout the study. No mortality was observed throughout the study.
Ophthalmoscopy was not performed in this study.
FOB and Motor Activity:
Neither home cage nor open field observations revealed any indication of treatment related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
Comparing the single intervals with the control groups, no significant deviations were measured for motor activity neither in males nor in females.
Body weight and body weight gain:
In the first and second week of treatment males of the high dose group showed significant body weight gain reduction in combination with a significantly reduced food consumption in the second week (see Table B.6.8-116, Table B.6.8-117, Figure B.6.8-26). Although not significantly altered, a tendency to impaired body weight development was also observed in female animals at that time, but the effect on food consumption was even more pronounced. As a consequence the concentration in the diet was reduced to 8000 ppm from study day 18 onwards in both sexes. In males the dose reduction prevented the further increase in effects on body weight development but the animals did not recover throughout the rest of the study period. The female animals however, seemed to recover as demonstrated by the comparable body weight of controls and high dose on day 21, however in the last week of treatment

there was again the tendency to decreased body weight development in relation to the decreased food consumption observed. Overall the high dose males showed a 16.8 % reduction in body weight and a 37.7 % reduction in body weight gain at the end of the 28-day study period, in females the reduction was 5.5 % and 9.4 % for body weight and body weight gain, respectively (see Table B.6.8-116). The significantly decreased body weight change value in male animals and the significantly increased body weight change value in female animals of the 1200 ppm on study day 21 were assessed as being incidental and not related to treatment as no dose response relationship occurred.

Table B.6.8-116: Mean body weight of rats administered ethylester derivate of M656H062 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1200	4000	12000/8000[1]	0	1200	4000	12000/8000[1]
Body weight [g]								
- Day 0	160.3	159.3	161.7	159	134.9	129.1	129.1	129.5
- Day 7	201.3	200.5	199.8	179.9**	142.1	145.2	139.6	139
- Day 14	240.3	242.8	238.3	200.2**	161.5	164.3	157.1	150.2
- Day 21	270.2	255.5	264.8	218.9**	168.9	177.3	165.7	167.7
- Day 28	283.7	278.7	276.7	235.9**	184.7	188.6	178.8	174.5
Δ% (compared to control)[#]		-1.8	-2.5	-16.8		2.1	-3.2	-5.5
Body weight gain [g]								
d 0 -> 7	41	41.2	38.1	20.9**	7.2	16.1	10.5	9.5
d 0 -> 14	80	83.4	76.6	41.2**	26.6	35.2	28	20.8
d 0 -> 21	109.9	96.2*	103.1	60**	34	48.2*	36.6	38.2
d 0 -> 28	123.4	119.4	115	76.9**	49.8	59.5	49.7	45.1
Δ% (compared to control)[#]		-3.2	-6.8	-37.7		19.5	-0.2	-9.4

1 As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.

* $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided).

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means).

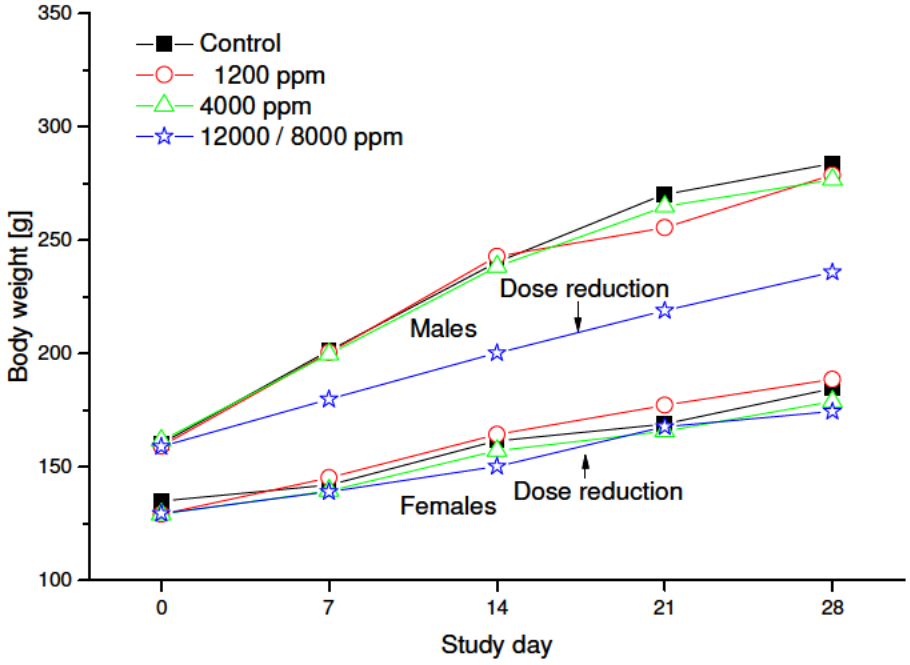


Figure B.6.8-26: Body weight development of rats administered ethylester derivate of M656H062 for at least 28 days

Food consumption and compound intake:
In the high dose males and females food consumption was clearly decreased between study days 11 to 14. After the reduction of the diet concentration on study day 18 the food consumption in males was in the comparable range to control group values with a trend to compensatory higher values in the week after the dose reduction (see Table B.6.8-117). However, in females where the compensatory increase in food consumption after decrease of the dose level was even more pronounced during week 3 (day 18 to 21) lower food consumption was again noticed thereafter in the fourth measurement (day 25 to 28). Overall the effects on food consumption were considered related to treatment.

Table B.6.8-117: Mean food consumption of rats administered the ethylester derivative of M656H062 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1200	4000	12000/ 8000	0	1200	4000	12000/ 8000
Food consumption [g]								
- Day 4-7	18.0	19.4	19.5	22.0	13.7	14.7	12.9	19.7
- Day 11-14	20.7	22.1	19.9	11.1	15.3	23.5	16.7	6.2
- Day 18-21	20.9	17.9	18.1	23.2	15.8	17.0	15.3	21.1
- Day 25-28	24.0	24.0	20.3	20.0	17.1	17.9	16.8	11.5
Total	83.6	83.4	77.8	76.3	61.9	73.1	61.7	58.5

The mean daily test substance intake over the study period calculated on the values for week 1 to 4 for the 1200 and 4000 ppm dose group and calculated for the 12000/8000 ppm dose group on the weeks 1 to 2 for 12000 ppm and on weeks 3 to 4 for 8000 ppm respectively is shown in the following (see Table B.6.8-118).

Table B.6.8-118: Calculated intake of ethylester derivative of M656H062

Test group	Concentration in the vehicle (ppm)	Mean daily test substance intake (mg/kg bw/day)	
		Males	Females
1	1200	103	131
2	4000	323	385
3	12000	1065	1096
	8000	763	767

Water consumption:
No test substance related, adverse changes with regard to water consumption were observed.
Blood analysis:
Haematological findings:
At the end of the study in rats of both sexes of the high 12000 and 8000 ppm group prothrombin time (HQT: Haepatoquick's test) was significantly shortened (see Table B.6.8-119). However, in both gender prothrombin time mean was within the historical control ranges whereas those of the controls were below this range. Thus this alteration was considered incidental and not related to treatment.
The isolated finding of significantly increased reticulocytes in males of the high dose group was considered incidental and not treatment related as it was within the historical control range and no other parameters of the red-blood cell system were affected (see Table B.6.8-119).

Table B.6.8-119: Selected haematology findings in rats administered ethylester derivative of M656H062 for at least 28 days (group means)

Sex		Males				Females			
Dose	[ppm]	0	1200	4000	12000/ 8000	0	1200	4000	12000/ 8000
	[mg/kg bw/day]		103	323	1065/ 763		131	385	1096/ 767
Reticulocytes	[%]	1.8	2.0	1.3	2.5*	2.1	1.8	2.2	1.7
		Historical control: 1.1-3.2							
Prothrombin time	[sec]	42	39.4	38.7	35.4**	37.2	37	34.6	34.4*
		Historical control: 33.3-39.6				Historical control: 30.3-36.7			

*p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

Clinical chemistry findings:
At the end of the study in males of the 12000/8000 ppm high dose group, γ-glutamyl transferase activities and cholesterol levels were increased and urea and total bile acid levels were decreased. In female animals of the high dose group triglyceride levels were increased. These were considered treatment-related adverse findings. γ-Glutamyl transferase activities and higher cholesterol levels indicated an altered liver cell metabolism most probably due to liver enzyme induction. This was confirmed by decreased ALT activities (see Table B.6.8-120) in these individuals.
TBA was already lower in males of the mid dose group (4000 ppm), but in this test group it was the only altered parameter and, therefore, this change was regarded as treatment related but not adverse (ECETOC, 2002a, ASB2014-8405).
In male animals of low and high dose group alanine aminotransferase (ALT) activities were decreased. The alteration was not dose dependent and even in the high dose group the activity decrease was not toxicologically relevant as the values were not below 50 % (see UK PSD, 2007a, ASB2014-8414). In male animals of high dose group chloride levels were marginally above the historical control range (see Table B.6.8-120). However, this was the only altered electrolyte level in these individuals.

Therefore, this change was regarded as incidental and not treatment related.
In females of the mid and high dose group (4000 as well as 12000/8000 ppm) cholesterol, total protein and globulin levels were higher and glucose levels were lower compared to controls. However, none of the mentioned parameters were changed dose dependently. All means were within historical control ranges except for cholesterol in the mid dose group which was marginally above this range (see Table B.6.8-120). Inorganic phosphate levels were higher in females of the 4000 ppm dose group, but the alteration was not dose dependent. These changes in females were therefore regarded incidental and not treatment related.

Table B.6.8-120: Selected clinical chemistry findings in rats administered ethylester derivative of M656H062 for at least 28 days (group means)

Sex		Males				Females			
Dose	[ppm]	0	1200	4000	12000/ 8000[1]	0	1200	4000	12000/ 8000[1]
	[mg/kg bw/day]		103	323	1065/ 763		131	385	1096/ 767
Bile acids, total	[µmol/L]	29.6	16.8	11.1**	6.6**	14.8	12.3	11	4,6
Cholesterol	[mmol/L]	1.89	2.24	2.05	2.64*	1.38	1.34	2.11**	1.95*
						Historical control: 0.95-1.96			
Triglycerides	[mmol/L]	0.86	1.19	0.99	1.15	0.50	0.40	0.75	1.24*
Protein, total	[g/L]	60.64	61.46	63.58	57.98	59.9	59.75	63.39*	62.38*
						Historical control: 58.40-66.83			
Globulins	[g/L]	22.26	23.05	23.93	21.54	20.46	20.56	23.35**	22.37**
						Historical control: 21.07-27.67			
Urea	[mmol/L]	5.83	5.67	5.62	4.43*	5.42	6.40	5.40	5.69
Glucose	[mmol/L]	5.80	6.67	5.89	5.61	5.76	5.13	4.63**	5.00*
						Historical control: 4.07-6.72			
ALT	[µkat/L]	0.79	0.63*	0.67	0.51**	0.53	0.57	0.53	0.45
γ-GT	[nkat/L]	0	0	0	40**	0	1	3	4
Cl	[mmol/L]	100.7	99.9	100.5	104.1*	102.4	102.6	103.2	102.6
		Historical control: 99.2-104.0							

1 As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
*$p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Urinalysis:
In rats of both sexes of the 4000 ppm as well as 12000/8000 ppm group as well as in males of the 1200 ppm group urobilinogen levels in the urine were increased (see Table B.6.8-121). This finding was not accompanied by increased bilirubin levels in serum or urine. Red blood cell parameters (red blood cell counts, haemoglobin and haematocrit levels) were also in the normal range. Total bile acid levels were lower at least in males of the 4000 ppm group as well as 12000/8000 ppm group indicating a reduced synthesis or more probably an increased excretion of conjugated bile acids via bile but a

reduced intestinal reabsorption. Higher urobilinogen levels in the urine demanded a higher rate of biliary excretion of conjugated bilirubin followed by an increased urobilinogen formation and intestinal absorption. Higher urobilinogen levels in the urine per se without any other findings in clinical pathology or in anatomical pathology of the kidneys and/or liver cannot be regarded as adverse, but as an adaptive effect due to the increased conjugation rate in the liver.
In females of the 1200 and 4000 ppm group pH values of the urine were lower compared to controls, but the decrease was not dose dependent and therefore it was regarded as incidental and not treatment related.

Table B.6.8-121: Selected urinalysis findings in rats administered ethylester derivative of M656H062 for at least 28 days (group means)

Sex		Males				Females			
Dose	[ppm]	0	1200	4000	12000/ 8000[1]	0	1200	4000	12000/ 8000[1]
	[mg/kg bw/day]		103	323	1065/ 763		131	385	1096/ 767
pH	[µmol/L]	6.5	5.8*	5.4*	5.7	5.9	5.7	5.7	5.6
Urobilinogen	[mmol/L]	1	3**	3**	3**	1	2	2**	2*

1 As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Necropsy:
Organ weight:
Terminal body weights of treated rats were significantly (-17 %) reduced in high dose male rats (Table B.6.8-122).
The only statistically significant weight effect considered treatment related was the dose dependent increase in absolute and relative liver weights in both male and female animals. The effects were statistically significant in males in all dose groups, while effects were seen in the mid and high dose group in females only. The absolute liver weights of treated males were within the historical control range. Relative liver weights in test group 2 and 3 were above the historical control range while the relative weight of test group 1 males was within that range. The weight increase was therefore assessed as treatment related in test groups 2 and 3 only. Because of concurrent findings in clinical pathology (see Table B.6.8-120, Table B.6.8-121) indicating an altered liver cell metabolism, the liver weight increases in rats of both sexes of the 12000/8000 ppm group (see Table B.6.8-122) and the moderate centrilobular liver cell hypertrophy in females of this group (see Table B.6.8-123 below) were regarded as adverse. The slight liver weight increases with only minimal histological findings and without any clinical pathological changes in rats of the 4000 ppm group however, were evaluated as adaptive effect.
Secondary to the decrease in body weight and, therefore, only indirectly related to treatment, there was a decrease in absolute kidneys and prostate weights and also an increase in relative brain weight in high dose group (see Table B.6.8-123). The statistically significant increases in absolute and relative brain and spleen weights in low dose males did not show a dose response relationship and therefore were considered to be incidental.

Table B.6.8-122: Selected mean absolute and relative organ weights of rats administered the ethylester derivative of M656H062 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000/ 8000[1]	0	1200	4000	12000/ 8000[1]
[mg/kg bw/day]		103	323	1065/ 763		131	385	1096/ 767
Terminal body weight	257.48	263.28	251.3	214.38**	165.82	170.68	163.56	159.46
[% of control]	100	102	98	83	100	103	99	96
Brain weight, absolute [g]	1.878	2.01*	1.968	1.808	1.792	1.8	1.816	1.81
[% of control]	100	113	107	91	100	1000	101	101
Brain weight, relative [%]	0.73	0.764*	0.784	0.844**	1.082	1.057	1.114	1.136
[% of control]	100	105	107	116	100	98	103	105
Kidney weight, absolute [g]	1.822	1.924	1.876	1.602*	1.338	1.346	1.348	1.32
[% of control]	100	106	103	88	100	101	101	99
Kidney weight, relative [%]	0.707	0.731	0.748	0.747	0.808	0.79	0.824	0.827
[% of control]	100	103	106	106	100	98	102	102
Liver weight, absolute [g]	6.552	7.304**	7.794**	7.882**	4.72	4.646	5.224*	5.3*
[% of control]	100	111	119	120	100	98	111	112
	Historical control: 6.402-10.131							
Liver weight, relative [%]	2.548	2.776*	3.102**	3.682**	2.849	2.728	3.196*	3.324**
[% of control]	100	109	122	145	100	96	112	117
	Historical control: 2.245 % to 3.091 %							
Spleen weight, absolute [g]	0.43	0.526**	0.454	0.398	0.332	0.336	0.338	0.304
[% of control]	100	122	106	93	100	101	102	92
Spleen weight, relative [%]	0.167	0.2*	0.18	0.185	0.2	0.197	0.207	0.191
[% of control]	100	119	108	111	100	99	104	95
Prostate weight, absolute [g]	0.53	0.48	0.478	0.36*	-	-	-	-
[% of control]	100	91	90	68	-	-	-	-
Prostate weight, relative [%]	0.206	0.182	0.19	0.167	-	-	-	-
[% of control]	100	89	93	81	-	-	-	-

1 As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Gross and histopathology:
The isolated macroscopic findings (focus in adrenal gland of one mid dose male animal and in one high dose female animal, focus in epididymidis of one mid dose male animal, kidney cyst in one low dose male animal and pelvic dilation of the kidney in one low dose male and one female control group animal) belong to the spectrum of background lesions and were considered to be incidental in nature and not related to treatment. Histopathological correlates were determined for the adrenal foci in the mid dose male and the high dose female in form of accessory cortical tissue and for the epididymal focus in the 4000 ppm group identified as a spermatogenic granuloma.
A histopathological correlate of centrilobular hypertrophy was found for the liver weight increases in

females of the mid and high dose group only and without any adverse histopathological findings (e.g. necrosis, fatty change, degeneration). Given the severity of the findings the effects in the high dose group were considered as adverse while the effects in the mid dose were considered adaptive.
The follicular hypertrophy/hyperplasia determined in males and females of the mid and high dose group (see Table B.6.8-123) was considered to be treatment related. In the 12000/8000 ppm group, this finding was graded slight in all males and 4 of 5 females, while it was graded minimal in 2 of 5 males and 1 of 5 females of the 4000 ppm group. This finding was also assessed as treatment related and secondary to enzyme induction in the liver. Due to the low incidence and grading the effect in the 4000 ppm group was considered adaptive.

Table B.6.8-123: Selected histopathological findings of rats administered ethylester derivative of M656H062 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000/ 8000[1]	0	1200	4000	12000/ 8000[1]
[mg/kg bw/day]		103	323	1065/ 763		131	385	1096/ 767
Number of animals evaluated	5	5	5	5	5	5	5	5
Liver								
- hypertrophy centrilobular	0	0	0	0	0	0	4	4
minimal	-	-	-	-	-	-	4	
severe	-	-	-	-	-	-		4
Thyroid								
- hypertrophy/hyperplasia, follicular	0	0	2	5	0	0	1	4
minimal	-	-	2		-	-	1	
moderate	-	-		5	-	-		4

1 As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

All other histopathological findings were either single observation, were equally distributed between control and treated groups or displayed no dose-response relationship. Therefore, these findings were considered to be incidental.

Conclusion:

The administration of ethylester derivate of M656H062 via the diet to male and female Wistar rats for 4 weeks test substance related adverse signs of toxicity at concentrations of 12000 ppm in males leading to a concentration reduction in males and females to 8000 ppm from day 18 of treatment onwards. Treatment related adverse effects were noticed after administered 12000/8000 ppm as indicated by impaired body weight development and food consumption, altered clinical chemistry parameters, increased absolute and relative liver weights and histopathological findings in livers and thyroids of male and/or female rats indicative for liver enzyme induction. The findings in the 4000 ppm group - increased absolute and relative liver weight - without any associated alteration in clinical chemistry or and only minimal histopathological changes was considered to be treatment related but not adverse. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 4000 ppm in male (323 mg/kg bw/day) and in female (385 mg/kg bw/day) Wistar rats.

B.6.8.2 Supplementary studies on the active substance

B.6.8.2.1 Haemoglobin binding potential

Studies evaluated in the original monograph of dimethenamid-P by the RMS in September, 2000 (ASB2010-10566):

Data point: CA 5.8.2/1

Report: [redacted] 1992 (TOX1999-448)
Investigation of the Potential of a Covalent Binding of [^{14}C]-dimethenamid (SAN 582 H) or its Derivatives to Rat and Human Haemoglobin
[redacted]
unpublished, 14 September 1992, BASF RegDoc.# 92/12484
(Experimental work: June – July 1992)

Guideline(s): No

Deviations: No

GLP: Yes (laboratory certified by Eidgenössisches Departement des Innern, Berne, Switzerland)

Acceptability: The study is considered to be acceptable.

Materials and methods:

Test Material: ^{14}C-radiolabelled dimethenamid, thiophene ring labelled, batch no. RA 683-2, radiochemical purity: 98.4 %.
Test System: Rat blood (3 adult female Wistar rats) and human blood (45 year old man)
Experiment 1 - determination of methaemoglobin:
As part of a liver enzyme study as discussed in section B.6.8.2.2 ([redacted], 1994 TOX1999-449), Wistar rats were administered unlabelled racemic dimethenamid via gavage in corn oil for 4 days at dosages of 0, 25, 100, 200 and 400 mg/kg. At the end of the treatment period, blood samples were collected. Methaemoglobin levels were determined and reported as part of this study.
Experiment 2 - haemoglobin binding:
Blood samples were collected from a human volunteer and Wistar rats. The packed red blood cell component was obtained and haemolysed chemically. The haemolysed blood components were incubated with 1 µL (0.2 µCi) ^{14}C-dimethenamid for 15 minutes at 37 °C in a shaking water bath. The incubation mixture was then separated into globin and haem fractions and the radioactivity counted.

Results:

Blood samples from Wistar rats treated for four consecutive days with various concentrations of dimethenamid did not indicate an increase in methaemoglobin.
Dimethenamid bound strongly to rat haemoglobin. However, no incorporation of dimethenamid into human haemoglobin was detected. Further investigation demonstrated that in rats the binding to haemoglobin was almost exclusively to the globin portion and very little radioactivity was found in the haem portion. The difference in haemoglobin binding between humans and rats is explained by the difference in three dimensional structure between the 2 species. It is known from the literature that the cysteine residue ß-125 in rat haemoglobin is accessible for chemical substitution, but in human haemoglobin the sequence does not contain a cysteine residue in position 125.

Conclusion:

Dimethenamid did not induce methaemoglobin in rat blood following a 4-d treatment. Dimethenamid

was shown to bind to rat haemoglobin, primarily to the globin portion, but no binding was demonstrated using human blood. It appears that the interaction between dimethenamid and haemoglobin is a species specific reaction. This binding is irrelevant for humans.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

B.6.8.2.2 Liver enzyme induction

Data point:	KCA 5.8.2
Report:	[redacted]., 1994 (TOX1999-449) Investigations of Liver Enzyme Induction by dimethenamid (SAN 582 H) in Rats [redacted] unpublished, 10 February 1994, BASF RegDoc.# 94/11897 (Experimental work: June 1992 – August 1993)
Guideline(s):	Special study, no guideline available.
Deviations:	Not applicable.
GLP:	Yes (laboratory certified by Eidgenössisches Departement des Innern, Berne, Switzerland)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

To determine liver enzyme levels, racemic dimethenamid was administered to groups of 6 male Sprague-Dawley rats via gavage in corn oil at dose levels of 0, 25, 100, 200 and 400 mg/kg bw for 4 consecutive days. A second group of 6 male rats were treated with dimethenamid at a dose level of 400 mg/kg bw/d for 4 days and then allowed a 4 day recovery period. The animals were examined daily for symptoms and mortality. Body weights were recorded daily and food consumption was determined for the total treatment period and recovery period. At the end of the treatment period urine was collected for standard analyses and then the animals were sacrificed and blood samples were drawn. All animals were subjected to a gross pathological examination and the liver, brain and kidneys were weighed. Samples of the liver were frozen for enzyme analysis.
Plasma levels of the following parameters were determined:

Alanine aminotransferase (ALAT)	Total Cholesterol
Aspartate aminotransferase (ASAT)	Bilirubin
Alkaline phosphatase (ALP)	Urea
Lactate dehydrogenase (LDH)	Fasting glucose
γ-glutamyl transferase (GGT)	Creatinine
Na, K, Cl, K, P	Total protein

The following parameters were determined from liver samples obtained:

Total Cytochrome P450-content (CYP)	Total glutathione (GSH)
Ethoxyresorufin-O-deethylase (EROD)	Glutathione-S-transferase (GST)
Pentoxyresorufin-O-dealkylase (PROD)	UDP-glucuronyl-transferase (UDPGT)
NADPH-cytochrome P450 reductase (NCPR)	

Standard urinalysis parameters were also investigated.

Results:

Findings noted in the study are summarised in Table B.6.8-124.

Table B.6.8-124: Results of clinical, organ weight, blood chemistry and liver enzyme analyses

Parameter Investigated	Dose levels (mg/kg bw/d)				
	0	25	100	200	400
Clinical					
Body weight gain (day 0–4) [g, mean ± SD]	+1.5 ± 2.4	+3.1 ± 1.9	+0.9 ± 3.6	-0.2 ± 3.0	-7.5 ± 4.2**
Organ weight					
Abs. liver wt [g, mean ± SD; % control]	7.5 ± 0.3	95 %	123 %**	133 %**	150 %**
Rel. liver wt [% bw, mean ± SD; % control]	3.9 ± 0.1	96 %	125 %**	131 %**	159 %**
Blood chemistry					
ALAT [IU/L, mean ± SD; % control]	26.6 ± 5.6	120 %	130 %	146 %	141 %*
Urinalysis					
Urine volume [mL, mean ± SD; % control]	4.7 ± 1.1	95 %	106 %	122 %	152 %*
Urine protein [score, mean ± SD; % control]	1.83 ± 0.41	109 %	91 %	91 %	64 %
Urine creatinine [mM, mean ± SD; % control]	7.6 ± 1.7	96 %	87 %	76 %	57 %**
Urine urea [mM, mean ± SD; % control]	353 ± 112	125 %	146 %	147 %	160 %*
Liver chemistry					
Total CYP [nmol/mg, mean ± SD; % control]	0.90 ± 0.09	105 %	134 %	139 %	155 %*
PROD [pmol/mg, mean ± SD; % control]	3.9 ± 2.1	148 %	380 %**	661 %*	565 %**
EROD [pmol/mg, mean ± SD; % control]	13 ± 6	110 %	220 %	234 %	249 %$
NCPR [nmol/mg, mean ± SD; % control]	135 ± 12	127 %*	148 %*	163 %*	241 %**
GST [µmol/mg, mean ± SD; % control]	0.84 ± 0.08	118.5 %*	148 %**	186 %**	253 %**
UDPGT [µmol/mg, mean ± SD; % control]	48 ± 11	116 %	116 %	139 %*	187 %**
Total GSH [nmol/mg, mean ± SD; % control]	27.1 ± 6.7	97 %	92 %	105 %	67 %

* = $p < 0.05$; ** = $p < 0.01$ (statistically different from control value, Dunnett's test);
$ = $p < 0.05$ (statistically significant from control value, Mann-Whitney U test)

One animal died due to a gavage error and was replaced. No other mortality and no clinical signs were observed. Food consumption was higher for all treatment groups compared to control. At 400 mg/kg bw/d, body weight gain was significantly decreased (body weight loss).
Absolute and relative liver weights were increased at doses from 100 to 400 mg/kg bw/d. The only blood liver enzyme change was increased alanine aminotransferase at the high dose. At the high dose,

urine volume was decreased and urine protein, creatinine and urea were decreased.
The liver enzyme analysis demonstrated significant changes with treatment with dimethenamid. Total cytochrome P450 and specifically PROD and EROD were increased with treatment. UDP-glucuronyl transferase was also increased at the 2 highest dose levels. Dimethenamid also induced an increase in glutathione s-transferase and NADPH reductase levels at all treatment levels. However, these changes were slight at the low dose. In addition, the induction of these enzymes represent a physiological adaptation in the liver to remove the chemical and are not an adverse effect. In the absence of liver weight change or other liver enzyme changes at the low dose, the slight changes on these 2 enzyme systems is not considered an adverse effect.
Glutathione was decreased at the high dose. This indicates that the glutathione conjugation pathway was saturated at the high dose level.
All parameters investigated returned to control or near control levels following the four day recovery period.

Conclusion:

Oral administration of racemic dimethenamid to rats for 4 days induced several liver enzyme systems. It was demonstrated that the metabolism of dimethenamid involves oxidation steps mainly by cytochrome P450 dependent enzymes and glutathione conjugation and glucuronidation. Upon cessation of treatment, the observed liver effects were almost fully reversible within a 4-day recovery period.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.

B.6.8.3 Immunotoxicity

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	CA 5.8.2/1
Report:	[redacted] 2013f (ASB2014-8422) BAS 656-PH - Immunotoxicity study in female C57BL/6J Rj mice - Administration via the diet for 4 weeks 2013/1028329 Dates of experimental work: 10/02/2012 - 03/06/2013
Guideline(s):	EPA 870.7800
Deviations:	No relevant deviations
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	The study is considered to be acceptable.

Materials and methods:

The immunotoxic potential of dimethenamid-P in female C57BL/6J Rj mice was analysed using dietary dose levels of 0, 500, 1500 and 4000 ppm (corresponding to mean intake levels of 120, 385 and 1167 mg/kg/d, respectively) for 28 days.

Test Material:	dimethenamid-P
Description:	liquid/ brown, clear
Lot/Batch #:	COD-001509, dimethenamid-P (BAS 656-PH):
Purity:	95.9 %
Stability of test compound:	The test substance was stable over the study period (Expiry date Oct. 01, 2013).

Vehicle control:	Rodent diet
Positive control:	Cyclophosphamide monohydrate (CPA)
Description:	Solid / white
Lot/Batch #:	SLBC0666V
Purity:	102.3 % (according to supplier)
Stability of test compound:	According to the supplier the positive control substance was stable over the study period (Expiry date March 2015).
Vehicle for CPA:	Drinking water
Test animals:	
Species:	Mouse
Strain:	C57BL/6J Rj
Sex:	Female
Age:	41 ± 1 days at delivery; approx. 49 ± 1 days at start of administration
Weight at dosing:	18.2 ± 0.6 g
Source:	Centre d´Elevage R. Janvier, Route des Chênes Secs - B.P. 5, 53940 Le Genest St Isle, France
Acclimation period:	8 days
Diet:	Kliba maintenance diet for mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*
Water:	Tap water in bottles, *ad libitum*
Housing:	Pairwise in polycarbonate cages type M III with wire cover (Becker & Co., Castrop-Rauxel, Germany) with dust free wooden bedding. Mouse tunnel (red, transparent) and Nestlets NES 3600 (PLEXX b.v.; Elst Netherlands) were added for enrichment.
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15/hour
Photo period:	12 h light/12 h dark (06:00 - 18:00/18:00 - 06:00)
Dates of experimental work:	10/02/2012 - 03/06/2013. (In life dates: 10-Oct-2012 (start of administration) to 08-Nov-2012 (necropsy)).

Animal assignment and treatment:

Dimethenamid-P was administered to groups of 8 female mice at dietary concentrations of 0, 500, 1500 and 4000 ppm for 28 days. Additionally 8 female mice were treated orally (gavage) with 10 mg cyclophosphamide monohydrate (CPA; positive control substance) per kilogram per day. CPA was administered as a solution in drinking water at a volume of 10 mL/kg. The administered volume was determined based on the most recently determined body weights.

The animals were assigned to the treatment groups by means of computer generated randomisation list based on body weights.

On day 23 of the study all animals received a single intraperitoneal injection (0.5 mL) of a sheep red blood cell (SRBC)-suspension containing 4 x 10^8 cells/mL

Test substance preparation and analysis:

The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Diet preparations were performed once before the start of administration.

Analyses performed using a comparable batch prior to the start of the administration period revealed that the test substance was stable in the diet for up to 46 days.

Homogeneity and concentration analyses of the diet preparations were

performed at the beginning of the administration period for all concentrations. No test article was determined in control diets.

Table B.6.8-125: Results of homogeneity and concentration control analysis of dimethenamid-P in rodent diet

Nominal Dose level [ppm]	Sampling	Concentration Mean ± SD# [ppm]	Relative standard deviation [%]	Mean of nominal concentration [%]
500	Oct. 09, 2012	479.5 ± 19.9	4.2	95.9
1500	"	1389.8 ± 19.3	1.4	92.7
4000	"	3966.7 ± 101.7	2.6	99.2

Values may not calculate exactly due to rounding of figures.

Relative standard deviations of the homogeneity of the dimethenamid-P samples were in the range of 1.4 to 4.2 %, which indicate the homogenous distribution of dimethenamid-P in the diet preparations. The actual (mean) average test substance concentrations were in the range of 92.7 to 99.2 % of the nominal concentrations confirming the correctness of the concentrations.
The positive control substance preparation (CPA in drinking water) was prepared once at the beginning of the study, split in daily aliquots and deep frozen at -18 °C. The mixtures were applied when reaching room temperature. The concentration control of the CPA solution was performed at the beginning of the study. Since the CPA formulation in drinking water was a solution a homogeneity analysis was redundant.

Table B.6.8-126: Results of concentration control analysis of CPA in drinking water

Nominal Concentration [mg/mL]	Sampling/Analysis	Analytical concentration [mg/mL]	Mean of nominal concentration [%]
1	Oct. 08, 2012/Feb. 19, 2013	0.928	92.8

The actual CPA concentrations was 92.8 % of the nominal concentration confirming the correctness of the concentration.
The stability analysis conducted revealed the stability of the CPA solution for 32 days when stored frozen and for 7 days when stored ambient. Indirectly the concentration control analysis revealed a stability of CPA solution for 134 days when stored frozen (see Table B.6.8-126).
Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table B.6.8-127: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change and food efficiency	For test substance and the vehicle control groups: A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means For the vehicle and positive control groups: A comparison of the dose group with the control group was performed using the t-test (two-sided) for the hypothesis of equal means

Table B.6.8-128: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians.

Table B.6.8-129: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.

Observations:
The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily.

1. abnormal behaviour during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. faeces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable.
Body weight:
The body weight of the animals was determined before the start of the administration period (in order to randomise the animals), at the start of the treatment (day 0), and once weekly thereafter.
Food consumption, food efficiency and compound intake:
Food consumption was determined once weekly on a cage basis and calculated as mean food consumption in grams per animal and day.
Food efficiency was calculated based upon individual values for body weight and mean weekly food consumption per cage group of animals:

$$Food\ efficiency\ at\ day\ x = \frac{BW_x - BW_y}{FC_{y\ to\ x}}\ x\ 100$$

with BWx and BWy body weight [g] at day x and day y (last weighing date before day x), FC y to x as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Substance intake for day } x = \frac{FC_x \times C}{BW_x}$$

with FCx as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BWx as body weight on day x of the study (in g).

Water consumption:

Individual water consumption was observed by daily visual inspection of the water bottles for any overt changes in the volume. No water consumption values were recorded.

Analysis of the primary immune response:

Blood was drawn in the morning from non-fasted, isoflurane anesthetised animals after decapitation (in case the blood volume from retro-orbital sampling was insufficient). The blood sampling procedure and the subsequent analysis of the blood samples were carried out in a randomised sequence.

The assays of serum parameters were performed under internal laboratory quality control conditions with reference controls to assure reliable test results.

Primary T-cell dependent antibody response (anti-SRBC IgM ELISA):

Plasma samples from all SRBC immunised animals were analysed for their specific anti SRBC-IgM titer in an ELISA (cat. No. 4200-1, Life Diagnostics Inc, West Chester, USA). Each sample was diluted 1:101. SRBC-IgM concentrations outside the standard curve range were measured in a second test run with an appropriate dilution. Generally, two in-house anti-SRBC positive serum were used for a standard curve. The ELISA was measured with a Sunrise MTP-reader (Tecan AG, Maennedorf, Switzerland), and evaluated with the Magellan-Software of the instrument producer.

Sacrifice and pathology:

The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).											
C	W	H		C	W	H		C	W	H	
✓	✓		kidneys	✓	✓		spleen		✓		body (anesthetised animals)
✓	✓		liver	✓	✓		thymus				

No histopathological examinations were performed.

Results:

Observations:

Clinical signs of toxicity:

No clinical signs were observed throughout the study.

Mortality:

No mortality was observed in this study.

Body weight and body weight gain:

Mean body weight of animals of the high dose (4000 ppm) was significantly lower by -7.2 % on study day 14 (see Table B.6.8-130, Figure B.6.8-27). Consequently, the body weight change value was significantly lower by -97.1 % on study day 14. These findings occurred in the absence of a similar pattern in mean food consumption, and therefore, were considered to be treatment related, direct adverse systemic effects of dimethenamid-P.

In contrast, the increased body weight change in the mid dose (1500 ppm) on study days 21 and 28 was considered to be incidental due to lacking dose-response relationship. Impaired body weight development was observed in CPA treated mice. Absolute mean body weight and body weight gain were reduced by 9 % and 103.1 %, respectively.

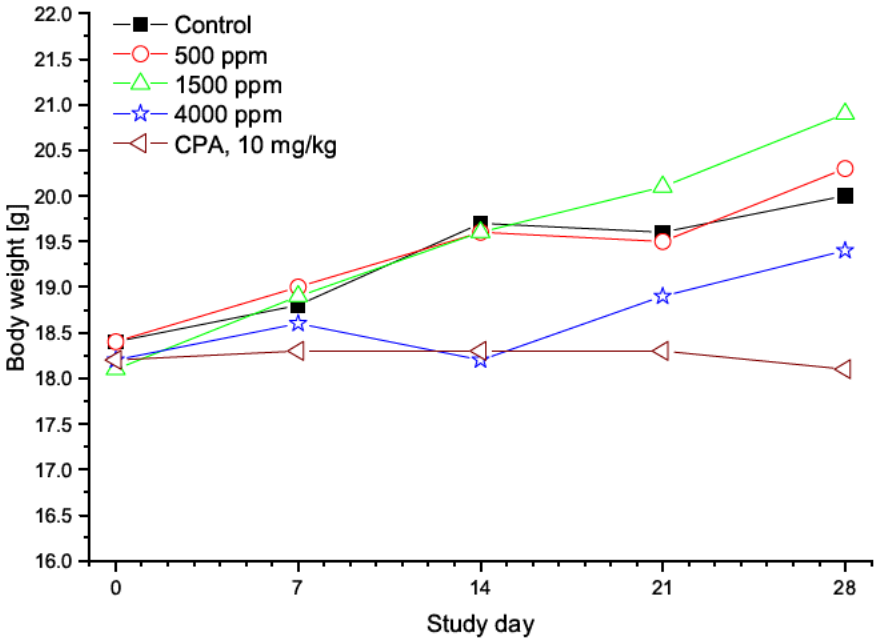


Figure B.6.8-27: Body weight development of mice administered dimethenamid-P for 28 days

Table B.6.8-130: Mean body weight of mice administered BAS 656 PH or cyclophosphamide (CPA) for 28 days

Treatment	Dimethenamid-P				CPA
Dose level	0	500	1500	4000	10 mg/kg
	ppm	ppm	ppm	ppm	
Body weight [g]					
- Day 0	18.4	18.4	18.1	18.2	18.2
- Day 7	18.8	19	18.9	18.6	18.3
- Day 14	19.7	19.6	19.6	18.2 **	18.3**
- Day 21	19.6	19.5	20.1	18.9	18.3*
- Day 28	20	20.3	20.9	19.4	18.1**
Day 28 Δ% (compared to control)#		1.6	4.6	-2.6	-9.0
Overall body weight gain Day 0 to day 28 [g]	1.6	1.8	2.8 **	1.2	0**
Δ % (compared to control)#		14.1	75.8	-23.4	-103

Values may not calculate exactly due to rounding of figues
* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnet-test, two sided)

Food and Water consumption and compound intake:
Food consumption was significantly increased from study day 14 to 21 for mice of test group 3 (4000 ppm) (see Table B.6.8-131). However, food spilling was observed prior to the determination of food consumption on study day 21 in one cage of the test group. Thus, the finding was assessed as

being not toxicologically relevant.

Table B.6.8-131: Mean cumulative food consumption of mice administered dimethenamid-P or cyclophosphamide (CPA) for 28 days

Treatment	Dimethenamid-p				CPA
Dose level	0	500	1500	4000	10 mg/kg
	ppm	ppm	ppm	ppm	
Food consumption [g/animal*day]					
- Day 0 to 7	3.9	3.8	4.1	4.9	3.7
- Day 7 to 14	4.2	5.2	5.1	4.8	4.7
- Day 14 to 21	4.5	4.8	5.6	6.5**	5.4
- Day 21 to 28	5.5	4.8	5.3	5.5	4*
Cumulative food consumption [g/animal]					
- Day 0 to 28[§]	126.7	130.2	140.7	151.9	124.6
Δ % (compared to control)[#]		3 %	11 %	20 %	-2 %

[§] Values were calculated based on mean daily food consumption
[#] Values may not calculate exactly due to rounding of figures
* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnet test, two sided)

The food consumption of the CPA treated animals was statistically reduced on days 21 and 28. This value was still within the normal range typical for the strain of mice. Taking into account the observed effects on body weight for CPA a treatment relation can however not be excluded.
The mean daily test substance intake over the entire study period was calculated and is shown in the following table:

Table B.6.8-132: Calculated intake of dimethenamid-P

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)
		Females
1	500	120
2	1500	385
3	4000	1167

No treatment related effects on water consumption were noted.
Immunological analyses:
Analysis of the primary T-cell dependent immune response:
The SRBC specific IgM titers of the plasma samples from the animals treated with dimethenamid-P were not relevantly altered as compared to the vehicle control group, whereas the SRBC titers were lower in mice of the positive control group (CPA, 10 mg/kg bw/d) (see Table B.6.8-133).

Table B.6.8-133: Analysis of the specific primary (IgM) immune response to SRBC in mice treated with dimethenamid-P or cyclophosphamide for 28 days

Treatment		Dimethenamid-P				CPA
Dose	[ppm]	0	500	1500	4000	
	[mg/kg bw/day]		120	385	1167	10
Specific IgM Titer (U/mL)						
- Mean ± SD		3604	3788	2176	2389	277**
		±	±	±	±	±
		2568	3697	1315	1755	113
- Median		2826	2576	1597	1988	242
- min. value		551	1938	1149	47	177
- max value		8305	12195	4353	5867	528

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test. two sided)

Even though the means seem to indicate a trend towards a decreased immune response, the immune response was not considered to be affected by treatment. This is due to the following reasons:
There was no dose response relationship.
The determined values show a high variability of the immune response within animals of the same group, as evident by the fact that the statistical evaluation did not show a statistically significant difference.
Except for one value of the low dose group (that was above) and one value in the high dose group (that was below), all individual immune responses in dimethenamid-P treated groups were within the range of individual responses in control animals (see Table B.6.8-133).
Necropsy:
Terminal body and organ weight:
Mean terminal body weights of the dimethenamid-P groups were not affected by treatment (see Table B.6.8-134).
Absolute liver weights were significantly increased in the mid and high dose group administered 1500 and 4000 ppm, respectively, by 19 and 37 %. Correspondingly the relative liver weights were increased by 7 and 41 %, respectively. These weight increases were considered to be treatment related.
Absolute and relative thymus weights were significantly increased by 29 % and 33 % in the high dose group (see Table B.6.8-134). These weight changes were however considered to be incidental as they were clearly within the historical control range for these parameters (see Table B.6.8-134).
Relative kidney weights were slightly increased (+15, +7, +11 %) when compared to control. The significantly increased kidney weights were considered to be incidental due to the lacking dose-response relationship.
In the positive control group however, the terminal body weights were significantly decreased. Moreover, treatment related and statistically significant decrease of absolute and relative terminal body and absolute spleen weight were observed in the animals with the positive control substance CPA (see Table B.6.8-134).

Table B.6.8-134: Mean absolute and relative organ weights of female mice treated with dimethenamid-P or cyclophosphamide for at least 28 days

Sex		Dimethenamid-P				CPA
Dose	[ppm]	0	500	1500	4000	
	[mg/kg bw/day]		120	385	1167	10
Terminal body weight	[g]	17.175	17.2	17.375	16.638	15.863**
	[% of control]	100	100	101	97	92
Kidney, absolute	[g]	0.243	0.279	0.26	0.27	0.229
	[% of control]	100	115	107	111	94
Kidney, relative	[%]	1.405	1.627	1.507*	1.616**	1.448
	[% of control]	100	116	107	115	103
Liver, absolute	[g]	0.758	0.816	0.901**	1.039**	0.684**
	[% of control]	100	108	119	138	90
Liver, relative	[%]	4.411	4.735*	5.191**	6.231**	4.315
	[% of control]	100	107	118	141	98
Spleen, absolute	[g]	0.064	0.063	0.066	0.063	0.053*
	[% of control]	100	98	104	98	82
Spleen, relative	[%]	0.364	0.361	0.378	0.367	0.331
	[% of control	100	99	104	101	91
Thymus, absolute	[mg]	41.125	45.125	48.125	53.0*	44.5
	[% of control]	100	110	117	129	108
	[mg]	Historical control: 38.200-66.600 (mean 44.993)				
Thymus, relative	[%]	0.239	0.263	0.277	0.318**	0.286
	[% of control]	100	110	116	133	119
	[%]	Historical control: 0.222-0.353 (mean 0.265)				

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Gross pathology:
No gross pathological lesions were observed in this study.

Conclusion:

Under the conditions of this study, in the presence of systemic toxicity, BAS 656-PH did not reveal any signs of immunotoxicity (by T-cell dependent antigen response) when administered via the diet over a period of 4 weeks to female mice. Thus, the NOAEL for immunotoxicity was set to 4000 ppm (1167 mg/kg bw/d), the highest dose tested (limit dose). The NOAEL for systemic toxicity was 500 ppm corresponding to 120 mg/kg bw/day in female C57BL mice.
The oral administration of the positive control substance CPA (10 mg/kg bw/d) led to findings indicative of immunotoxicity by T-cell dependent antigen response. This was represented by significantly lower SRBC IgM antibody titers as well as reduced spleen weights. Thus, assay sensitivity was verified in the present immunotoxicity study performed in female C57BL/6J Rj mice.

B.6.8.4 Studies on endocrine disruption

A separate evaluation of potential endocrine disruption was not a data requirement at the time of Annex I inclusion of dimethenamid-P. However, this endpoint is considered intrinsically covered by the respective pivotal toxicity studies on racemic dimethenamid and dimethenamid-P.
The data package of dimethenamid-P does not indicate a potential of dimethenamid-P to affect the oestrogen or androgen system.

Peer-reviewed Literature:

Data point:	CA 5.8.3/1
Report:	Shah I. et al., 2011a (ASB2014-8446) Using nuclear receptor activity to stratify hepatocarcinogens 2011/1295091
Guideline(s):	No
Deviations:	No
GLP:	No
Acceptability:	The study is considered to be supplementary.

Executive Summary of the Literature:
As part of the ToxCast program dimethenamid-P was tested for activity on human constitutive androstane receptors (CAR/NR1I3), pregnane X receptor (PXR/NR1I2), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptors (PPAR/NR1C), liver X receptors (LXR/NR1H), retinoic X receptors (RXR/NR2B) and steroid receptors (SR/NR3) *in vitro*. Dimethenamid-P was allocated to group D (negative for liver tumour progression). Dimethenamid-P was positive in activation assays for ATG_PPARg_Trans, ATG_PXRE_CIS, CLZD_CYP2B6, CLZD_CYP3A4, NCGC_AR_Antagonist, NCGC_PXR_Agonist_human.
Dimethenamid-P was not associated with the activation of other assays targeting the same molecular endpoints. For example dimethenamid-P was negative in the other assays targeting the androgen receptor system (e.g. ATG_AR_Trans, NCGC_AR_Agonist, NVS_NR_hAR) the oestrogen receptor system, or other assays linking a chemical to activation of CAR, PPAR or PXR.
In total it becomes apparent that the provided data on dimethenamid-P provides contradictory information not supported by the pivotal animal studies conducted and at best allows a linkage between dimethenamid-P and the CAR/PXR system, which would be in line with the *in vivo* observations of enzyme induction and liver response.

Data point:	CA 5.8.3/2
Report:	Reif D.M. et al., 2010a (ASB2014-8447) Endocrine profiling and prioritisation of environmental chemicals using ToxCast data 2010/1231552
Guideline(s):	No
Deviations:	No
GLP:	No
Acceptability:	The study is considered to be supplementary.

Executive Summary of the Literature:
This publication illustrates a profiling tool developed on the ToxCast database to prioritise chemicals with regard to endocrine disruption evaluation/testing as a decision support tool. Thus this priorisation tool was applied also to dimethenamid being part of the ToxCast program. The priorisation tool

focused on oestrogen, androgen and thyroid pathways and thus incorporated those screening assays of the ToxCast program considered relevant for putative endocrine profiles. In addition it incorporated external molecular pathway databases i.e. Kyoto Encyclopaedia of Genes and Genomes (KEGG), Ingenuity software and the Online Mendelian Inheritance in Men repository. The tests in which dimethenamid showed an activity are the same as described in the publication of Shah et al., 2011 (ASB2014-8446). The so-called ToxPi profile for dimethenamid showed no alert for thyroid or oestrogen receptor pathways but a limited alert for androgen receptor pathways as well as for other nuclear receptors (not specified). Also a limited activity for the KEGG pathways is shown. The publication does not provide an absolute ranking but in visual comparison to activity alerts for other compound the ranking of activities for dimethenamid is low. No linkage between dimethenamid-P and other endpoints was provided.

Data point:	CA 5.8.3/3
Report:	Sipes N.S. et al., 2013a (ASB2014-8448) Profiling 976 toxcast chemicals across 331 enzymatic and receptor signalling assays 2013/1371960
Guideline(s):	No
Deviations:	No
GLP:	No
Acceptability:	The study is considered to be supplementary.

Executive Summary of the Literature:
Summary report on the ToxCast program. The publication makes some general statements on the progress of the ToxCast program. The data provided for dimethenamid-P is the same as in Shah et al. (2011, ASB2014-8446). No linkage between dimethenamid-P and other endpoints is provided.

B.6.9 Medical data and information

B.6.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Information evaluated in the original monograph of dimethenamid-P from September, 2000 (ASB2010-10566):

A survey was conducted in 1992 to determine if there were any adverse health effects in personnel handling racemic dimethenamid and dimethenamid products.

Data point:	KCA 5.9
Report:	Rataj M., 1992 Human Exposure Experience with Frontier® herbicide (SAN 582) SANDOZ Agro, Inc., Des Plaines, Illinois 60018 unpublished, 15 April 1992, BASF RegDoc.# 92/12480, TOX1999-450
Guideline(s):	Human survey study, no guideline available
Deviations:	No
GLP:	No (Not subject of GLP regulations)
Acceptability:	The study is considered to be supplementary.

Materials and Methods

Through interviews or responses to information requests, the experiences of three groups of Sandoz Agro personnel involved in the manufacture, formulation, and actual use (mixing/loading and application) of Frontier® were documented.
Sandoz Agro's Beaumont Texas manufacturing facility produced three batches of Frontier® herbicide in 1991 and 1 batch in 1992. Between 5 - 10 people were involved in the process which included charging raw materials, process sampling and manually filling containers with the final formulated material. In 1991 approx. 750 1-litre containers and 475 0.5-litre containers were filled and in 1992 800 4-litre containers were filled. Employees wore protective gloves and clothing.
Approx. 5 - 7 people in the Research and Development Formulations group handled dimethenamid and formulations of dimethenamid between 1986 and 1992. Activities included material transfer, drying and packaging. Employees wore protective gloves and clothing and often were working under protective hoods.
During product development field trials, 20 people worked with dimethenamid products between 1984 and 1991. Activities including mixing, loading and application of the formulation product for application and spraying of the product diluted in water. Most applications were made with a backpack hand sprayer and some applications were with a tractor-mounted sprayer. These activities were conducted 7 – 15 times per year. Personnel wore protective gloves and clothing during handling of the product.

Results:

For all three groups surveyed, there were no cases of skin irritation, skin rash or other signs of allergic response. In addition, no general signs of adverse health effects were reported.

Conclusion:

Interviews and information requests were made with 50 people handling dimethenamid and dimethenamid formulated products over a 7-year period. There were no reported cases of skin irritation, skin sensitisation or other adverse health effects.

Data submitted with the dossier for the Renewal Assessment Report:

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of dimethenamid. Thus, the medical monitoring program is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.
The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to dimethenamid-P exposure have not been observed (BASF DocID 2014/1095679, 30/Mar/2014; Jilderda, 2014, ASB2014-8378).

B.6.9.2 Data collected on humans

Data submitted with the dossier for the Renewal Assessment Report:

Some cases of irritation of the eyes have been registered in the BASF internal clinical incident log exposed to dimethenamid in combination with other products. It is not clear whether dimethenamid was the cause for these irritations (BASF DocID 2014/1095679, 30/Mar/2014; Jilderda, 2014, ASB2014-8378).

B.6.9.3 Direct observations

Information evaluated in the original monograph of dimethenamid-P from September, 2000 (ASB2010-10566):

During experimental testing of dimethenamid and dimethenamid formulations, and during manufacture and field use of the dimethenamid containing Frontier® herbicide, no negative experiences affecting the health of workers were reported. No other poisoning incidents are known to the applicant.

Data submitted with the dossier for the Renewal Assessment Report:

Some cases of slight irritation of the skin, eyes or respiratory tract (including rhinitis and cough), head ache, nausea and dizziness have been reported to BASF in persons exposed to dimethenamid in combination with other active substances. These reports could not be verified, and it is not clear whether dimethenamid was the cause for these irritations. In one case sensitisation was discussed, which could not be verified (BASF DocID 2014/1095679, 30/Mar/2014; Jilderda, 2014, ASB2014-8378).

B.6.9.4 Epidemiological studies

Data submitted with the dossier for the Renewal Assessment Report:

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties (BASF DocID 2014/1095679, 30/Mar/2014; Jilderda 2014, ASB2014-8378).

B.6.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Data submitted with the dossier for the Renewal Assessment Report:

Specific signs of toxicity or specific clinical test methods are not known to BASF SE (BASF DocID 2014/1095679, 30/Mar/2014; Jilderda, 2014, ASB2014-8378).

B.6.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

Data submitted with the dossier for the Renewal Assessment Report:

See safety data sheet/precautions; symptomatic and supportive treatment, no specific antidote known (BASF DocID 2014/1095679, 30/Mar/2014; Jilderda, 2014, ASB2014-8378).

B.6.9.7 Expected effects of poisoning

Data submitted with the dossier for the Renewal Assessment Report:

Expected effects were derived for acute and subacute studies in animals (BASF DocID 2014/1095679, 30/Mar/2014; Jilderda, 2014, ASB2014-8378).

B.6.9.8 Medical Data - Annex

Data submitted with the dossier for the Renewal Assessment Report:

A search in the literature databases listed below - restricted to "pps=human" and "ct d human" - has been performed on March 11th, 2014 via DIMDI-host for the following terms (BASF DocID 2014/1095679, 30/Mar/2014; [redacted] 2014, ASB2014-8378):

- Dimethenamid*
- CAS 90717-03-6 and 163515-14-8

Medline 66 (NLM)
Medline alert (NLM)
Embase 74 (Elsevier)
Embase alert (Elsevier)
Cochrane Library-Central
Biosys (Thomson Reuters)
gms (German Medical Science)
IPA International Pharmaceutical Abstracts (Thomson Reuters)
Deutsches Aerzteblatt (Aerzteverlag)
Cross check via Internet available databases:
CHEMID (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)
PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)
Toxnet (http://toxnet.nlm.nih.gov/)

These searches revealed no relevant documents.

B.6.10 References relied on

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.1.1	[redacted]	1992	Absorption, distribution, metabolism and excretion of (^{14}C) SAN 582 in rat after single and mutiple doses (addendum to: SAN 582 H metabolism in rat; BASF RegDoc# 89/11026) [redacted] BASF RegDoc.# 92/12428 GLP, unpublished BVL2862833 TOX1999-406	Y	N	Not applicable	BASF	Y relevant IIA. 5.1.1.1 [5.1/01]
KCA 5.1.1	[redacted]	1992	SAN 582H: Determinatin of the presence of sulfonate metabolite in mice [redacted] 60018 BASF RegDoc.# 92/12445 GLP, unpublished BVL-1902716 TOX1999-407	Y	N	Not applicable	BASF	Y relevant IIA. 5.1.1.1 [5.1/02]
KCA 5.1.1	[redacted]	1992	SAN 582H: Addendum to determine sulfoxide of thiogylcolic acid conjugate in mouse excreta [redacted] 60018 BASF RegDoc.# 92/12446 GLP, unpublished BVL-1902757 TOX1999-408	Y	N	Not applicable	BASF	Y relevant IIA. 5.1.1.1 [5.1/03]
KCA 5.1.1	[redacted]	1992	SAN 582H: Determination of the presence of plant metabolites in rat [redacted] 60018 BASF RegDoc.# 92/12448 GLP, unpublished BVL-1902731 TOX1999-409	Y	N	Not applicable	BASF	Y relevant IIA. 5.1.1.1 [5.1/04]
KCA 5.1.1	[redacted]	1993	Qualitative investigations of the in-vitro (liver and kidney) metabolism of dimiethenamid (SAN 582 H) [redacted] BASF RegDoc.# 93/11765 GLP, unpublished BVL-1902732 TOX1999-410	Y	N	Not applicable	BASF	Y relevant IIA. 5.1.1.1 [5.1/05]
KCA 5.1.1/1	[redacted]	2014	Excretion and metabolism of ^{14}C - Dimethenamid-P (BAS 656 H) after oral administration in rats 2012/1194996 [redacted] GLP, unpublished BVL-2630070 ASB2014-8383	Y	Y	New data for AIR3 renewal	BASF	N II A 5.1
KCA 5.1.1/2	[redacted]	2012	^{14}C -BAS 656 H - Study on bile excretion in rats 2012/1021081 [redacted] GLP, unpublished BVL-2630071 ASB2014-8384	Y	Y	New data for AIR3 renewal	BASF	N II A 5.1

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.1.2/1	[redacted]	2002	Comparison of *in vitro* metabolism of enantiomers of BAS 656 H (dimethenamid) 2002/1004042 [redacted] GLP, unpublished BVL-2630073 ASB2014-8385	N	Y	New data for AIR3 renewal	BASF	N
KCA 5.1.2/2	[redacted]	2014	Comparative in-vitro-metabolism with ^{14}C -BAS 656-PH 2013/1337274 [redacted] GLP, unpublished BVL-2630074 ASB2014-8386	N	Y	New data for AIR3 renewal	BASF	N
KCA 5.2.1	[redacted]	1996	Acute oral toxicity study with SAN 1289 H technical in rats [redacted] BASF RegDoc.#96/11087 GLP, unpublished BVL-1902756 TOX1999-413	Y	N	Not applicable	BASF	Y relevant IIA. 5.2.1 [5.2/01]
KCA 5.2.1	[redacted]	1991	Acute oral toxicity study of SAN 582H technical (K/E) in rats [redacted] BASF RegDoc.# 91/11940 GLP, unpublished BVL-1902747 TOX1999-414	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.2.1 [5.12/01]
KCA 5.2.2	[redacted]	1996	Acute dermal toxicity study with SAN 1289 H technical in rabbits [redacted] BASF RegDoc.#96/5395 GLP, unpublished BVL-1902712 TOX1999-451	Y	N	Not applicable	BASF	Y relevant IIA. 5.2.2 [5.2/02]
KCA 5.2.2	[redacted]	1991	Acute dermal toxicity study of SAN 582H technical (K/E) in rabbits [redacted] BASF RegDoc.#91/11942 GLP, unpublished BVL-1902741 TOX1999-452	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.2.2 [5.12/02]
KCA 5.2.3	[redacted]	1996	An acute (4-hour) inhalation toxicity study of SAN 1289 H technical in the rat via nose-only exposure [redacted] BASF RegDoc.# 96/5397 GLP, unpublished BVL-1902782 TOX1999-415	Y	N	Not applicable	BASF	Y relevant IIA. 5.2.3 [5.2/03]
KCA 5.2.3	[redacted]	1986	4-hour acute inhalation toxicity study with SAN 582 H in rats [redacted] BASF RegDoc.# 86/11166 GLP, unpublished BVL-1902775 TOX1999-453	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.2.3 [5.12/03]

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.2.3/1	[redacted].	2012	BAS 656-P H - Acute inhalation toxicity (nose only) study in the rat 2011/1171036 [redacted] GLP, unpublished BVL-2630075 ASB2014-8387	Y	Y	New data for AIR3 renewal	BASF	N IIA 5.2.3
KCA 5.2.4	[redacted]	1996	Primary dermal irritation study with SAN 1289 H technical in rabbits [redacted] BASF RegDoc.#96/5406 GLP, unpublished BVL-1902771 TOX1999-416	Y	N	Not applicable	BASF	Y relevant IIA. 5.2.4 [5.2/04]
KCA 5.2.4	[redacted]	1988	Primary dermal irritation study in rabbits with SAN 582H technical [redacted] BASF RegDoc.# 88/11363 GLP, unpublished BVL-1902725 TOX1999-454	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.2.4 [5.12/04]
KCA 5.2.5	[redacted]	1996	Primary eye irritation study with SAN 1289 H technical in rabbits [redacted] BASF RegDoc.#96/5396 GLP, unpublished BVL-1902746 TOX1999-417	Y	N	Not applicable	BASF	Y relevant IIA. 5.2.5 [5.2/05]
KCA 5.2.5	[redacted]	1988	Primary eye irritation study in rabbits with SAN 582H technical [redacted] BASF RegDoc.# 88/11364 GLP, unpublished BVL-1902750 TOX1999-455	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.2.5 [5.12/05]
KCA 5.2.6	[redacted]	1996	Closed-patch repeated insult dermal sensitisation study with SAN 1289 H technical in guinea pigs (Buehler Method) [redacted] BASF RegDoc.#96/11088 GLP, unpublished BVL-1902770 TOX1999-418	Y	N	Not applicable	BASF	Y relevant IIA. 5.2.6 [5.2/06]
KCA 5.2.6	[redacted]	1995	Contact Hypersensitivity to Dimethanamid Technical in Albino Guinea Pigs- Maximisation-Test BASF RegDoc.# 95/11324 GLP, unpublished BVL-2896751 TOX2000-1560	Y	Y	New data for AIR3 renewal	BASF	N
KCA 5.2.7/1	[redacted].	2013	BAS 656-P H - *In vitro* 3T3 NRU phototoxicity test 2013/1110119 [redacted]. GLP, unpublished BVL-2630077 ASB20148388	N	Y	New data for AIR3 renewal	BASF	N

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.3.1	[redacted]	1996	A 4-week range-finding study of SAN 1289 H in rat via dietary administration [redacted] BASF RegDoc.#96/11147 GLP, unpublished BVL-1902745 TOX1999-419	Y	N	Not applicable	BASF	Y relevant IIA. 5.3.1.1 [5.3/01]
KCA 5.3.1	[redacted]	1987	SAN 582H: 5 weeks pilot feeding study in rats [redacted] BASF RegDoc.#87/11227 GLP, unpublished BVL-1902763 TOX1999-468	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.7 [5.12/18]
KCA 5.3.2	[redacted]	1996	A subchronic (3-month) toxicity study of SAN 1289 H in the rat via dietary administration [redacted] BASF RegDoc.#96/5420 GLP, unpublished BVL-1902769 TOX1999-421	Y	N	Not applicable	BASF	Y relevant IIA. 5.3.2.1 [5.3/03]
KCA 5.3.2	[redacted]	1988	SAN 582 H: 13-week dose-range finding study in CD-1 mice [redacted] BASF RegDoc.#88/11360 GLP, unpublished BVL-1902768 TOX1999-422	Y	N	Not applicable	BASF	Y relevant IIA. 5.3.2.2 [5.3/04]
KCA 5.3.2	[redacted]	1986	SAN 582 H: 13 week oral toxicity study in dogs [redacted] BASF RegDoc.#86/11159 GLP, unpublished BVL-1902739 TOX1999-423	Y	N	Not applicable	BASF	Y relevant IIA. 5.3.2.3 [5.3/05]
KCA 5.3.2	[redacted]	1986	SAN 582 H: 13 week oral toxicity study in dogs [redacted] BASF RegDoc.#86/11178 GLP, unpublished BVL-1902713 TOX1999-424	Y	N	Not applicable	BASF	Y relevant IIA. 5.3.2.3 [5.3/06]
KCA 5.3.2	[redacted]	1987	SAN 582 H toxicity to rats by repeated dietary administration for 13 weeks followed by a 4-week withdrawal period [redacted] BASF RegDoc.# 86/11183 GLP, unpublished BVL-1902718 TOX1999-457	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.3.1 [5.12/07]
KCA 5.3.2	[redacted]	1999	Statement: Review of substance-related findings in the liver after administration of dimethenamid (racemice and s-form) over 3 months to rats [redacted] BASF RegDoc.#99/10270 Not GLP, unpublished BVL-1902751 TOX1999-467	N	N	Not applicable	BASF	Y relevant IIA. 5.12.7 [5.12/17]

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.3.3	[redacted]	1990	SAN 582 H: 3-week repeated dermal limit test in rabbits [redacted] BASF RegDoc.#90/11142 GLP, unpublished BVL-1932670 TOX1999-420	Y	N	Not applicable	BASF	Y relevant IIA. 5.3.1.4 [5.3/02]
KCA 5.4.1	Wagner V., Coffman N.	1996	Salmonella/escherichia coli plate incorporation mutagenicity assay: SAN 1289 H MICROBIOLOGICAL Associates, Rockville, Maryland, USA BASF RegDoc.#96/5403 GLP, unpublished BVL-1902779 TOX1999-425	N	N	Not applicable	BASF	Y relevant IIA. 5.4.1.1 [5.4/01]
KCA 5.4.1	Engelhardt G., Hoffmann H.	1997	Salmonella typhimurium/escherichia coli reverse mutation assay with s-dimethenamid technical BASF Aktiengesellschaft, Ludwigshafen, Germany BASF RegDoc.#97/10622 GLP, unpublished BVL-1902766 TOX1999-426	N	N	Not applicable	BASF	Y relevant IIA. 5.4.1.1 [5.4/02]
KCA 5.4.1	Engelhardt G., Hoffman H.	1997	Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with s-dimethenamid BASF Aktiengesellschaft, Ludwigshafen, Germany BASF RegDoc.#97/10621 GLP, unpublished BVL-1902744 TOX1999-427	N	N	Not applicable	BASF	Y relevant IIA. 5.4.1.1 [5.4/03]
KCA 5.4.1	Wagner V., Klug M.	1997	Bacterial reverse mutation assay: SAN 1289 H technical MICROBIOLOGICAL Associates, Rockville, Maryland, USA BASF RegDoc.#97/5271 GLP, unpublished BVL-1902729 TOX1999-428	N	N	Not applicable	BASF	Y relevant IIA. 5.4.1.1 [5.4/04]
KCA 5.4.1	[redacted]	1996	CHO/HGPRT mutation assay: SAN 1289 H technical [redacted] BASF RegDoc.#96/5404 GLP, unpublished BVL-1902780 TOX1999-429	N	N	Not applicable	BASF	Y relevant IIA. 5.4.1.2 [5.4/05]
KCA 5.4.1	[redacted]	1996	Unscheduled DNA synthesis assay in rat primary hepatocytes [redacted] BASF RegDoc.#96/5399 GLP, unpublished BVL-1902730 TOX1999-431	N	N	Not applicable	BASF	Y relevant IIA. 5.4.1.4 [5.4/07]
KCA 5.4.1	Haworth L., Lawlor T.	1989	Mutagenicity test on SAN 582 H in the ames salmonella/ microsome reverse mutation assay Hazleton Laboratories America, Inc., USA BASF RegDoc.# 89/11032 GLP, unpublished BVL-1902777 TOX1999-459	N	N	Not applicable	BASF	Y relevant IIA. 5.12.5.1 [5.12/09]

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.4.1	[redacted]	1986	*In vitro* mammalian cell gene mutation (HGPRT Locus) assay with V79 Chinese hamster cells [redacted] BASF RegDoc.# 86/11167 GLP, unpublished BVL-1902762 TOX1999-460	N	N	Not applicable	BASF	Y relevant IIA. 5.12.5.2 [5.12/10]
KCA 5.4.1	[redacted]	1989	Mutagenicity test on SAN 582H: in the rat primary hepatocyte unscheduled DNA synthesis assay [redacted] BASF RegDoc.# 89/11033 GLP, unpublished BVL-1902720 TOX1999-463	N	N	Not applicable	BASF	Y relevant IIA. 5.12.5.4 [5.12/13]
KCA 5.4.1	[redacted]	1986	SAN 582 H: micronucleus test in bone marrow cell of the mouse [redacted] BASF RegDoc.# 86/11168 GLP, unpublished BVL-1902742 TOX1999-465	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.5.5 [5.12/15]
KCA 5.4.1/1	[redacted].	2013	BAS 656-PH - *In vitro* gene mutation test in L5178Y mouse lymphoma cells (TK +/- locus assay, microwell version) 2013/1003738 [redacted]. GLP, unpublished BVL-2630079 ASB2014-8389	N	Y	New data for AIR3 renewal	BASF	N II A 5.4.1
KCA 5.4.2	[redacted]	1996	Micronucleus cytogenetic assay in mice: SAN 1289 H technical [redacted] BASF RegDoc.#96/5401 GLP, unpublished BVL-1902728 TOX1999-432	Y	N	Not applicable	BASF	Y relevant IIA. 5.4.2.1 [5.4/08]
KCA 5.4.2	[redacted]	1993	Study to evaluate the potential of SAN 582 H to induce unscheduled DNA synthesis in rat liver using an *in vivo*/*in vitro* procedure [redacted] 1993/11757 GLP, unpublished BVL-1902753 TOX2001-472	Y	N	Not applicable	BASF	Y relevant II 5.4.2
KCA 5.4.2/1	[redacted]	2014	BAS 656-PH - Micronucleus assay in bone marrow cells of the mouse 2014/1038343 [redacted]. GLP, unpublished BVL-2630081 ASB20148390	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.4.2
KCA 5.4.2/2	Grauert E., Kamp H.	2014	Analytical report - BAS 656-PH Concentration control analyses in corn oil 2014/1104188 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630082 ASB2014-8391	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.4.2

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.5	[redacted]	1988	SAN 582 H: 52 week oral toxicity study in dogs [redacted] BASF RegDoc.#88/11361 GLP, unpublished BVL-1913745 TOX1999-433	Y	N	Not applicable	BASF	Y relevant IIA. 5.5.1 [5.5/01]
KCA 5.5	[redacted]	1988	SAN 582 H: 52 week oral toxicity study in dogs. Addendum [redacted] BASF RegDoc.#88/11362 GLP, unpublished BVL-1913747 TOX1999-434	Y	N	Not applicable	BASF	Y relevant IIA. 5.5.1 [5.5/02]
KCA 5.5	[redacted]	1990	SAN 582 H: potential tumorigenic and toxic effects in prolonged dietary administration to rats [redacted] BASF RegDoc.#90/11138 GLP, unpublished BVL-1902723 TOX1999-435	Y	N	Not applicable	BASF	Y relevant IIA. 5.5.3 [5.5/03]
KCA 5.5	[redacted]	1990	Potential tumorigenic and toxic effects study of SAN 582 H in prolonged dietary administration to rats [redacted] BASF RegDoc.#90/11179 GLP, unpublished BVL-1902737 TOX1999-436	Y	N	Not applicable	BASF	Y relevant IIA. 5.5.3 [5.5/04]
KCA 5.5	[redacted].	1993	Review of ovarian neoplasia in Sandoz study SDZ335 (compound SAN 582H) Addendum to: SAN 582 H potential tumorigenic and toxic effects in prolonged dietary administration to rats [redacted] BASF RegDoc.#93/11798 GLP, unpublished BVL-1902738 TOX1999-437	Y	N	Not applicable	BASF	Y relevant IIA. 5.5.3 [5.5/05]
KCA 5.5	[redacted]	1990	SAN 582 H: Potential tumorigenic effects in prolonged dietary administration to mice [redacted] BASF RegDoc.#90/11139 GLP, unpublished BVL-1902752 Vol. 1-5 BVL-1902724 Vol. 6-8 TOX1999-438	Y	N	Not applicable	BASF	Y relevant IIA. 5.5.4 [5.5/06]
KCA 5.6.1	[redacted]	1989	SAN 582 H: Two-generation reproduction study in the rat [redacted] BASF RegDoc.#90/11140 GLP, unpublished BVL-1902765 TOX1999-439	Y	N	Not applicable	BASF	Y relevant IIA. 5.6.1 [5.6/01]

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.6.2	[redacted]	1996	Oral (gavage) developmental toxicity study of SAN 1289 H in rats [redacted] BASF RegDoc.#97/5274 GLP, unpublished BVL-1902736 TOX1999-440	Y	N	Not applicable	BASF	Y relevant IIA. 5.6.2.1 [5.6/02]
KCA 5.6.2	[redacted]	1988	Developmental toxicity (embryo/fetal toxicity and teratogenic potential) study of SAN 582 H administered orally (stomach tube) to New Zealand white rabbits [redacted] BASF RegDoc.#88/11376 GLP, unpublished BVL-1902735 TOX1999-441	Y	N	Not applicable	BASF	Y relevant IIA. 5.6.2.2 [5.6/03]
KCA 5.6.2	[redacted]	1987	Developmental toxicity (embryo/fetal toxicity and teratogenic potential) study of SAN 582 H administered orally via gavage to Crl:COBS CD (SD) BR presumed pregnant rats [redacted] BASF RegDoc.# 87/11225 GLP, unpublished BVL-1902761 TOX1999-458	Y	N	Not applicable	BASF	Y relevant IIA. 5.12.4.1 [5.12/08]
KCA 5.6.2	[redacted]	1991	Review of skeletal ossification delays in SAN 1289 developmental toxicity study [redacted] BASF RegDoc.# 1999/5049 Not GLP, unpublished BVL-1946873 TOX1999-469	N	N	Not applicable	BASF	Y relevant IIA. 5.12.7 [5.12/19]
KCA 5.7.1/1	[redacted]	2013	BAS 656-PH: Acute oral neurotoxicity study in Wistar rats - Administration via gavage 2013/1028330 [redacted]. GLP, unpublished BVL-2630084 ASB2014-8392	Y	Y	New data for AIR3 renewal	BASF	N
KCA 5.7.1/2	[redacted]	2013	BAS 656-PH - Repeated dose 90-day oral neurotoxicity study in Wistar rats - Administration via the diet 2013/1165818 [redacted] GLP, unpublished BVL-2630086 ASB2014-8393	Y	Y	New data for AIR3 renewal	BASF	N
KCA 5.8/34	Anonymous	2002	Recognition of, and differentation between, adverse and non-adverse effects in toxicology studies - Technical report No. 85 2002/1027057 Not GLP, published Source: European Centre for Ecotoxicology and Toxicology of Chemicals, Brussel December 2002, Technical report No. 85; ISSN-0773-6347-85 BVL-2630143 ASB2014-8405	N	N	Not applicable	LIT	N II A 5.8.1

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.8.1	[redacted]	1995	Dimethenamid oxalamide: Acute oral toxicity study in the rat [redacted] BASF RegDoc.# 95/11340 GLP, unpublished BVL-1902778 TOX1999-442	Y	N	Not applicable	BASF	Y relevant IIA. 5.8.1.1 [5.8/01]
KCA 5.8.1	[redacted]	1992	Acute oral toxicity study in rats: SAN 582 H sulfonate metabolite (M-27) [redacted] BASF RegDoc. # 92/12507 GLP, unpublished BVL-1902709 TOX1999-443	Y	N	Not applicable	BASF	Y relevant IIA. 5.8.1.1 [5.8/02]
KCA 5.8.1	Clare C.	1995	Dimethenamid oxalamide (M23): Reverse mutation in 5 histidine requiring strains of salmonella typhimurium Hazleton Laboratories Europe Ltd., Harrogate, Great Britain BASF RegDoc.# 95/11336 GLP, unpublished BVL-1902727 TOX1999-444	N	N	Not applicable	BASF	Y relevant IIA. 5.8.1.2 [5.8/03]
KCA 5.8.1	Clare C.	1995	Dimethenamid sulfonate (M27): Reverse mutation in 5 histidine-requiring strains of salmonella typhimurium Hazleton Laboratories Europe Ltd., Harrogate, Great Britain BASF RegDoc.# 95/11338 GLP, unpublished BVL-1902764 TOX1999-445	N	N	Not applicable	BASF	Y relevant IIA. 5.8.1.2 [5.8/04]
KCA 5.8.1	[redacted]	1998	Micronucleus assay in bone marrow cells of the mouse with reg. nr. 360/715/M23 [redacted] BASF RegDoc.# 98/10169 GLP, unpublished BVL-1902734 TOX1999-446	Y	N	Not applicable	BASF	Y relevant IIA. 5.8.1.2 [5.8/05]
KCA 5.8.1	[redacted]	1998	Micronucleus assay in bone marrow cells of the mouse with reg. nr. 360714/M27 [redacted] BASF RegDoc.# 98/10168 GLP, unpublished BVL-1902711 TOX1999-447	Y	N	Not applicable	BASF	Y relevant IIA. 5.8.1.2 [5.8/06]
KCA 5.8.1/1	[redacted].	2000	Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79/HPRT) with Reg.-No. 360 715/M23 2000/1000178 [redacted]. GLP, unpublished BVL-2630144 TOX2002-1990	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/2	[redacted].	2014	Reg.No. 5886780 (metabolite of BAS 656-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats administration via the diet 2013/1342918 [redacted] GLP, unpublished BVL-2630145 ASB2014-8415	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.8.1/3	[redacted].	2000	Gene mutation assay in Chinese Hamster V79 cells *in vitro* (V79/HPRT) with Reg.-No. 360 714/M27 2000/1000179 [redacted] GLP, unpublished BVL-2630147 TOX2002-1991	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/4	[redacted]	2014	Reg.No. 360714 (metabolite of BAS 656 H, dimethenamid) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2013/1342917 [redacted] GLP, unpublished BVL-2630148 ASB2014-8416	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/5	Class T.	2013	Analytical report - Homogeneity and concentration control of dimethenamid metabolite M27 (Reg.No. 360714) in vehicle 2013/1413980 PTRL Europe, Ulm, Germany Fed.Rep. GLP, unpublished BVL-2630149 ASB2014-8450	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/6	Woitkowiak C.	2014	Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2014/1018061 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630151 ASB2014-8451	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/7	[redacted]	2014	Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P): *In vitro* cell mutation assay at the Thimidine Kinase Locus (TK+/-) in mouse lymphoma L5178Y cells 2014/1018062 [redacted] GLP, unpublished BVL-2630153 ASB2014-8458	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/8	[redacted]	2014	Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P): Micronucleus test in chinese hamster V79 cells *in vitro* 2014/1018063 [redacted] GLP, unpublished BVL-2630155 ASB2014-8465	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/9	[redacted]	2014	Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse 2014/1094090 [redacted] GLP, unpublished BVL-2630157 ASB2014-8470	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/10	Becker M., Landsiedel R.	2014	Analytical report - Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for externa studies 2014/1092435 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630159 ASB2014-8477	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/11	Grauert E., Kamp H.	2014	Analytical report - Reg.No. 5296352 (metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in dimethylsulfoxide / polyethylenglycol (3+7, v/v) 2014/1101994 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630161 ASB2014-8482	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/12	Schulz M., Landsiedel R.	2008	Reg.No. 360 712 (metabolite M31 of BAS 656 H) - Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) 2008/1064992 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-1902749 ASB2010-6897	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/13	[redacted]	2008	Reg.No. 360 712 (metabolite M31 of BAS 656 H) - *In vitro* gene mutation test in CHO cells (HPRT locus assay) 2008/1051510 [redacted]. GLP, unpublished BVL-1902773 ASB2008-7224	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/14	[redacted]	2008	Reg.No. 360 712 (metabolite M31 of BAS 656 H) - *In vitro* chromosome aberration assay in V79 cells 2008/1063692 [redacted] GLP, unpublished BVL-2630167 ASB2008-7223	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/15	[redacted]	2008	Amendment No. 1 to the report: Reg.No. 360 712 (metabolite M31 of BAS 656 H) - *In vitro* chromosome aberration assay in V79 cells 2008/1070759 [redacted] GLP, unpublished BVL-2630169 ASB2014-8430	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/16	[redacted]	2013	Reg.No. 360712 (metabolite of BAS 656 H) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2013/1042165 [redacted] GLP, unpublished BVL-2630171 ASB2014-8417	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/17	Woitkowiak C.	2013	Reg.No. 395234 (metabolite of BAS 656 H, dimethenamid) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2013/1113379 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630173 ASB2014-8452	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/18	Woitkowiak C.	2014	Reg.No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhy murium/Escherichia coli, reverse mutation assay 2013/1361332 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630175 ASB2014-8453	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/19	[redacted]	2013	Reg.No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P): *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1246089 [redacted] GLP, unpublished BVL-2630177 ASB2014-8459	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/20	[redacted]	2013	Reg.No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* micronucleus test in chinese hamster V79 cells 2013/1246088 [redacted]. GLP, unpublished BVL-2630179 ASB2014-8466	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/21	[redacted]	2013	Reg.No. 5917262 (metabolite of BAS 656-PH dimethenamid-P) - Micronucleus assay in bone marrow cells of the mouse 2014/1001781 [redacted] GLP, unpublished BVL-2630181 ASB2014-8471	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/22	Grauert M., Kamp H.	2014	Reg. No. 5917262 (metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in 30 % dimethyl sulphoxide + 70 % polyethylenglycol (v+v) 2014/1098005 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630183 ASB2014-8483	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/23	Becker M., Landsiedel R.	2014	Reg. No. 5917262 (metabolite of BAS 656-PH, Diemethenamid-P) - Plasma analysis for external studies 2014/1092436 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630185 ASB2014-8478	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/24	Woitcowiak C.	2013	Reg.No. 5917261 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhymurium/Eschericia coli, reverse mutation assay 2013/1361403 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630187 ASB2014-8487	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/25	[redacted].	2013	Reg.No. 5917261 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1246091 [redacted] GLP, unpublished BVL-2630189 ASB2014-8460	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/26	[redacted]	2013	Reg.No. 5917261 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* micronucleus test in Chinese hamster V79 cells 2013/1246090 [redacted] GLP, unpublished BVL-2630191 ASB2014-8467	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/27	[redacted]	2014	Reg. No. 5917261 (metabolite of BAS 655-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018092 [redacted] GLP, unpublished BVL-2630193 ASB2014-8418	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/28	Woitkowiak C.	2014	Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhymurium/Escherichia coli reverse mutation assay 2013/1397766 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630195 ASB2014-8454	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/29	[redacted].	2014	Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P): *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2014/1018055 [redacted] GLP, unpublished BVL-2630197 ASB2014-8461	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/30	[redacted]	2014	Reg.No. 5917260 (metabolite of BAS 656, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse 2014/1018032 [redacted] GLP, unpublished BVL-2630199 ASB2014-8472	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/31	Grauert M., Kamp H.	2014	Reg. No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in sterile water 2014/1098002 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630201 ASB2014-8484	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/32	Becker M., Landsiedel R.	2014	Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for external studies 2014/1092434 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630203 ASB2014-8479	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/33	[redacted]	2014	Reg.No. 5917260 (metabolite of BAS 656-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018064 [redacted] GLP, unpublished BVL-2630205 ASB2014-8419	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/34	Woitkowiak C.	2014	Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1363556 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630207 ASB2014-8455	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/35	[redacted].	2013	Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1246093 [redacted] GLP, unpublished BVL-2630209 ASB2014-8462	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/36	[redacted]	2013	Amendment No. 1 - Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1404743 [redacted]. GLP, unpublished BVL-2630211 ASB2014-8463	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/37	[redacted]	2013	Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* micronucleus test in Chinese hamster V79 cells 2013/1246092 [redacted] GLP, unpublished BVL-2630213 ASB2014-8468	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/38	[redacted]	2014	Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse 2014/1005221 [redacted] GLP, unpublished BVL-2630215 ASB2014-8473	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/39	Grauert E., Kamp H.	2014	Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in sterile water 2014/1098011 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630217 ASB2014-8485	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/40	Becker M., Landsiedel R.	2014	Analytical report - Reg. No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for external studies 2014/1092437 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630219 ASB2014-8480	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/41	[redacted]	2014	Reg.No. 5920718 (metabolite of BAS 656-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018065 [redacted] GLP, unpublished BVL-2630221 ASB2014-8420	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/42	Woitkowiak C.	2012	Reg.No. 5749263 (metabolite of BAS 656 H, dimethenamid) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2012/1220415 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630223 ASB2014-8456	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/43	[redacted]	2013	Reg.No. 5749263 (metabolite of BAS 656 H, dimethenamid) - *In vitro* gene mutation test in CHO cells (HPRT locus assay) 2013/1282610 [redacted]. GLP, unpublished BVL-2630225 ASB2014-8488	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/44	[redacted]	2013	Reg. No. 5749263 (metabolite of BAS 656 H, dimethenamid) - Micronucleus assay in bone marrow cells of the mouse intraperitoneally administration 2012/1205857 [redacted] GLP, unpublished BVL-2630227 ASB2014-8474	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/45	Woitkowiak C.	2013	Reg.No. 5936274 (derivate of metabolite of BAS 656-PH, dimethenamid-P) - Salmonella typhymurium / Escherichia coli reverse mutation assay 2013/1373303 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630229 ASB2014-8457	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/46	[redacted]	2013	Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - *In vitro* cell mutation assay at the Thymidine Kinase Locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1307624 [redacted] GLP, unpublished BVL-2630231 ASB2014-8464	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/47	[redacted]	2014	Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P): *In vitro* micronucleus test in chinese hamster V79 cells 2013/1307623 [redacted]. GLP, unpublished BVL-2630233 ASB2014-8469	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/48	[redacted]	2014	Reg.No. 5936274 (derivate of metabolite of BAS 656-PH, dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse 2014/1028628 [redacted], [redacted] GLP, unpublished BVL-2630235 ASB2014-8475	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/49	Becker M., Landsiedel R.	2014	Analytical report - Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - Plasma analysis for external studies 2014/1092433 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630237 ASB2014-8481	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/50	Grauert E., Landsiedel R.	2014	Analytical report - Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - Concentration control analyses in corn oil 2014/1101991 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished BVL-2630239 ASB2014-8486	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/51	[redacted]	2014	Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018066 [redacted]. GLP, unpublished BVL-2630241 ASB2014-8421	Y	Y	New data for AIR3 renewal	BASF	IIA. 5.8.1
KCA 5.8.1/52	Anonymous	2014	Export files of (Q)SAR-profiling modules of the OECD toolbox for dimethenamid-P metabolites 2014/1089828 Not GLP, unpublished BVL-2630243 ASB2014-8407	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/53	Anonymous	2014	*In vitro* AMES mutagenicity model with metabolic activation S9 - OASIS/TIMES QSAR analysis of dimethenamid-P metabolites AMES prediction 2014/1088460 Not GLP, unpublished BVL-2630245 ASB2014-8408	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/54	Anonymous	2014	*In vitro* AMES mutagenicity model with metabolic activation S9 - Amendment to OASIS/TIMES QSAR analysis of dimethenamid-P metabolites - Prediction for AMES mutagenicity 2014/1088478 Not GLP, unpublished BVL-2630247 ASB2014-8409	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/55	Anonymous	2014	*In vitro* chromosomal aberration with metabolic activation S9 - OASIS/TIMES QSAR analysis of dimethenamid-P metabolites prediction for chromosomal aberration *in vitro* 2014/1088461 Not GLP ,unpublished BVL-2630249 ASB2014-8410	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/56	Anonymous	2014	*In vitro* chromosomal aberration with metabolic activation S9 - Amendment to OASIS/TIMES QSAR analysis of dimethenamid-P metabolites prediction for chromosomal aberration 2014/1088479 Not GLP, unpublished BVL-2630251 ASB2014-8411	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/57	Serafimova, R., Todorov, M., Pavlov, T., Kotov, S., Jacob, E., Aptula, A., Mekenyan, O.	2013	Q(SAR) model reporting format (QMRF) ames mutagenicity with S9 2013/1414242 Assen Zlatarov University, Bourgas, Bulgaria Not GLP, unpublished BVL-2630253 ASB2014-8489	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/58	Mekenyan, O., Todorov, M., Serafimova, S., Aynur, A., Finking, R., Jacob, E.	2013	Q(SAR) Model Reporting Format (QMRF) *In vitro* chromosomal aberration with S9 2013/1414460 Not GLP, published Source: Chem. Res. Toxicol., 1927-1941 (2007) BVL-2679638 ASB2014-8490	N	N	Not applicable	LIT	N IIA. 5.8.1
KCA 5.8.1/59	Esdaile D.J.	2014	Metabolites of BAS 656 H - Structure-activity hazard identification screen using Derek 2012/1107265 CiToxLAB Hungary Ltd., Veszprem, Hungary Not GLP, unpublished BVL-2630257 ASB2014-8491	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/60	Anonymous	2014	Prediction and applicability domain analysis for models: Mutagenicity model (CAESAR) (version 2.1.9) - Mutagenicity SarPy model (version 1.0.4-BETA) - VEGA QSAR analysis of dimethenamid-P metabolites 2014/1088457 Not GLP, unpublished BVL-2630259 ASB2014-8412	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.1/61	Anonymous	2014	Prediction and applicability domain analysis for models: Mutagenicity model (CAESAR) (version 2.1.9) - Mutagenicity SarPy model (version 1.0.4-BETA) - Amendment to VEGA QSAR Analysis of dimethenamid-P metabolites 2014/1088458 Not GLP, unpublished BVL-2630261 ASB2014-8413	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1

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KCA 5.8.1/62	Anonymous	2002	Recognition of, and differentiation between, adverse and non-adverse effects in toxicology studies - Technical report No. 85 2002/1027057 Not GLP, published Source: European Centre for Ecotoxicology and Toxicology of Chemicals, Brussel December 2002, Technical report 85, ISSN-0773-6347-85 BVL-2630265, ASB2014-8405	N	N	Not applicable	LIT	N IIA. 5.8.1
KCA 5.8.1/63	Anonymous	2007	PSD guidance document - Toxicological significance of reduced levels of serum ALT and/or AST in animal studies 2007/1070125 Not GLP, unpublished BVL-2630263 ASB2014-8414	N	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.1
KCA 5.8.2	[redacted]	1992	Investigation of the potential of acovalent binding of ^{14}C -dimethenamid (SAN 582 H) or its derivatives to rat and human haemoglobin [redacted] BASF RegDoc.# 92/12484 GLP, unpublished BVL-1902760 TOX1999-448	N	N	Not applicable	BASF	Y relevant IIA. 5.8.2 [5.8/07]
KCA 5.8.2	[redacted]	1994	Investigations of liver enzyme induction by dimethenamid (SAN 582 H) in rats [redacted] BASF RegDoc.# 94/11897 GLP, unpublished BVL-1902717 TOX1999-449	Y	N	Not applicable	BASF	Y relevant IIA. 5.8.2 [5.8/08]
KCA 5.8.2/1	[redacted]	2013	BAS 656-PH - Immunotoxicity study in female C57BL/6J Rj mice - Administration via the diet for 4 weeks 2013/1028329 [redacted] GLP, unpublished BVL-2630268 ASB2014-8422	Y	Y	New data for AIR3 renewal	BASF	N IIA. 5.8.2
KCA 5.8.2	Cramer, G.M., Ford, R.A., Hall, R.L.	1978	Review Section: Estimation of toxic hazard - A decision tree approach Not GLP, published BVL-2895545, CHE2006-1120	N	N	-	LIT	N
KCA 5.8.2	Kroes, R.; Renwick, A.G., Cheeseman, M., Kleiner, J. et al.	2003	Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet Not GLP, published BVL-2895566 TOX2004-1275	N	N	-	LIT	N
KCA 5.8.2	Munro, I.C., Ford, R.A., Kennepohl, E., Sprenger, J.G.	1996	Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern Not GLP, published BVL-2895579 TOX2004-1274	N	N	-	LIT	
KCA 5.8.2	Melching-Kollmuß, S., Honarvar, N.	2010	Dimethenamid-P – Proposal for toxicolgical assessment of non-relevant metabolites in two lysimeter studies 2009/1026962 ASB2011-12734 BVL-3057025	N	N	-	BASF	N

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 5.8.3/1	Shah I. et al.	2011	Using nuclear receptor activity to stratify hepatocarcinogens 2011/1295091 Not GLP, published Souce: PLoS ONE, 2011, 6(2), e14584 BVL-26302670 ASB2014-8446	N	N	Not applicable	LIT	N
KCA 5.8.3/2	Reif D.M. et al.	2010	Endocrine profiling and prioritisation of environmental chemicals using ToxCast data 2010/1231552 Not GLP, published Source: Environmental Health Perspectives Volume 118, Number 12, December 2010: 1714-1720; doi: 10.1289/ehp.1002180 BVL-2630272 ASB2014-8447	N	N	Not applicable	LIT	N
KCA 5.8.3/3	Sipes N.S. et al.	2013	Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays 2013/1371960 Not GLP, published Source: Chem. Res. Toxicol., 2013, 26, 878-895 BVL-2630274 ASB2014-8448	N	N	Not applicable	LIT	N
KCA 5.9.1	Rataj M.	1992	Human exposure experience with frontier herbicide (SAN 582) SANDOZ Agro, Inc., Des Plaines, Illinois 60018 BASF RegDoc.# 92/12480 Not GLP, unpublished BVL-1902726 TOX1999-450	N	N	Not applicable	BASF	Y relevant IIA. 5.9.1 [5.9/01]
	Anonymous	2002	Reporting table, dimethenamid-P (Hb) section 4, 11587/ECCO/BBA/01, rev. 3 page 57 ASB2015-560	N	N	-		
	ECHA	2013	Opinion proposing harmonised classification and labelling at EU level of dimethenamid-P (ISO) CLH-O-0000003037-80-03/F ASB2015-2797	N	N	-	LIT	
	EFSA	2012	Scientific Opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment EFSA Journal 2012;10(07): 2799 ! EFSA-Q-2008-756 ASB2012-10281	N	N	-	LIT	
	EFSA	2012	Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC) EFSA Journal 2012;10(7):2750 ! EFSA-Q-2008-747 ASB2015-2796	N	N	-	LIT	
	EU Commission	2003	Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC Sanco/221/2000 –rev.10- final ASB2012-3097	N	N	-	LIT	
	Germany	2000	Dimethenamid-P (Monograph) GLP: N, published ASB2010-10566	N	N	-	LIT	