European Commission

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BAS 750F
(Mefentrifluconazole)

Volume 3 – B.6 (AS)

Rapporteur Member State: United Kingdom
Co-Rapporteur Member State: France & Austria

Version History

When	What
April 2017	Initial DAR

Table of contents

B.6. TOXICOLOGY AND METABOLISM DATA

Since the ISO proposed name of menfentrifluconazole has not been confirmed at the time of this evaluation, the active substance will be referred to as BASF 750 F throughout this report.

BAS 750 F is a new fungicide belonging to the chemical group of triazoles. Owing to its unique isopropanol moiety, it is proposed that it belongs to a new sub-group of triazole fungicides: the isopropanol azoles. It is used for the control of *Septoria tritici* in cereals (barley, oat, rye, triticale and wheat). BAS 750 F is a sterol biosynthesis inhibitor that belongs to the sub-group of demethylation inhibitors. Its primary mode of action is the blocking of ergosterol biosynthesis through the inhibition of cytochrome P450 sterol 14α-demethylase (CYP51). The depletion of ergosterol and accumulation of non-functional 14α-methyl sterols results in the inhibition of growth and also in cell-membrane disruption.

BAS 750 F (LS 5834378) is a racemic mixture of an (R)-enantiomer (LS 5934591) and an (S)-enantiomer (LS 5934588) in a 1:1 ratio. Both enantiomers as the racemate showed biological activity on all tested pathogens. All regulatory toxicology studies used the racemate in a 1:1 ratio. Plant and livestock metabolism studies indicate that consumers will either be exposed to a 50:50 enantiomer ratio (poultry and plants) or to residues with higher levels of the R-enantiomer (livestock). An investigation into the mammalian toxicity of the enantiomers demonstrated that the S-enantiomer was more toxicologically active than the R-enantiomer (see section B.6.8.3). In a metabolism study in rats (see section B.6.1.1.4), rapid metabolism of the racemate resulted in a shift from the 1:1 ratio to higher R-enantiomer ratios, comparable to livestock, and thus indicated preferential metabolism of the S-enantiomer in mammals. Thus, the proposed human health risk assessment of BAS 750 F, which is based on toxicological studies with the racemic mixture, covers the realistic human exposure scenario and adequately considers the isomeric composition of BAS 750 F.

The minimum purity of the active substance is specified to be ≥ 97.0 %. All toxicological studies used a technical-grade active ingredient with purity of 95.5 to 99.4 %. No individual significant impurity is present in the specified material or the batches used in the toxicological studies at a concentration greater than 2.3 %. All toxicologically-relevant impurities, on the basis of their hazardous properties, are present only in trace amounts and do not impact the classification and labelling of BAS 750 F. The batches used for the most important toxicity tests are relevant to the technical material currently being marketed (see volume 4).

Since this is a new active substance, the studies presented have been conducted for the purpose of EU (and global) approval and have not previously been evaluated. All study protocols followed the respective OECD test guidelines, unless stated otherwise.

Table B.6. Batches of BASF 750 F used in toxicology studies

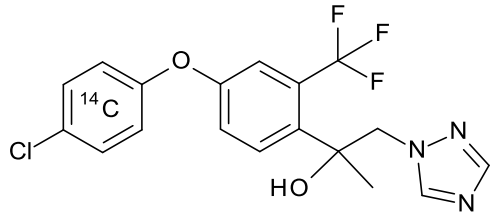
	COD-001662	COD-001740	L84-176	L85-12	COD-001880	01651-181
A.S. %	95.5 %	98.8 %	97.7 %	99.4 %	98.8 %	97.9 %
Study reports	CA 5.1.1/2 CA 5.3.1/3 CA 5.3.2/1 CA 5.6.2/2	CA 5.1.1/1 CA 5.1.1/2 CA 5.1.1/4 CA 5.2.1/1 CA 5.2.2/1 CA 5.2.3/1 CA 5.2.4/2 CA 5.2.5/3 CA 5.2.6/1 CA 5.3.2/3 CA 5.4.1/4 CA 5.4.1/6 CA 5.4.2/1 CA 5.5/1 CA 5.5/3 CA 5.6.1/1	CA 5.2.4/1 CA 5.2.5/1 CA 5.2.5/2 CA 5.3.1/1 CA 5.6.2/1	CA 5.8.3/1	CA 5.2.7/1 CA 5.4.1/1 CA 5.3.1/4 CA 5.3.2/5 CA 5.3.3/1 CA 5.7.1/1	CA 5.4.1/3 CA 5.4.1/5 CA 5.4.1/7

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

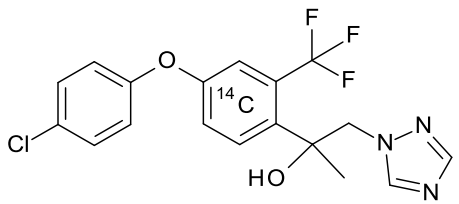
The absorption, distribution, excretion and metabolism of BASF 750 F have been investigated in rats by the oral route, with further investigation of plasma kinetics after intra-venous administration. Plasma kinetics has also been investigated in mice. A comparative *in vitro* metabolism study has been provided.

For these studies, the active substance was radiolabelled in the chlorophenyl ring (C-label), in the trifluoromethylphenyl ring (TFMP-label) or in the triazole moiety (T-label). The molecular structures and the positions of the labels are shown below.

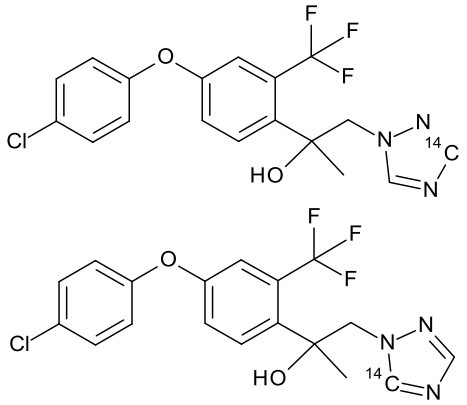
C label [Chlorophenyl-U-C14-]-BAS 750 F



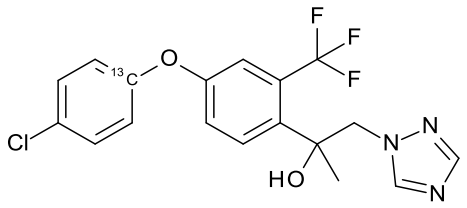
TFMP label [Trifluoromethyl-ring-U-C14-]-BAS 750 F



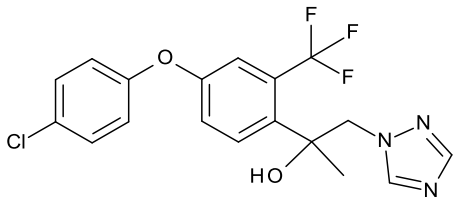
T label [Triazole-3(5)-C14-]-BAS 750 F



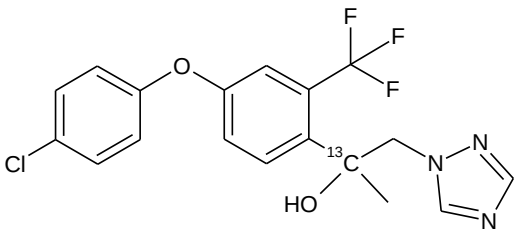
C label [Chlorophenyl-1-C13-]-BAS 750 F



BAS 750 F, unlabelled



TFMP label [Trifluoromethylphenyl-^{13}C]-BAS 750 F



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

The absorption, distribution, metabolism, elimination and plasma kinetics of BAS 750 F were investigated in male and female rats. The study was divided into three separate studies and employed three radiolabels (C-, TFMP- and T-label). The biokinetic parts were performed separately for the C- /

TFMP-label and the T-label, whilst the metabolism part was performed for all three labels. Additional investigations into plasma kinetics were conducted in mice.

Table B.6.1.1.1. Summary of toxicokinetics, metabolism and distribution studies

Route, guideline, GLP status, reference	Species	Radiolabel	Dose level	Analysed parameters
Oral (gavage) OECD 417, GLP Report CA 5.1.1/1 [redacted], 2015 a (2015/1208128)	Rat, Wistar	14C-Chlorophenyl, radiochemical purity 99.3% Trifluoromethyl-ring-U-14C, radiochemical purity 97.9-98.3% Chemical purity 98.8%	Single dose: 5 & 180 mg/kg Repeated dose: 14 daily administrations of unlabelled BASF 750 F, 1 administration of substance with C-label, all 180 mg/kg	Absorption Distribution Excretion Plasma kinetics
Oral (gavage), intra-venous OECD 417, GLP Report CA 5.1.1/2 [redacted], 2016 a (2015/1078847)	Rat, Wistar	Triazole-3(5)-C14, radiochemical purity > 98% Chemical purity 95.5-98.8%	<u>Mass balance, excretion, distribution</u> Single oral dose: 5 & 180 mg/kg Repeated oral dose: 14 daily administrations of unlabelled BASF 750 F, 1 administration of substance with T-label, all 180 mg/kg <u>Plasma kinetics</u> Single oral dose: 5, 40, 120, 360 mg/kg Single i.v. dose: 0.4 mg/kg	Absorption Distribution Excretion Plasma kinetics
Oral (gavage) OECD 417 Report CA 5.1.1/3 [redacted] 2016 a (2015/1107610)	Rat, Wistar	14C-Chlorophenyl, radiochemical purity 99.3% Trifluoromethyl-ring-U-14C, radiochemical purity 97.9-98.3% Triazole-3(5)-C14, radiochemical purity > 98% Samples taken from studies above	Samples taken from studies above	Excretion Metabolism
Oral (gavage) OECD 417 Report CA 5.1.1/4 [redacted], 2014 a (2014/1018105)	Mouse, C57BL/6 JRj, at least 4/sex/group	Triazole-3(5)-C14, radiochemical purity > 95%	Single doses of 10, 50, 75 mg/kg bw	Plasma kinetics

In the series of rat studies (reports 5.1.1/1, 5.1.1/2 and 5.1.1/3), groups of Wistar rats were gavaged with a single dose of radiolabelled BASF 750 F, or with a single dose of radiolabelled material

following 14 consecutive daily doses of unlabelled material, as detailed in Table B.6.1.1.2. One of the studies also used four oral dose groups to investigate potential saturation effects. The test material was prepared in 0.5% carboxy methylcellulose (CMC); 14C-labelled BASF 750 F was mixed with unlabelled and stable-isotope (13C) BASF 750 F to produce the required specific activity. Samples of excreta, blood and tissues were collected as detailed below. Radioactivity was measured by liquid scintillation counting, with solubilisation of blood and tissues and solubilisation then combustion of faeces. The results from oral and i.v. administration are presented below.

Table B.6.1.1.2. Design of the toxicokinetic studies in rats

Dose type, (group)	Dose level (mg/kg)	Experiment Report	Radiolabel	Dose route	Group size	Samples	Time Hrs
[redacted] 2015 a, report CA 5.1.1/1							
Low (1)	5	PK (5.1.1/1)	14C C-label	Oral	3 / sex	Blood	0.5 to 168
High (2)	180				3 / sex		
Low (4)	5	Excretion (5.1.1/1) Metabolism (5.1.1/3)	14C C-label		4 / sex	Urine, faeces, exhaled air, organs/tissue	0 to 168
High (10)	180				4 / sex		
Multiple (7)	180				4 / sex	Urine, faeces, organs/tissues	
High (14)	180		14C TFMP-label		4 / sex	Urine, faeces, exhaled air, organs/tissues	
Low (5)	5	Bile study (5.1.1/1) Metabolism (5.1.1/3)	14C C-label		4 / sex	Bile, urine, faeces	0 to 168
High (6/13)[1]	180				4 / sex		
High (12)	180		14C TFMP-label		4 / sex		
Low (8)	5	Distribution (5.1.1/1)	14C C-label		12 / sex	Organs / tissues	0 to 53
High (9)	180				12 / sex		
[redacted] 2016 a, report CA 5.1.1/2							
Low (7)	5	Excretion Distribution (5.1.1/2) Metabolism (5.1.1/3)	14C – T-label	Oral	4 / sex	Urine, faeces, exhaled air, organs/tissues	0 to 168
High (6)	180				4 / sex		
Multiple (8)	180				4 / sex		
Saturation (1-3, 5)	360, 120, 40, 5,	PK (5.1.1/2)			4 / sex / group	Blood	0 to 168
Low (4)	0.4			i.v.	6 / sex		
Low (10)	5	Bile study (5.1.1/2) Metabolism (5.1.1/3)		Oral	6 M / 10 F	Urine, faeces, bile	0 to 72
High (9)	180				11 M / 6 F		
Low (12)	5	Distribution (5.1.1/2)			12 / sex	Organs / tissues	M = 1, 4, 18, 24 F = 1, 2, 4, 24
High (11)	180				12 /sex		M = 1, 24, 36, 48 F = 1, 8, 24, 34

PK = plasma kinetics

[1] As group 6 was incomplete, additional animals were dosed and named group 13 – all procedures were the same as for group 6.

In the mouse plasma kinetics study, 14C-labelled BASF 750 (T-label) was administered in 0.5 % CMC and mixed with unlabelled test material to achieve the required specific radioactivity. Blood samples were collected from before until 168 hours after dose administration.

B.6.1.1.1. Plasma kinetics

Rats

The plasma kinetics of BASF 750 F were investigated in male and female rats after single oral doses of 5 and 180 mg/kg (14C C-label) or, to investigate potential saturation effects, after single oral doses of 5, 40, 120 or 360 mg/kg (14C T-label). In a further experiment, a single low-dose (0.4 mg/kg) i.v. administration of 14C T-labelled substance was employed to obtain information on the kinetics of BASF 750 F where there was 100% systemic bioavailability.

Following a single oral dose of 5 mg/kg, the maximum plasma concentrations occurred at 1.2 and 0.5 hours in males and females, respectively. The initial half-life was calculated to be 7.7 hours in males and 2.6 hours in females, with terminal half-lives of 85.7 and 62.1 hours (the latter based on one animal) for males and females, respectively. Following a single oral dose of 180 mg/kg/d, the maximum plasma concentration occurred at 5.5 and 0.7 hours in males and females, respectively. The initial half-life was 12.9 hours in males and 4.0 hours in females, whilst the terminal half-lives were 87.7 hours and 78.3 hours for males and females, respectively. At both doses, the mean blood-plasma ratios indicated that there was no significant distribution into red blood cells. The AUC values indicated that internal exposure was clearly correlated with the dosing regimen. After a single oral dose of both 5 and 180 mg/kg, the internal dose (indicated as AUC0→∞) for males was approximately twice that for females.

The plasma kinetic parameters for the C-label are presented in Table B.6.1.1.3.

Table B.6.1.1.3. Plasma kinetic parameters (C-label)

	Dose [mg/kg bw]	**cmax [µg Eq/g]**	**Tmax [h]**	**Tlast [h]**	**initial half-life [h]**	**terminal half-life [h]**	**AUC0→168 [µg Eq*h/g]**	**AUC0→∞ [µg Eq*h/g]**
Male	5, p.o.	2.04	1.2	168	7.68	85.7 [1)]	34.9	39.6 [1)]
	180, p.o.	62.5	5.5	168	12.9	87.7 [1)]	1650	1810 [1)]
Female	5, p.o.	1.67	0.5	168	2.56 [1)]	62.1 [1), 2)]	15.7	15.3 [1), 2)]
	180, p.o.	49.9	0.7	168	3.99 [1)]	78.3 [1), 3)]	845	807 [1), 3)]

1) approximation
2) n=1
3) n=2

In the studies with the T label, the maximum plasma concentrations occurred directly after i.v. administration and between one to 24 hours after oral administration. After i.v. administration, the residues of 14C-BASF 750 F were < 0.01 µg Eq/g at sacrifice (168 hours), with a terminal half-life of 12.5 hours in males and 10.0 hours in females. Following oral administration of the T-label, the plasma kinetic data indicated that there was rapid absorption that led to a dose-dependent increase in maximum plasma concentrations; the maximum plasma concentrations generally occurred one hour post-dosing. The observation of a second C_{MAX} value at a later time (8 or 24 hours) for defined dose levels indicated that there was a potential for enterohepatic recirculation of the substance and/or its metabolites. Radioactivity declined rapidly after dosing, with there being 0.01 / 0.00 µg Eq/g in the 5 mg/kg group and 0.26 / 0.13 µg Eq/g in the 360 mg/kg group at sacrifice (168 hours) in males and females, respectively. As was the case with the C-label, the internal doses (AUC) of the T-label were higher for males than for females in all the dose groups. A comparison of the AUC of oral (5 mg/kg bw) versus intravenous (0.4 mg/kg bw) administration yielded dose-corrected ratios of about 80 % and

111 % for males and females, respectively, indicating that a high oral uptake was likely. A ratio of > 100 % for the females provided evidence of an oral metabolic first-pass effect, leading to metabolites in the systemic circulation with a lower distribution volume than the parent compound.

The plasma kinetic parameters for the T-label are presented in Table B.6.1.1.4.

Table B.6.1.1.4. Plasma kinetic parameters (T-label)

Dose [mg/kg bw]	**Route, no. of doses**	**Cmax [µg Eq/g]**		**Tmax [h]**		**half life [h]**		**AUC [µg Eq x h/g]**	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
0.4	1x i.v.	1.35	1.17	Directly	Directly	12.46	10.03	3.6	1.3
5	1x oral	3.04	2.07	1	1	43.83	34.11	38	17
40	1x oral	23.08	13.77	1	1	20.43	41.94	296	119
120	1x oral	53.73	34.37 20.41	1	1 8	17.39	58.38	886	467
360	1x oral	57.08 55.96	20.32 29.19	1 24	1 8	30.05	38.72	2629	1148

Mice

The plasma kinetics of BASF 750 F were investigated in mice that were dosed by gavage with a single administration of 10, 50 or 75 mg/kg bw of active substance (T-label).

The maximum plasma concentrations showed a clear dose dependency and were achieved 0.5 to 8 hours post-dosing. The observation that more than one C_{max} value was present in the high-dose groups of males and females, in the mid-dose group of males as well as in the low dose group of females indicated a potential enterohepatic (re)circulation of the test substance and/or potential metabolites thereof. The internal dose (indicated by the AUC) was clearly correlated to the oral dose administered. The ratio between the AUC-values and the chosen dose levels was slightly under-proportional to the oral doses for males, indicating a reduced absorption of the test substance with increased dose, but was approximately proportional for female mice. Generally, a comparable time course of radioactivity was found for blood as for plasma in both sexes, with the tendency to slightly higher blood/plasma ratios at later sampling time points, indicating that parts of the test substance and/or its metabolites may have been bound to blood constituents.

Table B6.1.1.5. Plasma kinetic parameters in mice after single oral gavage dosing

Sex	**Dose [mg/kg bw]**	**c_{max} [µg Eq/g]**	**T_{max} [h]**	**Terminal half-life [h]**	**$AUC_{0\rightarrow 168\ h}$ [µg Eq*h/g]**	**$AUC_{0\rightarrow\infty}$ [µg Eq*h/g]**
Male	**10**	5.66	8	80.4	147	151
	50	19.78; 19.18	1; 8	65.2	687	694
	75	24.80; 26.02; 26.85	0.5; 3 8	31.8	955	958
Female	**10**	3.98; 5.31	1; 4	54.2	126	127
	50	17.24	8	40.1	475	478
	75	21.48; 24.62	0.5; 8	34.6	1008	1012

B.6.1.1.1. Balance and excretion

The balance and excretion pattern of BAS 750 F was investigated in male and females rats dosed orally with single doses of 5 and 180 mg/kg active substance (C- and T-label) or multiple doses of 180 mg/kg active substance (14 daily administrations of unlabelled substance followed by a final dose of C- or T-labelled substance). An additional group was given a single high dose (180 mg/kg) of 14C TFMP-labelled substance. Urine was collected after 6, 12 and 24 hours and subsequently in 24-hour intervals up to 168 hours and faeces in 24-hour time intervals up to 168 hours. In the balance experiment of the low dose, the first two male animals were placed in closed metabolism cages in order to collect exhaled air for up to 48 hours. Since less than 2 % of the total radioactive dose was detected in exhaled air, all experiments were carried out in open systems, and the study authors concluded that exhalation was not an important excretion pathway for any of the labels. The experiment was terminated at 168 hours, at which time the remaining radioactivity was measured in tissues.

The excretion data obtained with the C- and TFMP-label were very similar (Table B.6.1.1.6). With both labels, the major excretion of ^{14}C-BAS 750 F occurred via the faeces for male and female animals of all the groups, i.e., after single high-, single low- and multiple high-dose administrations; in all cases, > 75 % of the administered radioactivity was excreted via this route. Urinary excretion was a minor route, with a maximum of 12.2 % of the administered radioactivity being excreted in the urine. The pattern of excretion after repeated oral administration showed similar amounts of radioactivity excreted in urine compared with the single-dose experiment, giving comparable kinetics after single and multiple dosing. Excretion was fast and had occurred to a major extent within 2-3 days after dosing.

Table B.6.1.1.6. Excretion balance (% of administered radioactivity, C- and TFMP-label)

Label	**[^{14}C] chlorophenyl label**						**TFMP label**	
Administration frequency	**Single**		**Single**		**15-days repeated**		**Single**	
Balance / excretion	**5 mg/kg bw**		**180 mg/kg bw**		**180 mg/kg bw**		**180 mg/kg bw**	
Time interval [h]	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Urine 0-6	1.63	2.01	0.407	0.305	0.580	2.40	0.671	0.866
Urine 6-12	1.78	2.58	0.779	0.622	0.911	1.38	1.47	1.25
Urine 12-24	2.89	2.58	1.82	2.03	1.61	2.45	3.06	3.01
Urine 24-48	1.74	2.44	1.85	2.25	1.04	2.36	3.04	3.21
Urine 48-72	0.550	1.64	0.808	1.24	0.617	1.10	0.913	1.07
Urine 72-96	0.167	0.476	0.364	0.949	0.129	0.444	0.244	0.320
Urine 96-120	0.076	0.199	0.101	0.383	0.069	0.221	0.101	0.200
Urine 120-144	0.073	0.166	0.051	0.150	0.058	0.115	0.064	0.145
Urine 144-168	0.053	0.142	0.036	0.131	0.030	0.083	0.042	0.100
Subtotal Urine	**8.95**	**12.2**	**6.22**	**8.06**	**5.05**	**10.6**	**9.61**	**10.2**
Feces 0-6	0.002	1.16	0.050	0.004	0.699	2.12	n.a.	0.840
Feces 6-12	1.09	2.66	0.576	0.246	11.2	1.56	4.18	4.30
Feces 12-24	26.2	41.4	31.0	32.0	40.5	34.1	37.4	50.2
Feces 24-48	49.4	32.4	34.2	26.9	28.2	41.0	26.3	23.4
Feces 48-72	9.90	7.92	16.0	16.8	4.72	7.32	6.16	4.49
Feces 72-96	2.04	2.07	4.01	7.63	0.934	1.94	1.03	0.842
Feces 96-120	0.600	0.820	0.953	1.20	0.277	0.529	0.309	0.194
Feces 120-144	0.176	0.193	0.294	0.480	0.098	0.173	0.123	0.137
Feces 144-168	0.097	0.105	0.126	0.312	0.071	0.127	0.154	0.052
Subtotal Feces	**88.9**	**87.7**	**86.9**	**85.5**	**86.3**	**80.4**	**75.7**	**84.5**
Cage wash	0.320	0.674	0.432	0.924	0.280	0.629	1.07	1.60
Plasma	0.013	0.003	0.011	0.003	0.013	0.003	0.008	0.004
Blood	0.018	0.005	0.017	0.005	0.020	0.005	0.013	0.006
Heart	0.001	0.000	0.001	0.000	0.001	0.000	0.000	0.000
Lung	0.001	0.000	0.001	0.000	0.001	0.000	0.001	0.001
Spleen	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Uterus	n.a.	0.000	n.a.	0.000	n.a.	0.000	n.a.	0.000
Pancreas	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Adipose tissue	0.002	0.001	0.010	0.002	0.006	0.004	0.005	0.002
Muscle	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Testes / Ovaries	0.002	0.000	0.002	0.000	0.002	0.000	0.002	0.000
Adrenals	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Thyroid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bone marrow	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Stomach	0.001	0.000	0.000	0.000	0.001	0.000	0.000	0.000
Liver	0.165	0.067	0.049	0.035	0.040	0.027	0.023	0.031
Brain	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Kidney	0.008	0.007	0.007	0.004	0.005	0.003	0.003	0.001
Carcass	0.161	0.119	0.118	0.180	0.073	0.075	0.107	0.096
Skin	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gut	0.004	0.004	0.004	0.003	0.002	0.002	0.003	0.003
Bone	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Stomach content	0.003	0.001	0.001	0.002	0.002	0.001	0.003	0.001
Gut content	0.037	0.043	0.054	0.046	0.025	0.023	0.024	0.025
Subtotal	**0.741**	**0.928**	**0.702**	**1.20**	**0.472**	**0.774**	**1.26**	**1.77**
Subtotal volatiles	**0.128**	**n.a.**	**0.057**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Total	**98.7**	**100.9**	**93.8**	**94.8**	**91.8**	**91.7**	**86.5**	**96.4**

n.a. = not applied

The excretion data for the T-labelled substance are presented in Table B.6.1.1.7. The time course of the amount of radioactivity found in urine and faeces indicated that excretion occurred predominantly within the first three (single oral dose of 180 mg/kg bw) to four days (single oral dose of 5.0 mg/kg bw) after administration. With all the dose regimes excretion was mainly via the faeces, with faecal excretion being higher in females than males. In particular, urinary excretion was noticeably higher in the single low-dose males (41 %) than in the other groups. The pattern of excretion after repeated oral high-dose administration showed slightly higher amounts of radioactivity excreted in urine (23 % versus 19 % of administered dose for males and 17 % versus 11 % of administered dose for females, respectively) than in the single high-dose experiment, giving an indication that minor changes in kinetics / metabolism occurred after multiple dosing.

Table B.6.1.1.7. Excretion balance (% of administered radioactivity, T-label)

		5 mg/kg bw				**180 mg/kg bw**				**(14+1) 180 mg/kg bw**			
		male		**female**		**male**		**female**		**male**		**female**	
Balance/Excretion			***(cum.)***		***(cum.)***		***(cum.)***		***(cum.)***		***(cum.)***		***(cum.)***
Urine	0 - 6 h	4.44	*4.4*	3.49	*3.5*	0.71	*0.7*	1.07	*1.1*	1.32	*1.3*	0.96	*1.0*
	6 - 12 h	8.40	*12.8*	3.58	*7.1*	1.65	*2.4*	1.71	*2.8*	2.08	*3.4*	1.56	*2.5*
	12 - 24 h	11.10	*23.9*	4.27	*11.3*	5.61	*8.0*	3.27	*6.1*	7.12	*10.5*	4.55	*7.1*
	24 - 48 h	10.93	*34.9*	2.74	*14.1*	6.61	*14.6*	3.13	*9.2*	7.51	*18.0*	5.77	*12.8*
	48 - 72 h	3.64	*38.5*	0.72	*14.8*	2.62	*17.2*	0.96	*10.1*	2.88	*20.9*	2.52	*15.4*
	72 - 96 h	1.19	*39.7*	0.29	*15.1*	0.95	*18.2*	0.22	*10.4*	0.95	*21.9*	0.79	*16.2*
	96 - 120 h	0.74	*40.4*	0.12	*15.2*	0.38	*18.5*	0.12	*10.5*	0.37	*22.2*	0.20	*16.4*
	120 - 144 h	0.37	*40.8*	0.08	*15.3*	0.15	*18.7*	0.07	*10.6*	0.20	*22.4*	0.16	*16.5*
	144 - 168 h	0.19	*41.0*	0.05	*15.3*	0.06	*18.7*	0.07	*10.6*	0.10	*22.5*	0.09	*16.6*
Subtotal Urine		**41**		**15**		**19**		**11**		**23**		**17**	
Feces	0 - 24 h	18.94	*18.9*	35.18	*35.2*	33.79	*33.8*	35.2	*35.2*	36.70	*36.7*	23.03	*23.0*
	24 - 48 h	30.10	*49.0*	37.46	*72.6*	34.25	*68.0*	39.5	*74.7*	28.99	*65.7*	40.34	*63.4*
	48 - 72 h	6.71	*55.8*	10.23	*82.9*	10.12	*78.2*	12.6	*87.3*	6.02	*71.7*	12.42	*75.8*
	72 - 96 h	2.14	*57.9*	3.42	*86.3*	2.40	*80.6*	1.92	*89.2*	1.18	*72.9*	4.79	*80.6*
	96 - 120 h	0.63	*58.5*	0.45	*86.7*	0.63	*81.2*	0.72	*90.0*	0.82	*73.7*	0.88	*81.5*
	120 - 144 h	0.20	*58.7*	0.22	*87.0*	0.12	*81.3*	0.09	*90.0*	0.10	*73.8*	0.30	*81.8*
	144 - 168 h	0.04	*58.8*	0.07	*87.0*	0.03	*81.3*	0.04	*90.1*	0.02	*73.8*	0.36	*82.1*
Subtotal Feces		**59**		**87**		**81**		**90**		**74**		**82**	
Cage wash		**0.60**		**0.18**		**0.11**		**0.55**		**0.47**		**0.30**	

Balance/Excretion	5 mg/kg bw		180 mg/kg bw		(14+1) 180 mg/kg bw	
	male	female	male	female	male	female
Blood cells	0.01	0.01	0.00	0.00	0.00	0.00
Plasma	0.00	0.00	0.00	0.00	0.00	0.00
Lung	0.00	0.00	0.00	0.00	0.00	0.00
Heart	0.00	0.00	0.00	0.00	0.00	0.00
Spleen	0.00	0.00	0.00	0.00	0.00	0.00
Kidney	0.00	0.00	0.00	0.00	0.00	0.00
Adrenals	0.00	0.00	0.00	0.00	0.00	0.00
Testes/Ovaries	0.00	0.00	0.00	0.00	0.00	0.00
Uterus	---	0.00	---	0.00	---	0.00
Muscle	0.00	0.00	0.00	0.00	0.00	0.00
Brain	0.00	0.00	0.00	0.00	0.00	0.00
Adipose Tissue	0.00	0.00	0.00	0.00	0.00	0.00
Bone	0.00	0.00	0.00	0.00	0.00	0.00
Bone marrow	0.00	0.00	0.00	0.00	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00
Pancreas	0.00	0.00	0.00	0.00	0.00	0.00
Stomach cont.	0.00	0.00	0.00	0.00	0.00	0.00
Stomach	0.00	0.00	0.00	0.00	0.00	0.00
Gut cont.	0.03	0.03	0.01	0.01	0.01	0.03
Gut	0.01	0.01	0.01	0.01	0.01	0.01
Liver	0.07	0.09	0.01	0.02	0.01	0.02
Skin	0.30	0.06	0.11	0.04	0.18	0.13
Carcass	0.76	0.15	0.09	0.08	0.09	0.08
Subtotal Tissues	**1.18**	**0.35**	**0.23**	**0.16**	**0.30**	**0.27**
Total recovery	**101.54**	**102.90**	**100.42**	**101.41**	**97.16**	**99.28**

With all three labels, excretion was fast, more or less complete with no evidence of accumulation and occurred to a major extent within three days after oral dosing, predominantly by the faecal route. The excretion of the C- and TMFP-labels showed similar patterns for males and females. For the triazole (T) label, however, the different excretion patterns for male and female animals, especially in the low dose group in which there was noticeably higher urinary excretion for male than for female animals, indicated differences in kinetics/metabolism between the sexes.

B.6.1.1.2. Biliary excretion

Biliary excretion was investigated in bile-duct-cannulated rats dosed orally with a single dose of 5 (C- or T-labels) or 180 mg/kg bw (C-, TFMP-, T-labels) BAS 750 F. Bile, urine and faeces were collected up until 72 to 168 hours after administration.

For the C-label, based on the amounts of radioactivity excreted via bile and urine and the radioactive residues found in cage wash and carcass, oral absorption of ^{14}C-BAS 750 F in rats was dose-dependent, with slightly lower bioavailability at the higher dose level (Table B6.1.1.8); the oral bioavailability was calculated to be about 78 % and 85 % of the administered dose at 5 mg/kg bw and about 67 % and 64 % of the administered dose at 180 mg/kg bw for males and females, respectively. After administration of 5 mg/kg bw, most of the administered radiolabel was excreted within the first 24 hours in both males (9 % via the urine and 66 % via bile) and females (21 % via the urine and 61 % via the bile). For the TFMP-label, excretion via bile in males and females was found to be 56 % and 55 % of the administered radioactivity 18 hours after administration of 180 mg/kg bw of active substance. Total excretion of radioactivity via bile at 168 h post-dosing was 59 % and 60 % of dose for males and females, respectively.

A slight difference was observed in oral absorption at 180 mg/kg bw between the C-label and the TFMP-label; the study authors attributed this to experimental variability (formulation and inter-animal differences) rather than a label-related difference.

Table B6.1.1.8. Bile excretion balance (% of administered radioactivity, C- and TFMP label)

Label	**[^{14}C] chlorophenyl -BAS 750 F**					**trifluormethylring-U-C^{14} - BAS 750 F**	
Administration frequency	**Single 5 mg/kg bw**		**single 180 mg/kg bw**			**single 180 mg/kg bw**	
	male	**female**	**male 1)**	**female 1 2)**	**female 2 3)**	**male**	**female**
Subtotal Urine	**10.1**	**18.4**	**34.4**	**15.5**	**10.2**	**11.3**	**10.7**
Subtotal Feces	**6.83**	**6.43**	**11.4**	**4.39**	**16.7**	**30.7**	**26.7**
Cage wash	0.220	0.287	0.492	0.246	0.191	0.575	0.250
Stomach content	0.000	0.000	0.002	0.000	0.000	0.000	0.000
Stomach	0.001	0.001	0.001	0.000	0.000	0.000	0.000
Gut content	0.001	0.004	0.204	0.002	0.001	0.002	0.003
Gut	0.003	0.002	0.007	0.001	0.002	0.001	0.001
Carcass	0.191	0.155	0.169	0.053	0.072	0.148	0.077
Subtotal	**0.416**	**0.449**	**0.875**	**0.302**	**0.267**	**0.726**	**0.331**
Subtotal Bile	**67.0**	**61.4**	**31.9**	**40.0**	**53.6**	**58.6**	**59.6**
Total	**84.3**	**86.6**	**78.7**	**60.2**	**80.8**	**101.3**	**97.3**
Bioavailability	**77.5**	**85.2**	**67.1**	**55.8**	**64.1**	**70.7**	**70.7**

1) Animal nos.19, 21, 126 and 127; animals 126 and 127 had a recovery of radioactivity <80 % (72 and 74 % respectively)
2) Animal nos. 69 and 71; both animals showed low recovery of radioactivity, possibly caused by problems with bile cannula
3) Animal nos. 128, 129 and 130

For the T-label, bile, urine and faeces were collected up to 72 hours after administration of a single dose. After a dose of 5 mg/kg bw, excretion via bile was found to be 71 % and 74 % of the administered radioactivity in males and females, respectively. Total excretion of radioactivity via urine after 72 hours was 11 % for males and 10 % for females (Table B6.1.1.9). After a dose of 180 mg/kg bw, mean excretion via bile was 42 % and 46 % of the administered radioactivity in males and females, respectively. Mean total excretion of radioactivity via urine after 72 hours was 7 % for males and 11 % for females. Based on the amounts of radioactivity excreted via bile and urine, combined with the radioactive residues found in cage wash and carcass, the oral absorption of ^{14}C- BAS 750 F in rats was calculated to be 84 % and 85 % of the administered low dose (5.0 mg/kg bw), and 50 % and 58 % of the administered high dose (180 mg/kg bw) for males and females, respectively.

Table B6.1.1.9. Bile excretion balance (% of administered radioactivity, T-label)

	5 mg/kg bw		**180 mg/kg bw**	
Balance/Excretion	**male**	**female**	**male**	**female**
Subtotal Urine	**11**	**10**	**7**	**11**
Subtotal Bile	**71**	**74**	**42**	**46**
Subtotal Feces	**14**	**8**	**38**	**24**
Cage wash	**0.51**	**0.26**	**0.30**	**0.72**
Stomach cont.	0.01	2.01	2.62	4.29
Stomach	0.01	0.15	0.14	0.18
Gut cont.	0.10	0.54	1.53	1.47
Gut	0.01	0.08	0.10	0.11
Carcass	0.88	0.38	1.06	0.95
Total recovery	**97.45**	**95.33**	**91.83**	**88.73**
Bioavailability	**84**	**85**	**50**	**58**

Taking together the information from the three labels, recovery values from urine and bile combined with those of the cage wash and carcass generally demonstrated that approximately 85 % of an orally administered single dose of 5 mg/kg bw was absorbed; this dose is in the same order of magnitude as the point of departure from the study used to estimate the AOEL. An oral absorption value of 100 % will therefore be used in the calculation of the AOEL.

The bile-excretion experiments with the three labels demonstrated that excretion of the active substance was fast and occurred to a major extent via the biliary pathway. Compared with the balance experiments, mean urinary excretion of the T-label was generally lower in bile-duct-cannulated rats, demonstrating that there was a partial reabsorption of the triazole moiety into the systemic circulation in intact animals; this finding correlates with the indication of enterohepatic recirculation in the plasma-kinetic experiments with the T-label.

B.6.1.1.3. Distribution

The tissue distribution of BAS 750 F was investigated in rats following single oral administrations of 5 and 180 mg/kg bw substance (C- and T-label). Three animals were sacrificed at each of four time-points, which corresponded to the following time-points in plasma kinetics: maximum plasma concentration (MPC), ½ MPC, ¼ MPC and 1/8 MPC.

Following a single oral dose of 5 mg/kg bw (C-label), the highest tissue residues within one hour of dosing (excluding the gastrointestinal tract and its contents) were detected in the plasma, liver, adrenal glands and kidney. Following a single oral dose of 180 mg/kg bw (C-label), the highest tissue residues within two hours of dosing (excluding the gastrointestinal tract and its contents) were detected in the liver and adrenal glands. With both doses, radioactive residue concentrations generally declined in organs and tissues in parallel with the radioactive residues in plasma. However, in the high-dose males, the residue concentrations at 53 hours were largely similar to those at 38 hours. As the study authors were not able to identify any errors or inconsistencies in data entry, dose administration or analysis, they attributed this observation to experimental variability.

Table B.6.1.1.10. Mean tissue concentration of radioactivity (in µg Eq/g tissue) after single oral administration of ^{14}C-BAS 750 F at a dose level of 5 mg/kg bw (C-label)

Label	**[^{14}C] chlorophenyl -BAS 750 F**							
Administration frequency	**single**				**single**			
Dose level	**5 mg/kg bw**				**5 mg/kg bw**			
	male animals				**female animals**			
Time after administration [h]	**T=1**	**T=7**	**T=20**	**T=34 [1)]**	**T=0.5**	**T=3**	**T=12**	**T=24**
Plasma	2.00	0.969	0.574	0.241	1.40	0.577	0.239	0.155
Blood	1.21	0.549	0.317	0.134	0.948	0.336	0.147	0.096
Heart	0.607	0.185	0.154	0.050	1.04	0.214	0.078	0.031
Lung	0.632	0.293	0.145	0.059	1.07	0.275	0.099	0.054
Spleen	0.329	0.105	0.054	0.026	0.630	0.138	0.040	0.021
Uterus	n.a.	n.a.	n.a.	n.a.	0.596	0.271	0.113	0.068
Pancreas	0.615	0.185	0.080	0.030	0.986	0.293	0.080	0.045
Adipose tissue	0.267	0.221	0.118	0.042	0.373	0.417	0.165	0.059
Muscle	0.169	0.068	0.033	0.015	0.307	0.086	0.024	0.016
Testes / Ovaries	0.232	0.193	0.118	0.041	1.04	0.287	0.106	0.052
Adrenals	1.31	0.298	0.120	0.041	3.32	0.535	0.155	0.057
Thyroid	0.349	0.154	0.065	0.031	0.674	0.109	0.040	0.024
Bone marrow	0.310	0.097	0.047	0.022	0.383	0.060	0.020	0.012
Stomach	22.4	1.83	0.347	0.041	15.6	4.46	0.313	0.132
Liver	12.7	5.60	2.47	1.03	9.72	4.16	2.11	1.29
Brain	0.256	0.042	0.012	0.005	0.777	0.090	0.014	0.006
Kidney	1.52	1.10	0.433	0.189	1.93	0.683	0.399	0.234
Carcass	0.192	0.118	0.056	0.025	0.284	0.139	0.053	0.044
Skin	0.124	0.116	0.064	0.028	0.153	0.104	0.036	0.021

Gut	4.80	4.61	1.84	0.626	7.39	4.71	2.45	1.83
Bone	0.102	0.051	0.028	0.014	0.118	0.049	0.020	0.011
Stomach content	413.9	18.8	0.707	0.128	164.8	49.6	3.03	3.23
Gut content	26.1	99.6	53.3	15.2	26.6	68.0	83.5	46.0

1) Animal 38 excluded from mean (too little formulation dosed)

Table B.6.1.1.11. Mean tissue concentration of radioactivity (in µg Eq/g tissue) after single oral administration of ^{14}C-BAS 750 F at a dose level of 180 mg/kg bw (C-label)

Label	**[^{14}C] chlorophenyl -BAS 750 F**							
Administration frequency	**single**				**single**			
Dose level	**180 mg/kg bw male animals**				**180 mg/kg bw female animals**			
Time after administration [h]	**T=2**	**T=22**	**T=38**	**T=53**	**T=0.5**	**T=4**	**T=17**	**T=24**
Plasma	58.8	22.1	11.2	11.2	57.0	23.1	7.71	5.50
Blood	42.7	12.9	5.88	6.94	35.1	12.1	4.54	3.20
Heart	42.0	6.39	2.64	2.78	47.7	18.3	3.05	1.69
Lung	41.2	5.49	2.40	2.73	44.0	18.1	2.98	1.95
Spleen	23.9	2.63	1.02	1.12	27.9	11.2	1.24	0.840
Uterus	n.a.	n.a.	n.a.	n.a.	23.0	13.2	3.36	2.32
Pancreas	61.6	5.60	2.12	1.81	64.0	29.5	3.04	1.70
Adipose tissue	19.0	6.24	0.570	0.702	20.5	66.6	6.09	3.66
Muscle	64.9	16.6	2.64	4.35	17.8	8.82	0.795	0.573
Testes / Ovaries	23.3	5.19	2.15	1.90	46.3	21.6	3.97	2.24
Adrenals	99.0	9.32	2.41	2.68	136.0	52.1	6.59	3.59
Thyroid	40.9	9.03	3.16	2.92	50.0	16.8	20.0	2.86
Bone marrow	26.3	4.15	1.31	1.60	29.6	10.7	3.15	0.818
Stomach	322.8	5.72	9.08	4.86	683.3 [1)]	385.7	18.1	20.4
Liver	207.2	93.1	16.1	21.0	212.4	84.8	39.4	30.1
Brain	29.7	0.976	0.229	0.235	39.6	13.5	0.638	0.277
Kidney	51.7	12.1	5.96	6.31	62.8	25.4	6.78	4.99
Carcass	12.0	2.50	0.873	1.41	8.87	4.79	1.71	1.21
Skin	15.0	2.62	0.882	1.27	5.58	3.18	1.12	0.836
Gut	178.2	83.7	17.8	24.6	119.8	123.4	115.6	78.7
Bone	8.70	1.39	0.565	0.784	7.93	3.33	1.41	0.864
Stomach content	1940.8	33.6	9.24	14.8	10657.4	1678.3	61.1	30.7
Gut content	2020.1	1243.1	346.5	330.4	1535.2	1744.6	1340.1	1134.5

1) Animal 89 excluded from mean (stomach weight unrealistically low)

One hour after administration of 5 mg/kg bw ^{14}C-BAS 750 F (T-label) to male and female rats, the highest tissue concentrations were found in the gastro-intestinal tract and its contents (Table B.6.1.1.12). Otherwise, the highest residues were measured in the liver and adrenal glands. With the exception of the gastro-intestinal tract and its contents, the highest residues one-hour after administration of 180 mg/kg bw were in the liver, adrenal glands, plasma, thyroid, kidney, pancreas, lung (females) and ovaries (Table B.6.1.1.13). With both doses, radioactive residue concentrations generally declined in organs and tissues from the one-hour time-point onwards and during the following 28 and 24 hours continued to decline in parallel with the radioactive residues in plasma.

Table B.6.1.1.12. Mean tissue concentration of radioactivity after single oral administration of ^{14}C-BAS 750 F at a dose level of 5 mg/kg bw (T-label)

Dose: 5 mg/kg bw	**Mean tissue concentration [µg Eq/g] Males**				**Mean tissue concentration [µg Eq/g] Females**			
Time after administration	**C_{max} 1 h**	**$C_{max}/2$ 4 h**	**$C_{max}/4$ 18 h**	**$C_{max}/8$ 28 h**	**C_{max} 1 h**	**$C_{max}/2$ 2 h**	**$C_{max}/4$ 4 h**	**$C_{max}/8$ 24 h**
Blood cells	0.88	0.55	0.33	0.20	0.30	0.12	0.14	0.04
Plasma	2.99	1.20	0.42	0.29	1.53	0.48	0.42	0.09
Lung	1.47	0.85	0.41	0.29	1.01	0.44	0.37	0.11

Dose: 5 mg/kg bw	Mean tissue concentration [µg Eq/g] Males				Mean tissue concentration [µg Eq/g] Females			
Heart	1.29	0.67	0.36	0.25	0.87	0.34	0.27	0.07
Spleen	0.76	0.61	0.40	0.26	0.55	0.36	0.32	0.08
Kidney	1.98	1.78	0.54	0.49	1.66	0.89	0.84	0.26
Adrenal glands	4.68	1.63	0.64	0.50	3.72	1.47	0.86	0.18
Testes/Ovaries	0.57	0.70	0.40	0.26	1.06	2.01	0.82	0.11
Uterus	---	---	---	---	0.64	1.50	0.76	0.14
Muscle	0.58	0.61	0.38	0.26	0.41	0.22	0.20	0.07
Brain	0.72	0.53	0.33	0.22	0.59	0.26	0.19	0.06
Adipose tissue	0.62	0.36	0.11	0.05	0.54	0.70	0.96	0.17
Bone	0.32	0.29	0.16	0.12	0.16	0.09	0.08	0.02
Bone marrow	1.07	0.79	0.48	0.34	0.97	0.39	0.34	0.10
Thyroid	2.71	1.42	0.84	0.68	2.53	0.84	0.64	0.14
Pancreas	1.36	0.74	0.53	0.24	1.33	1.37	1.09	0.09
Stomach content	131.04	78.59	1.16	2.41	94.05	71.23	41.77	0.28
Stomach	14.23	10.87	0.96	0.67	19.73	15.47	6.23	0.30
Gut content	43.59	49.46	13.02	8.05	34.33	32.87	63.74	11.88
Gut	6.42	7.42	2.97	1.00	15.48	23.05	12.18	2.08
Liver	16.35	6.74	1.64	1.07	9.13	4.24	4.14	0.68
Skin	0.69	0.57	0.39	0.27	0.48	0.31	0.23	0.07
Carcass	0.67	0.55	0.36	0.24	0.52	0.55	0.37	0.10

Table B.6.1.1.13. Mean tissue concentration of radioactivity after single oral administration of ^{14}C-BAS 750 F at a dose level of 180 mg/kg bw (T-label)

Dose: 180 mg/kg bw	Mean tissue concentration [µg Eq/g] Males				Mean tissue concentration [µg Eq/g] Females			
Time after administration	C_{max} 1 h	$C_{max}/2$ 24 h	$C_{max}/4$ 36 h	$C_{max}/8$ 48 h	C_{max} 1 h	$C_{max}/2$ 8 h	$C_{max}/4$ 24 h	$C_{max}/8$ 34 h
Blood cells	21.60	13.61	9.16	8.98	17.10	8.40	4.04	2.57
Plasma	70.96	18.03	11.20	10.43	52.86	17.32	6.93	2.85
Lung	47.87	15.63	10.10	11.21	70.49	20.16	6.24	3.15
Heart	45.94	14.37	9.17	18.55	48.22	18.09	5.13	2.30
Spleen	31.44	15.03	9.98	5.26	34.78	13.35	5.59	2.49
Kidney	65.36	22.06	13.37	13.54	69.81	26.98	8.90	4.13
Adrenal glands	123.94	26.44	12.71	13.15	144.30	52.10	11.04	4.70
Testes/Ovaries	22.00	14.45	9.46	9.61	52.19	25.67	7.89	2.34
Uterus	---	---	---	---	31.22	16.63	7.88	2.79
Muscle	22.69	13.67	8.88	8.85	23.06	9.62	4.35	2.15
Brain	37.38	12.61	8.27	8.70	47.77	15.09	3.59	1.86
Adipose tissue	22.82	3.44	1.46	3.88	40.50	44.88	2.83	1.18
Bone	6.99	5.46	2.33	3.23	8.09	2.82	1.92	0.63
Bone marrow	35.12	18.65	10.73	11.25	34.22	13.20	5.45	3.15
Thyroid	70.82	36.53	16.11	17.07	74.52	29.11	25.12	5.76
Pancreas	59.52	14.30	9.18	9.60	76.61	25.21	6.91	2.58
Stomach content	6558.92	52.53	54.38	38.23	4056.53	725.01	22.33	16.68
Stomach	671.23	33.77	19.33	22.86	1373.12	95.21	17.38	6.01
Gut content	1349.46	909.28	572.58	185.46	1273.02	2395.29	978.73	550.58
Gut	201.79	104.52	59.29	24.84	399.59	178.25	139.13	38.93
Liver	267.46	41.53	29.38	17.11	221.46	91.19	29.87	13.83
Skin	21.49	14.28	11.65	12.61	34.50	14.38	4.32	2.56
Carcass	24.87	15.86	12.08	18.24	28.59	14.99	6.45	3.64

Overall, the distribution experiments demonstrated that BASF 750 F (chlorophenyl and triazole moieties) was rapidly and widely distributed in rats after a single oral administration.

B.6.1.1.4. Metabolism

The metabolism of BAS 750 F in intact rats was investigated after single oral doses of 5 and 180 mg/kg bw (C-, TFMP- or T-labels) or multiple oral doses of 180 mg/kg/d (C- and T-labels). Metabolism in bile-duct-cannulated rats was also investigated after single doses of 5 and 180 mg/kg bw (C-, TFMP or T-labels).

Sixty-eight metabolites of BAS 750 F were identified by HPLC-MS/MS and nuclear magnetic resonance analysis. The proposed metabolic pathway is shown in Annex I, together with a summary of the identified and quantified metabolites and the proportions detected in different compartments. The identified metabolites comprised the phase I and phase II conversions of the parent compound tabulated below.

Table B.6.1.14. Summary of the metabolic conversions of BASF 750 F.

Phase I metabolism			
Step	**Conversion**	**Figure (Annex I)**	**Metabolites**
1	Hydroxylation: mono, di- and tri-hydroxylation including CL-shift	Figure B6.11.1, Figure B6.11.2., Figure B6.11.3, Figure B6.11.4, Figure B6.11.8.	M750F015, M750F016, M750F017, M750F078, M750F062
2	Methylation	Figure B6.11.2., Figure B6.11.7	M750F089
3	Cleavage of the ether group	Figure B6.11.6, Figure B6.11.8.	M750F003
4	Cleavage of the triazole ring from the parent	Figure B6.11.6, Figure B6.11.8.	M750F001
Phase II metabolism: conjugation of Phase I metabolites by:			
a	Sulfation	Figure B6.11.1, Figure B6.11.2., Figure B6.11.4, Figure B6.11.6, Figure B6.11.7, Figure B6.11.8.	M750F043, M750F048, M750F055, M750F057, M750F058, M750F059, M750F060, M750F066, M750F067, M750F071, M750F079, M750F082, M750F096, M750F097, M750F101, M750F098 (includes M750F060 with potential Cl-shift)
b	Glucuronidation	Figure B6.11.1, Figure B6.11.2., Figure B6.11.4, Figure B6.11.6, Figure B6.11.7, Figure B6.11.8.	M750F035, M750F044, M750F045, M750F046, M750F047, M750F049, M750F052, M750F054, M750F063, M750F108
c	GSH adduction and its decomposition products	Figure B6.11.4, Figure B6.11.5, Figure B6.11.7, Figure B6.11.8.	M750F048, M750F050, M750F052, M750F053, M750F055, M750F061, M750F065, M750F069, M750F075, M750F079, M750F084, M750F085, M750F087, M750F091

In both male and female rats, the unchanged parent compound was extensively excreted in faecal material (up to 35 % of the administered dose, all labels). All the metabolites detected in faeces had been cleaved from the parent compound or been once subject to hydroxylation. Besides the parent, all dose groups of all labels showed a comparable metabolite pattern, with M750F015 (ranging from 10 – 41 % of the dose) and M750F016 / M750F017 (ranging from 15 – 32 % of the dose) as the major components.

Predominately glucuronide and sulphate conjugates of mono-, di- or trihydroxylated BAS 750 F were excreted via urine. With the C-label, the main common urinary metabolites in males given a single dose of 180 mg/kg bw were detected in comparable amounts of up to 1 % of the dose, respectively: M750F049, M750F050 and M750F058 / M750F081. For female rats a broader spectrum of metabolites was detected, but despite this the overall metabolic pathway of the two sexes was

comparable in urine for the C-label and the different doses and dosing regimens. The urinary metabolite patterns with the TFMP- and T-labels were largely comparable. The main components in males and females given a single dose of 180 mg/kg bw were M750F054, M750F049 and M750F003 (up to 2.8 % of the dose) for the TFMP-label and M750F001 (9.6 % - 10.5 % of the dose), M750F071 and M750F054 (<5 % of the dose) for the T-label. For the dose groups given the T-label, generally the main compound detected was M750F001 (up to 20 % of the dose) and was higher in males than females.

The metabolite patterns in bile were comparable for samples of all labels for both male and female rats. Metabolites M750F035, M750F044, M750F045, M750F049 (including isomers) and M750F087 constituted the main proportions of radioactive residues in bile samples and were usually detected in large amounts within 0 - 6 hours (19-53 % of the applied dose for these metabolites combined). These metabolites are either once or twice hydroxylated parent compounds, which have been subsequently subjected to glucuronidation.

In tissues (liver, kidney, fat) and plasma collected one-hour after dose administration, the major portion of metabolites were detected as hydroxylated or unchanged parent compound; the unchanged parent ranged from 0.005 % to 2 % of the administered dose. Minor amounts were detected as glucuronide or sulphate conjugates of dihydroxylated parent compound. No sex-specific differences were observed in the residue composition of tissues and plasma. The portions of radioactive residues were highest for liver (up to 7.3 % of the dose) and lowest for plasma (< 0.1 % of the dose).

The relative amounts of the isomers were approximately 1:1 in the application formulation and remained so in the methanol extracts of faeces. In the methanol extracts of liver and kidney as well as in plasma, the ratio between S- and R-enantiomer shifted towards a higher relative amount of the R-enantiomer (see table below). It is noted, though, that the liver, kidney and plasma samples contained only minor amounts of radioactive residues (< 1 % of the administered dose). Notwithstanding, this data indicate that there is preferential metabolism and elimination of the S-enantiomer in rats.

Table B.6.1.15. Isomer ratio of BAS 750 F in matrices of rat metabolism studies (T-label)

		Males		Females	
Matrix [1]		**S-enantiomer [%]**	**R-enantiomer [%]**	**S-enantiomer [%]**	**R-enantiomer [%]**
1 x 5 mg/kg bw					
faeces	[0-24 h]	45.65	54.35	–	–
liver	[1 h]	34.59	65.41	18.85	81.15
kidney	[1 h]	24.21	75.79	13.99	86.01
1 x 180 mg/kg bw					
application formulation		–	–	51.55	48.45
faeces	[0-72 h]	49.42	50.58	51.01	48.99
plasma [1 h]		30.46	69.54	23.72	76.28
liver	[1 h]	31.28	68.72	20.50	79.50
kidney	[1 h]	26.92	73.08	18.88	81.12
(14+1) x 180 mg/kg bw					
faeces	[0-24 h]	50.97	49.03	49.67	50.33
	[24-48 h]	48.94	51.06	50.50	49.50

1) Enantiomer-specific analyses were representatively performed for different dose groups, time periods and matrices with a sufficient amount of the parent compound.

B.6.1.2. Comparative *in vitro* metabolism

A comparative *in vitro* metabolism study was performed to investigate the qualitative comparison of the metabolite patterns of ^{14}C-BAS 750 F formed after incubation with human, rat and mouse hepatocytes. The species rat and mouse were chosen to compare with human metabolism because they were used in the toxicological studies with BAS 750 F.

Table B.6.1.2.1. Summary of comparative *in vitro* metabolism study

Method Guideline, GLP status, reference	Species, strain, sex, no./group	Test substance, dose levels, duration of exposure	Results	Remarks
Comparative metabolism No guideline GLP Report CA 5.1.2/1 Funk *et al*., 2016 a (2015/1020123)	Rat / Wistar Mouse / C57BL/6 J Rj Human For all species, hepatocytes were from males & females mixed Triplicates of each experimental set-up.	T-, C- and TFMP-labelled ^{14}C-BASF 750 F. 1 µM incubated for 10, 30, 60 & 180 minutes at 37°C except rat samples of C- & TFMP-labels (0 and 180 minutes only) Negative controls & positive controls (10 µM testosterone or 7-ethoxycoumarin) included	Positive controls demonstrated the metabolic activity (phase I and II) of the hepatocytes from all species. No metabolism or degradation of BASF 750 F in negative controls. Two peaks detected in human & also rat samples (unchanged parent & biotransformation product). One peak (unchanged parent) in mouse samples.	Comparative *in vitro* metabolism Concentration chosen on basis of viability of human hepatocytes with 1 µM, 5 µM & 10 µM active substance

A comparative *in vitro* metabolism study has been performed with BAS 750 F. The objective of this study was the qualitative comparison of the metabolite patterns of ^{14}C-BAS 750 F formed after incubation with human, rat and mouse hepatocytes. The viability of human hepatocytes after incubation with 1 µM, 5 µM and 10 µM BAS 750 F (triazole label) was tested in order to select the appropriate concentration of the test item. Therefore, 250 µL of the respective application medium were incubated in a 24-well plate with 250 µL hepatocyte cell suspension at 37°C and 5% CO_2 for 180 min. The cell viability was determined using a luminescent cell viability assay. The viability of the human hepatocytes was highest (87 %) at a final concentration of 1 µM; this concentration was therefore chosen for each label.

The radiolabelled test item (T-, C- or TFMP-label) was incubated with hepatocytes from humans, rat or mouse (cells from males and females mixed in 1:1 ratio) for 10, 30, 60 or 180 min. The rat and mouse hepatocytes from males and females were mixed to be consistent with those from humans, which were purchased as a mixture of male and female cells. After the 180-minute incubation, the viability of the hepatocytes was determined and shown to be high (viability of treated hepatocytes > 79 % compared with untreated samples). Negative and positive controls were run in parallel to prove the absence of non-metabolic degradation and the metabolic activity of the hepatocytes (phase I and phase II metabolic reactions), respectively. The control experiments yielded the expected results. Samples were analysed by liquid scintillation counting and HPLC, with selected samples additionally being analysed by HPLC-MS.

The viability of rat, mouse and human hepatocytes after 180 minutes of incubation was acceptable. After the incubation of BAS 750 F with human hepatocytes, up to two peaks were detected in the radio-chromatograms. Both peaks represented more than 5 % of the applied radioactivity (AR). One of these signals represented the unchanged active substance BAS 750 F, whilst the second corresponded to a metabolite of BAS 750 F and was detected after an incubation period of 180 minutes. Both these

peaks were also detected in rat hepatocyte samples, with the metabolite peak occurring from 10 minutes of incubation. In rats, this metabolite peak was represented by two closely adjacent peaks in the HPLC-MS analyses. BAS 750 F was not detected in rat hepatocyte samples after an incubation period of 180 min. Additional peaks were observed in the rat hepatocyte samples that did not occur in the human samples. No significant biotransformation of the test item was observed after incubation with mouse hepatocytes. The peaks obtained from human hepatocytes are compared with the same peaks recorded in rat and mouse samples in the table below.

Table B.6.1.2.2. Comparison of peaks detected after incubation of human, rat and mouse hepatocytes with BAS 750 F

Incubation time [min]	Analyte / Peak	Human [Mean % AR]	Rat [Mean % AR]	Mouse [Mean % AR]
C-LABEL				
0	BAS 750 F	85.80	91.45	88.54
10	BAS 750 F	93.01	not applied	89.84
30	BAS 750 F	91.76		87.53
60	BAS 750 F	91.63		86.69 [1]
180	BAS 750 F Peak at 7.6 min	74.49 18.71	– 31.41	90.51 –
TFMP-LABEL				
0	BAS 750 F	93.51	88.07	92.57
10	BAS 750 F	93.66	not applied	96.33
30	BAS 750 F	90.70		94.59
60	BAS 750 F	92.83		91.67
180	BAS 750 F Peak at 7.6 min	70.31 21.47	– 31.95	89.75 [1] –
T-LABEL				
0	BAS 750 F	87.53	96.23	90.53
10	BAS 750 F Peak at 7.6 min	95.21 –	41.04 2.89	93.00 –
30	BAS 750 F Peak at 7.6 min	92.15 –	15.74 12.14	93.32 –
60	BAS 750 F Peak at 7.6 min	92.64 –	5.79 22.34	93.21 –
180	BAS 750 F Peak at 7.6 min	72.84 18.92	– 43.36	95.48 [2] –

1 Sum of mean % applied radioactivity values of two evaluated replicates.
2 Only replicate 3 was evaluated.

In conclusion, no unique human metabolite was observed. The metabolic degradation of BAS 750 F was quantitatively different but qualitatively the same in rat hepatocytes compared with human cells when incubated for up to 180 minutes. No metabolism was detected following incubation with mouse hepatocytes for up to 180 minutes.

B.6.1.3. Overall conclusion

The plasma kinetics of BAS 750 F in rats and mice demonstrated high absorption following oral administration, indicated potential enterohepatic recirculation of the triazole moiety, and showed fast excretion and a more-or-less linear correlation of the internal exposure to the oral dose. The biliary excretion data confirmed that oral absorption in rats was approximately 85 % following single low-dose administration; the same oral absorption value in humans will be assumed. In the absence of specific data, default inhalation absorption of 100 % is assumed. Dermal absorption was low (4 % for the formulation concentrate; see volume 3CP B6).

The biliary excretion experiments confirmed that excretion was fast, more or less complete and occurred to a major extent within three days after oral dosing in rats, predominantly by the faecal route. There was no evidence of accumulation. In addition to the information provided by the plasma kinetics data, evidence of enterohepatic recirculation of the triazole moiety was provided by the biliary-excretion investigations. The distribution experiments demonstrated that BAS 750 F was rapidly and widely distributed in rats after a single oral administration. The active substance was extensively and rapidly metabolized (see Annex I for the proposed metabolic pathway), resulting in rapid and extensive excretion (biliary and urinary routes). A preferential metabolism and elimination of the S-enantiomer in rats was indicated.

In a comparative *in vitro* metabolism study, one metabolite was detected following incubation with human hepatocytes, which was also detected in rat hepatocyte samples; hence, metabolism in hepatocytes of the two species was qualitatively similar. The study did not detect a unique human metabolite and so the rat metabolism study is concluded to provide results that are representative of human metabolism.

B.6.2. ACUTE TOXICITY

The acute toxicity of BAS 750 F has been investigated by the oral, dermal and inhalation routes. *In vitro* and *in vivo* irritation studies are available, whilst skin sensitisation has been investigated in a guinea pig maximisation test.

The RMS questioned the conduct of *in vivo* irritation studies when regulatory-acceptable *in vitro* alternatives are available, and a guinea-pig study without justification. The applicant's response referred to the need to meet global data requirements; to comply with the pesticide regulations of non-European countries, the acute dermal toxicity and *in vivo* irritation studies were conducted. The guinea pig maximisation test was used in preference to the murine local lymph node because of the current lack of acceptability of the latter as a stand-alone method in some major Asian countries.

B.6.2.1. Oral

The acute-toxic-class method has been used to investigate the acute oral toxicity of BAS 750 F.

Table B.6.2.1. Summary of the acute oral toxicity of BASF 750 F

Method Guideline, GLP status, reference	**Species, strain, sex, no./group**	**Test substance, dose levels, duration of exposure**	**LD50**	**Remarks**
OECD 423 (2001) (acute toxic class method) GLP Report CA 5.2.1/1 [redacted] 2013c (2013/1149656)	Rats / Wistar / 3 females / group Observation period: 14 days	2000 mg/kg/d suspended in corn oil Purity 98.8 %	> 2000 mg/kg	No deaths. Clinical signs included cowering position, impaired general state and piloerection between 2 and 5 hours after administration. No adverse macroscopic necropsy findings

In an acute oral toxicity study performed in accordance with the acute toxic class method (OECD 423), 2000 mg/kg BAS 750 F was administered to three fasted female rats. As no deaths occurred in this group, the result was confirmed in three additional animals at the same dose level.

None of the animals died. The observed clinical signs were indicative of general toxicity and did not give any indication of specific target-organ toxicity; moreover, they had resolved within a few hours of the dose being administered, and gross pathology did not reveal any adverse findings. The mean body weight of the animals increased throughout the study period within the normal range.

On the basis of this study, it is concluded that BAS 750 F is not acutely toxic by the oral route (LD50 > 2000 mg/kg) and does not meet the criteria for classification for acute oral toxicity or STOT-SE (please see CLH report).

B.6.2.2. Dermal

One acute dermal toxicity study is available, which was conducted in rats.

Table B.6.2.2. Summary of the acute dermal toxicity of BAS 750 F

Method Guideline, GLP status, reference	**Species, strain, sex, no./group**	**Test substance, dose levels, duration of exposure**	**LD50**	**Remarks**
OECD 402 (1987) GLP Report CA 5.2.2/1 [redacted] 2013b (2013/1149657)	Rat, Wistar, 5/sex Observation period: 14 days	5000 mg/kg suspended in corn oil, applied for 24 hours Purity 98.8 %	> 5000 mg/kg	No deaths. No signs of systemic toxicity or skin effects. No adverse macroscopic necropsy findings.

In an acute dermal toxicity study, rats were exposed to a single limit dose of 5000 mg/kg for 24 hours under a semi-occlusive dressing. The application area comprised at least 10 % of the total body surface area. At the end of the 24-hour exposure period, the dressing was removed and the application site was rinsed with warm water. Skin effects were monitored 30-60 minutes after removal of the dressing, weekly thereafter and on the last day of observation. There were no deaths, signs of systemic toxicity, local skin effects or adverse macroscopic findings at necropsy. The mean body weights of the animals increased within the normal range throughout the study period.

On the basis of this study, it is concluded that BAS 750 F is not acutely toxic by the dermal route (LD50 > 5000 mg/kg) and does not meet the criteria for classification for acute dermal toxicity or STOT-SE (please see CLH report).

B.6.2.3. Inhalation

A limit test to investigate the acute toxicity of BAS 750 F by the inhalation route has been conducted in rats.

Table B.6.2.3. Summary of studies to investigate the acute inhalation toxicity of BAS 750 F

Method Guideline, GLP status, reference	**Species, strain, sex, no./group**	**Test substance, dose levels, duration of exposure**	**LC50**	**Remarks**
Acute inhalation toxicity OECD 403 (2009) GLP Report CA 5.2.3/1 [redacted], 2014a (2014/1127433)	Rat, Wistar, 5/sex Head/nose exposure Observation period: 14 days	5.3 mg/l (analytical concentration) as a dust aerosol for 4 hours Purity 98.8 % Mass median aerodynamic diameters (MMADs) of 3.8 µm	> 5.3 mg/l	No deaths. Clinical signs included laboured breathing, abdominal respiration, respiratory sounds, encrusted eyes, red & colourless discharge and/or red crusts of the nose, poor general state, hunched posture, hyper-excitability, no defecation, piloerection and substance-contaminated fur; observed from 2 hours to 11 days after exposure. No adverse macroscopic necropsy findings.

In an acute inhalation study, rats were exposed in a head/nose-only system for four hours to a limit concentration of 5.3 mg/l of BAS 750 F as a dust aerosol. There were no deaths. General indications of toxicity and respiratory effects that are commonly associated with the inhalation route of exposure were observed between 2 hours and 11 days after the exposure. No clinical symptoms were recorded from day 12 onwards. Body weights increased as expected from day 3 onwards, and there were no adverse macroscopic findings upon necropsy. It was noted that the relative humidity (19 %) was less than that recommended in the test guideline (30-70 %) because of the need to use compressed air for dust generation; however, the study authors did not consider that this would influence the test results because of the relatively short exposure time.

On the basis of this study, it is concluded that BAS 750 F is not acutely toxic by the inhalation route (LC50 > 5.3 mg/l) and thus does not meet the criteria for classification for acute inhalation toxicity or STOT-SE (please see CLH report).

B.6.2.4. Skin irritation

Information on the skin irritation potential of BAS 750 F is available from a non-GLP *in vitro* study and a GLP-compliant rabbit study.

Table B.6.2.4. Summary of the skin irritation studies with BAS 750 F

Method Guideline, GLP status, reference	**Test system**	**Test substance, dose levels, duration of exposure**	**Results**
EpiDerm skin corrosion / irritation test OECD 431 Not GLP Report CA 5.2.4/1 Remmele, 2012a (2012/1367952)	Human reconstituted epidermis model exposed for 3 minutes & 1 hour (corrosion test) or 1 hour with 42 hours post-incubation (irritation test)	25 µl bulk volume (approx. 11 mg), minimally moistened with water Purity 97.7 %	Tissue viability values were comparable to the negative control (100-102% of the negative control values) for all experiments. The positive control substance (5% SDS for irritation test) resulted in reduced tissue viability (3% of the negative control) Not a skin irritant under the conditions of the study.
Acute dermal irritation / corrosion in rabbits OECD 404 (2002) GLP Report CA 5.2.4/2 [redacted] 2013a (2013/1150122)	Rabbit, New Zealand White, 3 females (step-wise procedure)	0.5 g minimally moistened with water applied to intact skin for 4 hours under semi-occlusive dressing. Purity 98.8 %	Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0 for erythema 0, 0, 0 for oedema Not a skin irritant.

An *in vitro* study with the EpiDerm™ human skin model was performed as a pre-test. The cell viability, as measured by dehydrogenase conversion of the yellow, water-soluble MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), into a blue formazan salt, was comparable between the BAS 750 F-exposed samples and the negative controls. The reductions in cell viability with the positive control substances demonstrated the sensitivity of the system. The test material did not show an irritant potential under the conditions of this study.

The *in vivo* study was conducted in a step-wise procedure; an initial animal that showed there were no severe skin lesions was supplemented with two additional rabbits. Slight erythema (grade 1) was observed in one of the three treated animals immediately after removal of the patch; this was reversible within one hour. No other cutaneous reactions were observed during the study. Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0 and 0.0 for both erythema and oedema.

On the basis of these studies, it is concluded that BAS 750 F was not a skin irritant (please see CLH report).

B.6.2.5. Eye irritation

The eye irritation potential of BAS 750 F has been investigated in two non-GLP *in vitro* tests and a GLP-compliant test in rabbits.

Table B.6.2.5. Summary of the eye irritation studies with BASF 750 F

Method Guideline, GLP status, reference	**Test system**	**Test substance, dose levels, duration of exposure**	**Results**
EpiOcular eye irritation test Not guideline or GLP Report CA 5.2.5/1 Remmele, 2012b (2012/1367953)	2 EpiOcular tissue samples	50 µl bulk volume (approx. 15 mg) minimally moistened with water, exposed for 90 minutes followed by 18 hours' post-incubation period Purity 97.7 %	Tissue viability values were 81% of the negative control. Positive control (methyl acetate) resulted in tissue viability of 20% of negative control value. No eye irritation potential.
Bovine corneal opacity & permeability test (BCOP) OECD 437 Not GLP Report CA 5.2.5/2 Remmele 2012c (2012/1367954)	Three bovine corneas	750 µl of 20% solution in water, exposed for 4 hours Purity 97.7 %	Mean *in vitro* irritancy scores (IVIS): BAS 750 F = -0.4 ± 2.1 Negative control = 5.5 ± 2.5 Positive control (20% imidazole) = 118.3 ± 3.6 Histopathology did not reveal findings that indicated eye damage. No serious eye damage potential.
Acute eye irritation in rabbits OECD 405 (2002) GLP Report CA 5.2.5/3 [redacted] 2013a (2013/1150121)	Rabbit, New Zealand White, 3 (step-wise procedure)	0.1 ml bulk volume (approx. 38 mg) applied in one eye for 24 hours, followed by rinsing with tap water. Purity 98.8 %	Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0 for corneal opacity 0, 0, 0 for iris lesions 0.3, 0.3, 0.7 for redness of the conjunctiva 0, 0, 0 for conjunctival chemosis All reactions reversible within 72 hours after application. Not an eye irritant in accordance with CLP criteria.

Two *in vitro* pre-tests were performed to evaluate the potential for corrosion / severe eye damage and eye irritation before an *in vivo* test was undertaken.

In an EpiOcular™ test, tissue destruction was determined by measurement of the metabolic activity of the tissue after exposure/post-incubation with a colorimetric test (reduction of MTT to its formazan salt). In the system employed, a substance was considered to be irritant if the mean relative tissue viability with the test material was less than or equal to 50 % of the negative control value. On this basis, the mean relative tissue viability obtained with BAS 750 F (81 %) was concluded to indicate that there was not an eye irritation potential.

In the BCOP assay, corneal opacity was measured quantitatively as the amount of light transmission through the cornea. Permeability was measured quantitatively as the amount of sodium fluorescein dye that passed across the full thickness of the cornea. Both measurements were used to calculate an *in vitro* irritancy score (IVIS) of the test substance (= mean opacity value + (15 x mean OD490)), which is used for the prediction of serious eye damage. In addition, histological evaluation was performed. A substance was considered to represent a risk of serious damage to the eyes if the IVIS was > 55. On

this basis, it was concluded that BAS 750 F did not represent a risk of serious eye damage (IVIS = -0.4).

The follow-up *in vivo* study was conducted in a step-wise procedure: an initial animal was used to establish a potential for severe lesions, which was subsequently supplemented with two additional animals. In addition to the readings at 1, 24, 48 and 72 hours, an additional examination was performed at 24 and 48 hours with the installation of fluorescein. There were no signs of gross toxicity, adverse clinical signs or abnormal behaviour following administration of the test substance. Slight conjunctival redness (grade 1) was noted in all three animals at hours 1 and 24 after application and persisted in one animal up to hour 48. Slight conjunctival chemosis (grade 1) was noted in one out of three animals 1 hour after application. Slight discharge (grade 1) was noted in two out of three animals 1 hour after application. Additional findings, for example injected scleral vessels in a circumscribed area, were noted in all animals at hour 1 and persisted in two animals up to 24 hours. No corneal lesions were detectable even after instillation of fluorescein performed 24 and 48 hours after application. The mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for corneal opacity, iris lesions and conjunctival chemosis and 0.3, 0.3 and 0.7 for redness of the conjunctiva. The ocular reactions were fully reversible within 72 hours after application.

It is thus concluded that BAS 750 F is not an eye irritant under the conditions of these studies (please see CLH report).

B.6.2.6. Skin sensitisation

The skin sensitisation potential of the active substance has been investigated in a guinea-pig maximisation test. The applicant has provided additional information from products that contain BAS 750 F to support sub-categorisation in accordance with CLP.

Table B.6.2.6.1. Summary of the skin sensitisation studies with BAS 750 F

Method, Guideline, GLP status, reference	**Species, strain, sex, no/group**	**Test substance, dose levels, duration of exposure**	**Results**
Guinea-pig maximisation test (GPMT) OECD 406 (1992) GLP Report CA 5.2.6/1 [redacted] 2013a (2013/1150123)	Guinea pigs, Dunkin-Hartley, females, 5 in control group & 10 in test group	Intra-dermal induction: 5% in paraffin oil or 5% in Freund's complete adjuvant/0.9% aqueous NaCl Topical induction: 60% in paraffin oil Challenge: 50% in paraffin oil Purity 98.8%	Responses after challenge: **24h** / **48h** / **24 or 48h** Control: 0/5 / 0/5 / 0/5 Test: 2/10 / 6/10 / 6/10 All positive responses consisted of grade 1 erythema. Positive control (separate study) α-hexylcinnamaldehyde = 7/10

The induction and challenge concentrations of BAS 750 F employed in the available GPMT were determined from a series of pre-tests. In the pre-tests, paraffin oil showed satisfactory results as a vehicle for intra-dermal injection, whereas other commonly-used vehicles (1% aqueous CMC, polyethylene glycol 400) were demonstrated to be unsuitable. The maximum injectable concentration of BAS 750 F was 5% (w/w) suspension, and hence was used for the intra-dermal induction. Two animals pre-treated with the adjuvant were used to determine the minimal irritating concentration of BAS 750 F for the topical induction and the maximum non-irritating concentration for the challenge application. Based on the results of this pre-test, a concentration of 60% (w/w) was selected for topical induction and a concentration of 50% (w/w) for topical challenge.

In the main test, intra-dermal injection of 5% (w/w) BAS 750 F in the adjuvant mixture caused skin irritation (grade 1 to 2) and necrosis in the test and control groups. The sites of injections with only BAS 750 F or vehicle showed none or a slight erythema but without necrosis. Necrosis was observed during the topical induction phase at the sites of adjuvant administration in both groups, but not at the injection sites without the use of adjuvant, nor did these sites show discernible erythema.

None of the control animals responded with skin reactions during the challenge. In the test group, 6 of 10 animals showed skin reactions in the form of discrete or patchy erythema (grade 1); two of these animals additionally presented with papules 24 and/or 48 hours after the challenge. The challenge treatment with the vehicle alone did not cause skin reactions in any animals of the test group. As 60 % of animals gave a positive response in this adjuvant test, it is concluded that a skin sensitisation potential was demonstrated under the conditions of the study.

Under the criteria of the CLP regulation, it is possible to classify substances in sub-category 1B when ≥ 30 % animals in a GPMT respond at > 1 % intra-dermal induction concentration, as was the case in the presented study. Sub-category 1A is applied when ≥ 30 % animals in a GPMT respond at ≤ 0.1 % intra-dermal induction concentration, or ≥ 60 % respond at > 0.1 % to ≤ 1% intra-dermal induction concentration. The data from the available study allow one to conclude that classification in at least sub-category 1B is warranted; however, since an intra-dermal induction concentration below 0.1 % was not tested, it is not possible to exclude a classification in sub-category 1A. In this situation, the guidance on the application of the CLP criteria[1] recommends that the default position of classification in category 1 be adopted, i.e., without sub-categorisation.

In a position paper (Stinchcombe, 2016a; report number CA 5.2.6/3; 2016/1028946), the applicant has compiled local lymph node assay (LLNA) data from several products that contain BAS 750 F and two that contain structurally closely-related synthesis intermediates to support a classification of the active substance in sub-category 1B for skin sensitisation.

Information was provided on the results of LLNAs in which six products were tested. Three of these products, which contained 10% BAS 750 F, were only tested at concentrations of up to 5 % because of skin irritation; all gave negative results. The LLNA results with the remaining three products are presented in the table below.

Table B.6.2.6.2. Summary of LLNA EC3 values obtained with BAS 750 F-containing products

Product	**BASF 750 F content (%)**	**Maximum test conc. (%)**	**Skin sensitisation**	**EC 3 (%)**	
				Product	BAS 750 F (calculated)
BAS 752 00 F	10	25	Yes	19.6	1.96
BAS 750 02 F	40	50	No	> 50	> 20
BAS 751 01 F	20	50	Yes	29.8	5.96

Two synthesis intermediates that share the same molecular backbone as BAS 750 F gave EC 3 values in the range of 11.7 to 23.4 %.

The applicant has concluded that these data demonstrate that BAS 750 F is a weak or moderate skin sensitiser and should be classified in sub-category 1B. The RMS notes the variability in the calculated EC 3 values and the difficulty in interpreting these values when different products, with different co-formulants, have been tested. Furthermore, the guidance on the application of the CLP criteria cautions that the current test methods are based on the application of a maximised dose, which can only be obtained by the use of the substance itself and not diluted in a mixture. The RMS also has

[1] Guidance on the application of the CLP criteria, version 4.1, June 2015, ECHA.

doubts about the suitability of using data on products to assess the skin sensitisation potential of the active substance. Therefore, the RMS proposes that BAS 750 F be classified as a skin sensitiser Category 1 without sub-categorisation (please see CLH report).

B.6.2.7. Photo-toxicity

Since the extent of absorption by BAS 750 F in the wavelength range of natural sunlight (290-790 nm) exceeded the ultraviolet/visible molar extinction/absorption coefficient threshold of 10 $L \times mol^{-1} \times cm^{-1}$ specified in Regulation 283/2013, an *in vitro* neutral red uptake (NRU) photo-toxicity study in Balb/c 3T3 cells was performed.

Table B.6.2.7. Summary of photo-toxicity study on BAS 750 F

Method, Guideline, GLP status, reference	Test system	Test substance, dose levels, duration of exposure	Results
In vitro 3T3 NRU photo-toxicity test OECD 432 (2004) GLP Report CA 5.2.7/1 Cetto & Landsiedel, 2015a (2015/1117503)	Balb/c 3T3 cells, clone A31 6 replicates per concentration / irradiation group Irradiation groups irradiated for 50 minutes at UVA intensity of 5 J/cm^2	0.5, 1.0, 2.2, 4.6, 10.0, 21.5, 46.4, 100 µg/ml in dimethyl sulphoxide, exposed for 1 hour Purity 98.6%	Cytotoxicity observed in absence and presence of UV/VIS irradiation at 100 µg/ml. EC50 = 73 µg/ml without irradiation. EC50 = 69.8 µg/ml with irradiation. Photo-irritation factor (PIF) = 1.1 (no photo-toxic potential). Positive control (chlorpromazine) PIF = 102.1 (photo-toxic potential)

The concentrations to be employed in an *in vitro* photo-toxicity test were determined from a pre-test for toxicity. In this pre-test, eight concentrations that ranged from 4.6 to 1000 µg/ml were investigated.

On the basis of the pre-test results, the highest concentration in the main test was selected as 100 µg/ml. Seven further doses with a diluting factor of $3\sqrt{10}$ were used to detect a possible dose-response relationship. The Photo-Irritancy-Factor (PIF) prediction model was used to assess the photo-toxic potential of the substance. At 100 µg/ml, in the absence and presence of UV/VIS irradiation, test-substance precipitation in the culture medium was observed at the end of treatment, as were changes in cell morphology (also observed with the positive control substance at the three highest concentrations). Clear cytotoxic effects, indicated by neutral red absorbance values of below 50 % of vehicle control values, were observed in the absence and the presence of UV/VIS irradiation at 100 µg/ml. The PIF value obtained with BAS 750 F (1.1) was below the cut-off value at which no photo-toxic potential is predicted (≤ 2).

In conclusion, under the conditions of this study BAS 750 F was not photo-toxic.

B.6.3. SHORT-TERM REPEATED-EXPOSURE TOXICITY

The short-term toxicity of BAS 750 F has been investigated via the oral route in rats, mice and dogs (28-day and 90-day studies; additionally, a one-year study in dogs) and via the dermal route in rats (28 days). No short-term inhalation toxicity studies were performed because BAS 750 F is not volatile (vapour pressure at 20 °C: 3.2 x 10^{-6} Pa; at 25°C: 6.5 x 10^{-6} Pa; at 55 °C: 3.1 x 10^{-4} Pa) and is not used as a fumigant or aerosol.

The NOAELs presented below are those proposed by the RMS. Where it differs, the NOAEL proposed by the applicant has been presented in the discussion and conclusion of each study. In

addition, the RMS has performed Benchmark-dose (BMD) analysis to provide information on the reliability of the proposed NOAELs.

BMDL values represent a specified-effect level rather than a 'no effect' level. The BMD approach makes extended use of the available dose-response data and so is a scientifically more advanced method compared with the NOAEL for deriving a reference point. It also provides a quantification of the uncertainties in the dose-response data, thus increasing the transparency and robustness of the risk assessment. In contrast, the NOAEL approach is completely dependent upon the dose-spacing in each study. In 2009, the Scientific Committee[2] recommended that EFSA Scientific Panels and Units apply the BMD approach to chemicals in food (pesticides, additives, contaminants). This position has recently been re-affirmed: 'the Scientific Committee considers that the use of the BMD approach is always better than the NOAEL approach to define a reference point; therefore the application of this guidance document is unconditional for EFSA and is strongly recommended for all parties submitting assessments to EFSA for peer-review' (Hardy *et al*. (2017) [*The EFSA Journal* 2017; 15(1):4658]). The Scientific Committee (2009) concluded that 'health-based guidance values derived using the BMD approach can be expected to be as protective as those derived from the NOAEL approach, i.e. on average over a large number of risk assessments. Therefore the default values for uncertainty factors currently applied remain appropriate and there is no need for any additional uncertainty factor'.

The RMS has conducted BMD analysis with Proast version 62.9 or 63.5. These versions have implemented the recommendation in the Scientific Committee guidance (2017) that the Akaike Information Criterion (AIC) be used to characterise the relative goodness-of-fit of different mathematical models to a dose-response dataset. Model averaging, which is also recommended in the draft guidance, was not fully implemented in the version of Proast available at the time of this evaluation, but was used for the analysis of quantal data. The Proast software accepts all plausible models then presents the lowest BMDL and highest BMDU from all these models. Data for male and female animals were assessed as separate co-variates. Proast analyses differences between the co-variates and, if a single curve describes all the sub-groups combined, presents one set of results. This combined analysis has the advantage of increasing the statistical power of the modelling and hence of improving the precision of the estimate (a smaller confidence interval is obtained). Therefore, where one BMDL / BMDU value for both males and females is presented in the analyses below, the values for the co-variates were combined by the software. If males and females showed different sensitivities to the analysed effect, the analysis resulted in sub-group-specific confidence intervals; these are presented as separate values.

Software-generated BMD reports are provided in Annex II. The response levels were based on guidance given in *The EFSA Journal* (2009), 1150, 1-72 and Chemicals Regulation Directorate (2013)[3]. In this review the response level for relative liver-weight increases has been set to 15 %, based on an assessment of normal biological variation in organ weights (JMPR, 2015[4]); for more explanation of the rationale behind this, see the discussion in section B.6.3.5.

[2] *The EFSA Journal* (2009), 1150, 1-72.

[3] Chemicals Regulation Directorate, Health & Safety Executive, UK; Investigation of the state of the art on identification of appropriate reference points for the derivation of health-based guidance values (ADI, AOEL and AAOEL) for pesticides and on the derivation of uncertainty factors to be used in human risk assessment. Supporting Publications 2013:EN-413. [169 pp.]. Available online: www.efsa.europa.eu/publications

[4] WHO, 2015: Pesticide residues in food: WHO Core Assessment Group on Pesticide Residues. Guidance document for WHO monographers and reviewersWHO/HSE/GOS/2015.1, 1-106 pp.

Table B.6.3.1. Summary of short-term toxicity studies with BAS 750 F

Study **Purity**	**Species**	**Doses**	**NOAEL (mg/kg bw/d) / BMDL**	**Main effects**
28-day oral (dietary) OECD 407 (2008) GLP Purity 97.7% Report CA 5.3.1/1 [redacted] 2015a (2014 / 1170747) CA 5.3.1/2: [redacted] 2015a (2015 / 1249664)	Rat, Wistar 5/sex/group	0, 500, 1500, 4000 ppm Equivalent to Males: 0, 47, 135, 388 mg/kg/d Females: 0, 47, 138, 334 mg/kg/d	**NOAEL** 135 (1500 ppm) **$BMDL_{15}$** 147 (relative liver weight)	<u>500, 1500 ppm</u>: No adverse effects <u>4000 ppm:</u> ↓ bw gain (by ~ 30 %** in males & females) and food intake (females); final bw ↓ by 15 % & 9 % in males & females, respectively ↓ albumin (females, by 7 %**), ↓ total bilirubin (females, by 66 %*), ↑ cholesterol (females, by 75 %**) ↑ relative liver wt in females (by 23 %**), ↓ absolute kidney wt in males (by 12 %*) ↑ liver cell hypertrophy (males & females, minimal severity)
28-day oral (dietary) OECD 407 (2008) GLP Purity 95.5% Report CA 5.3.1/3 [redacted] 2014a (2013 / 1110704)	Mouse, C57BL/6 Rj 5/sex/group	0, 30, 100, 300, 1000 ppm Equivalent to Males: 0, 4.8, 15.5, 47.9, 128 mg/kg/d Females: 0, 5.8, 18.5, 61.0, 145 mg/kg/d	**NOAEL** 18.5 (100 ppm) **$BMDL_{15}$** 15 (liver weight)	<u>30 ppm:</u> ↑ relative liver wt (by 12 %* but within historical-control range) & liver cell hypertrophy in males (5/5) <u>100 ppm:</u> ↑ relative liver wt (by 18 %*), liver cell hypertrophy in males (5/5) <u>300 ppm:</u> ↑ relative liver wt (by 22 %* in males & 33 %* in females) & liver cell hypertrophy (5/5 for males & females) <u>1000 ppm:</u> ↓ body weight gain (overall by 65 % in females; weight loss in males) & food intake (males & females), ↓ final body weights (by 13 % males, 6 % in females), ↑ ovary weight (absolute & relative, 63-70 %) ↑ ALT (males*), ↓ cholesterol (males**), ↓ glucose (females**). ↓ albumin (females*); marked ↑ liver wt (> 70 %**), hypertrophy, liver cell necrosis, oval cell & bile duct hyperplasia (males & females)

Table B.6.3.1. Summary of short-term toxicity studies with BAS 750 F

Study Purity	**Species**	**Doses**	**NOAEL (mg/kg bw/d) / BMDL**	**Main effects**
28-day oral (capsule) OECD 407 GLP Purity 98.6% Report CA 5.3.1/4 [redacted] 2015a (2014 / 1170748)	Beagle dog (range-finding study) 3/sex/group	Males: Days 1-2: 300 or 1000 mg/kg bw/d Days 7-35/36: 125 or 250 mg/kg bw/d Females: Day 1: 300 or 500 mg/kg bw/d Days 3-29/30: 125 or 250 mg/kg bw/d	No NOAEL Not suitable for setting BMDL	<u>≥ 300 mg/kg bw/d:</u> Severe clinical signs in all dogs (males and females) <u>250 (reduced from 1000 /500) mg/kg bw/d</u> 2-3 dogs/sex with delayed food intake; isolated vomiting, single occurrence of unsteady gait and poor general condition ↓ bw gain (-0.5** / -0.9* kg in males/females on days 0 – 14; overall -0.3* / -0.7** kg males/females), ↓ cholesterol (by 45 %) 1 female with ↑ AST & ALT, ↓ terminal bw (-12%) ↑ liver wt (relative ≥ 31 %) with hypertrophy and eosinophilic change of hepatocytes <u>125 (reduced from 300) mg/kg bw/d</u> 1 female with delayed food intake 1 male with vomiting on 3 days ↓ bw gain (-0.3* kg males, days 0 - 14), ↓ cholesterol (by 43 %) ↓ terminal bw (-5 to -7%) ↑ liver wt (relative ≥ 25%) in males & females with hypertrophy and eosinophilic change of hepatocytes
90-day oral (dietary) OECD 408 GLP Purity 95.5% Report CA 5.3.2/1 [redacted] 2015b (2015 / 1198721)	Rat, Wistar 10/sex/group	0, 400, 1200, 3600 ppm Equivalent to: Males: 0, 27, 76, 256 mg/kg bw/d Females: 0, 30, 91, 314 mg/kg bw/d	**NOAEL** 76 (1200 ppm) **$BMDL_{10}$** 91 (body-weight gain)	<u>440, 1200 ppm:</u> No adverse effects <u>3600 ppm:</u> ↓ bw gain (males: -11%, females: -20%**) ↑ ALP (males** & females**), ↑ cholesterol* + ↓ albumin** (females) ↑ relative liver wt (males: +11%**, females: +13%**) ↑ minimal hepatocellular hypertrophy (males & females)

Table B.6.3.1. Summary of short-term toxicity studies with BAS 750 F

Study Purity	Species	Doses	NOAEL (mg/kg bw/d) / BMDL	Main effects
90-day oral (dietary) OECD 408 GLP Purity 98.8% CA 5.3.2/2: [redacted], 2015a (2014 / 1046542) CA 5.3.2/3 – plasma analysis: Becker & Kamp, 2014a (2014/1177165) CA 5.3.2/4 – amendment to plasma analysis to correct typing error: Becker, 2015a (2015 / 1240217)	C57BL/6 Rj mouse 15/sex/dose	0, 10, 50, 250, 750 ppm Males: 0, 2, 11, 58, 174 mg/kg bw/d Females: 0, 3, 15, 67, 211 mg/kg bw/d	**NOAEL** 11 (50 ppm) **$BMDL_{15}$** 14 (relative liver weight)	<u>10 ppm:</u> No adverse effects <u>50 ppm:</u> ↑ Hb, Ht in males** ↓ cholesterol in males** <u>250 ppm:</u> In males, ↑ Hb, Ht, MCH, RBC & platelet counts (evidence of haemoconcentration) ↓ cholesterol in males** & females**, ↓ albumin/globulin ratio in females* ↑ relative liver wt (males: +38%**, females: +26%**) & hypertrophy ↑ liver cell necrosis (grade 1) in 2/10 males & cytoplasmic alteration (grade 1) in 4/10 males <u>750 ppm:</u> ↓ bw gain (consistent in males**, transient in females) ↑ platelet, ↓ relative eosinophil counts in females ↓ albumin/globulin ratio in males* & females** ↑ relative liver wt (males: +87%**, females: +67%**) ↑ liver cell necrosis + cytoplasmic alteration in males & females
90-day oral capsule OECD 409 (1998) GLP Purity 98.6% Report CA 5.3.2/5 [redacted] 2015a (2015 / 1000530)	Beagle dog 5/sex/dose	0, 15, 90, 180 mg/kg bw/d	**NOAEL** 90 **$BMDL_{15}$** 18 (relative liver weight)	<u>15, 90 mg/kg bw/d:</u> No adverse effects <u>180 mg/kg bw/d:</u> 1 male and 3 females with vomiting & delayed food intake ↓ food intake (females) (max. -7%, day 7) ↓ bw gain (days 0-91 males: -49.4%*, females: -59.6%), ↑ alkaline phosphatase (3 months, males* & females*) ↓ protein (males: 6 weeks**, females: 6 weeks* & 3 months*); ↓ creatinine (females, 6 weeks)

Table B.6.3.1. Summary of short-term toxicity studies with BAS 750 F

Study Purity	**Species**	**Doses**	**NOAEL (mg/kg bw/d) / BMDL**	**Main effects**
				↑ relative liver weight (males: +20%**)
12-month oral capsule OECD 452 GLP Batch COD-001880 Purity 98.8% [redacted] 2016 (2016 / 1000645)	Beagle dog 5 / sex/ dose	0, 10, 30, 150 mg/kg bw/d	**NOAEL** 30	<u>10 mg/kd bw/d:</u> No treatment-related adverse effects <u>30 mg/kd bw/d:</u> ↑ liver weight in males (relative +11 %) and females (relative + 18 %) <u>150 mg/kg bw/d:</u> ↓ bw (up to -11.6 % on days 301 & 336) and bw gain (max. -126.7 % on days 0-7) in females ↑ alkaline phosphatase in males & females; ↓ AST in males ↓ total protein (males), albumin (males & females), calcium (males & females), creatinine (females) ↓ absolute lymphocyte counts (males at 3 months) ↑ absolute & relative liver weight in males (relative +33 % *) and females (relative +31 %) Centrilobular or diffuse hepatocellular hypertrophy in 5/5 males & 5/5 females
			$BMDL_{15}$ 11 (relative liver weight)	
28-day dermal OECD 410 GLP Purity 98.6% Report CA 5.3.3/1 [redacted] 2015b (2014/1170751)	Wistar rat 10/sex/dose	0, 100, 300, 1000 mg/kg bw/d 6 hours/day on 5 days/week for 4 weeks (males: 21 applications; females: 22 applications) in 0.5 % carboxymethyl cellulose in drinking water	**NOAEL** ≥ 1000	No adverse effects at any dose

bw = body weight; * = statistically significant, $p \leq 0.05$; ** = statistically significant, $p \leq 0.01$

B.6.3.1. Oral 28-day studies

B.6.3.1.1. 28-day rat study

A 28-day range-finding study has been conducted in rats [redacted] 2015a). An amendment to the original report was subsequently produced ([redacted], 2015a) to correct some historical control data that had been wrongly reported. Concentrations of 0, 500, 1500 and 4000 ppm were administered to groups of 5 rats/sex/group; these concentrations corresponded to intakes in males/females of 0/0, 47/47, 135/138 and 388/334 mg/kg/d, respectively.

One male rat of the high-dose group died during blood collection on day 29; this death was not considered to be a consequence of exposure to BAS 750 F. All other animals survived, and there were no clinical signs of toxicity. Mean body weights were consistently lower in high-dose male animals throughout the treatment period; the body weight decrease attained statistical significance from study day 7 until study day 28, with a maximum of -15% on study day 28. In the high-dose female animals, mean body weights were significantly lower on days 14 and 28, with a maximum of -8.7% on day 28. These changes were consistent with the lower body-weight gains of both males and females throughout the study (see table below). The RMS regards the changes in body weight and body-weight gain to be related to treatment and adverse. No significant effects on mean body weights were observed in male and female animals of the low- and mid-dose groups. Data on food consumption were available from one cage of 5 rats per dose group and sex. In females, the relative changes of food intake were consistently decreased at 4000 ppm (-27%, -16%, -5.4% and -31% on days 7, 14, 21 and 28); no other groups were affected. The applicant noted that recorded mean food intake from a group of five animals housed in one cage was much less sensitive to detect a substance-related effect than the body-weight data, which was recorded for each animal individually.

Table B6.3.1.1. Body weight development in 28-day rat study

	Males				Females			
Dose level [ppm]	**0**	**500**	**1500**	**4000**	**0**	**500**	**1500**	**4000**
Body weight [g]								
Day 0	158.8	158.9	159.1	157.8	129.5	129.8	132.5	131.6
Day 28	300.2	293.0	289.3	**265.5****	185.6	184.5	188.8	**169.4***
Change %		-2.4	-3.6	**-14.6**		-0.6	1.7	**-8.7**
Overall body weight gain (g)	141.4	134.1	130.2	**98.7****	56.1	24.8	56.3	**37.8****
Change %		-5.2	-7.9	**-30.2**		-2.4	0.4	**-32.6**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

Haematology and urinalysis parameters were unaffected by exposure to BAS 750 F. Some clinical chemistry parameters showed treatment-related, statistically significant changes only in females. Albumin was slightly decreased at 4000 ppm (by 7 %), as was total bilirubin (by 66 %); however, the latter was thought by the study authors to reflect increased metabolism and excretion of bilirubin that was secondary to the induction of xenobiotic-metabolising enzymes and hence not adverse. Cholesterol was slightly increased in females at 1500 ppm (by 30 %) and 4000 ppm (by 75 %), although at the mid-dose level the value obtained was within the historical control range; thus the change at 1500 ppm cannot be confidently attributed to BAS 750 F.

Table B6.3.1.2. Clinical chemistry parameters (selected)

		Males				Females			
Dose level [ppm]		**0**	**500**	**1500**	**4000**	**0**	**500**	**1500**	**4000**
Cholesterol	[mmol/l]	1.72	1.74	1.96	2.08	1.05	1.07	1.37*	**1.84****
						Historical control range: 0.95 - 1.81			
Albumin	[g/l]	37.77	37.32	37.38	37.77	39.19	39.36	38.37	**36.31****
Total bilirubin	[µmol/l]	1.58	1.31	1.06	0.93	1.14	1.02	0.91	**0.39***

Historical control data from the test facility: 49 studies 28-day treatment of Wistar rats (Dec-2007 - Jul-2012)

Statistically significant changes of organ weights comprised reduced absolute kidney weights in high-dose group males and increased relative liver weights in high-dose group females (see table below). The increase in the relative liver weight in high-dose females was associated only with minimal hepatocellular hypertrophy upon histopathology and some changes in clinical-chemistry parameters (decreased albumin, increased cholesterol), indicating a slight impairment of liver function rather than clear adversity. The 12 % decrease in the mean absolute kidney weights of male rats at 4000 ppm was consistent with the 11 % decrease in terminal body weight; the RMS therefore concludes that this finding was secondary to the body-weight effects and not indicative of specific renal toxicity.

Table B.6.3.1.3. Organ weight findings (statistically significant) in 28-day rat study

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	%	Relative weight [% of bw]	%	Absolute weight	%	Relative weight [% of bw]	%
Terminal wt [g]	0	272.54				168.94			
	500	266.64	-2			169.08	±0		
	1500	265.34	-3			172.96	+2		
	4000	241.65	-11			157.92	-7		
Kidneys (g)	0	2.072		0.761		1.326		0.785	
	500	2.002	-3	0.752	-1	1.416	+7	0.837	+7
	1500	2.052	-1	0.773	+2	1.408*	+6	0.814	+4
	4000	**1.815***	**-12**	0.752	-1	1.308	-3	0.827	+5
Liver (g)	0	7.350		2.693		4.460		2.644	
	500	7.304	-1	2.736	+1	4.822	+8	2.840	+7
	1500	7.492	+2	2.818	+5	4.936	+11	2.849	+8
	4000	6.875	-6	2.844	+6	5.116	+15	**3.241****	**+23**

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

No treatment-related adverse findings were detected at gross necropsy. In the control and 4000 ppm groups, histopathology was conducted on the adrenal glands, kidneys, liver, spleen and thyroid glands. Hepatocellular centrilobular hypertrophy of minimal severity (grade 1) was observed at 4000 ppm. All five of the females at this dose were affected, but only two of the males (the incidence was 0 in both control groups). Since the hypertrophy in the males was not associated with liver-weight increases or clinical chemistry changes, its relationship to treatment is questionable. No histopathological findings were detected upon examination of the adrenal cortex, the adrenal medulla, the spleen or the thyroid glands. All other findings occurred either individually or were equally distributed between control and treatment groups and are thus not treatment related.

Discussion and conclusion

In conclusion, therefore, dietary administration of BAS 750 F to Wistar rats for 28 days resulted in reduced body-weight gains in males and females at 4000 ppm, resulting in lower body weights at the

end of the study; reduced feed consumption was additionally recorded in females. Indications of slight impairment of liver function were reported in females, with increased relative weight, centrilobular hepatocellular hypertrophy of minimal severity and slight alterations in clinical pathology parameters (albumin and cholesterol) at 4000 ppm; however, no adverse findings were noted at histopathology. The proposed NOAEL is thus 1500 ppm (135 / 138 mg/kg bw/d in males / females), based on reduced body-weight gain in both sexes, reduced feed intake in female rats, and indications of slight impairment of liver function in females at the LOAEL of 4000 ppm (388 / 334 mg/kg bw/d in males / females).

To refine the assessment, BMD analysis was performed on the parameters relative liver weight, body weight on day 28 and overall body-weight gain ratio (day 28 / day 1, individual data). For the rationale behind the choice of a BMR of 15 % for the increase in relative liver weight, see section B.6.3.5.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Relative liver weight	15 %	Males Females	462.0 146.8	Inf 395.2	- 2.7
Body weight day 28	5 %	Males Females	154.9	335.0	2.2
Overall body weight gain	10 %	Males Females	266.4	366.6	1.4

For the change in body weight, the BMR was set at 5 %, because a response level of 10 % was too high to enable a calculation. Most of the BMDL values obtained had a low level of uncertainty, as reflected in the BMDU / BMDL ratio. Therefore, although this was a range-finding study with small group sizes, some confidence can be attached to the BMDL values obtained, and they support the proposed NOAEL.

B.6.3.1.2. 28-day mouse study

In a 28-day range-finding study, BAS 750 F was administered via the diet to groups of five male and five female C57BL/6 J Rj mice at concentrations of 0, 30, 100, 300 and 1000 ppm over a period of 4 weeks (corresponding to intakes in males / females of 0/0, 4.8/5.8, 15.5/18.5, 47.9/61.0 and 128/145 mg/kg bw/d, respectively). The applicant provided historical control data from the test facility with the same strain: eight studies conducted between 2010 and 2011 for the clinical pathology, and six studies conducted between 2010 and 2013 for the pathology.

There were no deaths or clinical signs of toxicity. The mean body weights of males and females in the high-dose group were statistically significantly decreased throughout the study. At this dose, mice of both sexes lost weight during the first treatment week, which they regained in week 2. Thereafter, body-weight development stagnated in males but was comparable to controls in the females. At the end of the treatment period, the mean weight of males of the 1000 ppm group was almost the same as on day 0, whilst females at this dose had increased their initial weight, although not by as much as the control females (see table below). The study authors concluded that these weight changes were treatment-related, but could also have been partly attributed to reduced palatability of the feed during the first treatment week. This supposition was possibly supported by a consistent decrease in food consumption in high-dose males and females throughout the study period, but particularly on day 7 in males (-42 %) and day 14 in females (-45 %).

Table B.6.3.1.4. Body weight and body weight gain in 28-day mouse study

	Males					Females				
Dose level [ppm]	**0**	**30**	**100**	**300**	**1000**	**0**	**30**	**100**	**300**	**1000**
Body weight [g]										
- Day 0	20.9	20.6	21.2	21.4	21.1	17.6	17.6	18.1	18.1	18.1
- Day 28	24.2	25.5	25.2	26.0	**21.0****	20.3	20.1	21.4*	20.3	**19.0***
% change		5.5	4.3	7.4	**-13.1**		-0.9	5.4	0.4	**-6.2**
Overall body weight gain (g)	3.3	4.9	4.0	4.6	**-0.1****	2.6	2.5	3.2	2.2	**0.9****
% change		47	21	37	**-103**		-6	22	-15	**-65**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

Haematology investigations did not reveal any treatment-related changes in red-blood-cell parameters. The relative monocyte count value in males of the 1000 ppm group was marginally above the historical-control range (see table below). Absolute and relative monocyte counts were also higher than controls in females of all dose groups, but without a dose-response relationship. In females at 1000 ppm, relative neutrophil counts were decreased. Although this relative parameter was below the historical-control range, the absolute neutrophil counts in this test group were not significantly changed and were within the historical control range. Overall, the white-blood-cell parameters provide no or at most weak evidence of a treatment-related effect.

Table B.6.3.1.5. Changes in white-blood-cell parameters in 28-day mouse study

	Males					Females				
Dose level [ppm]	**0**	**30**	**100**	**300**	**1000**	**0**	**30**	**100**	**300**	**1000**
Neutrophil counts abs. [giga/l]	0.43	0.32	0.43	0.56	0.30	0.42	0.52	0.44	0.35	0.34
						Historical control range:0.31 – 1.34				
Monocytes abs. [giga/l]	0.02	0.02	0.07	0.09**	0.11*	0.02	0.07**	0.18**	0.15**	0.17**
	Historical control range: 0.02 – 0.13									
Monocytes rel. [%	0.3	0.5	1.4**	1.1**	2.6*	0.5	1.8*	4.4**	3.9**	3.5**
	Historical control range: 0.4 – 2.5									
Neutrophil counts rel. [%]	7.4	7.7	9.0	7.2	7.3	11.9	14.2	8.6	9.2	6.0**
						Historical control range: 14.1 – 30.4				

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon (two-sided)

The only treatment-related changes in clinical chemistry were in alanine aminotransferase (ALT) and cholesterol in males, and albumin and glucose in females (see table below). Decreased cholesterol in males and decreased albumin in females was recorded from 30 / 100 ppm; however, since in each instance these were the only clinical chemistry finding noted in the low- and high-dose groups, the applicant regards these not to be adverse. The RMS notes that liver weights were increased at all doses but without associated histopathology below 1000 ppm (see below), and agrees that, by themselves, these changes in cholesterol (males) and albumin (females) do not indicate an adverse effect. Other clinical chemistry changes were either within the historical control ranges or did not show a dose-response relationship (data not shown).

Table B.6.3.1.6. Changes in clinical chemistry parameters in 28-day mouse study

		Males					**Females**				
Dose level [ppm]		**0**	**30**	**100**	**300**	**1000**	**0**	**30**	**100**	**300**	**1000**
ALT	[µkat/L]	0.83	0.81	0.97	1.14	**2.08***	1.15	1.03	1.22	1.36	2.67
		Historical control range: 1.12 - 2.29									
Cholesterol	[mmol/l]	2.53	2.01*	1.51**	1.24**	**0.69****	2.04	1.32**	0.87**	0.68**	0.83**
Albumin	[g/l]	31.17	31.33	28.63**	27.87**	31.31	31.85	30.64	28.67*	28.16*	**27.69***
Glucose	[mmol/l]	7.54	7.21	6.96	7.29	5.70	7.79	7.90	6.83	5.95	**5.25****
Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)											

Treatment-related, specific organ weight changes (i.e., not secondary to body-weight change) in the liver were recorded in males from 30 ppm and in females from 300 ppm (see table below). The relative weights were outside the historical-control range from 100 ppm in males and 300 ppm in females. Although there was a statistically significant increase in absolute liver weight in females at 100 ppm, the relative increase was not statistically significant and, moreover, was within the historical control range. In females at 1000 ppm, the thymus weight (absolute and relative) was increased to a level that was outside the historical-control range. The absolute and relative ovary weight was also statistically significantly decreased at this dose, although a clear dose-response relationship was not evident. Other changes in organ weights were either not dose-related or were secondary to decreases in the body weight (change in absolute values but no statistically significant change in relative values; data not shown).

Table B.6.3.1.7. Treatment-related, specific changes in organ weights in 28-day mouse study

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	%	Relative weight [% of bw]	%	Absolute weight	%	Relative weight [% of bw]	%
Terminal weight [g]	0	20.875				17.56			
	30	21.48	+3			17.68	+1		
	100	21.54	+3			18.22	+4		
	300	22.37	+7			17.44	-1		
	1000	**18.32***	**-12**			**16.66***	**-5**		
Ovaries (mg)	0					13.36		0.076	
	30					11.78	-12	0.067	-13
	100					9.88	-26	0.054	-28
	300					12.08	-10	0.069	-9
	1000					**9.62***	**-28**	**0.058***	**-24**
Liver (mg)	0	864.6		4.067		784.8		4.469	
	30	**978.8***	**+13**	**4.552***	**+12**	827.4	+5	**4.680****	**+5**
	100	**1037.0****	**+20**	**4.818***	**+18**	**944.6***	**+20**	5.185	+16
	300	**1110.6***	**+28**	**4.958***	**+22**	**1035.8****	**+32**	**5.939****	**+33**
	1000	**1276.2****	**+48**	**6.959***	**+71**	**1282.2****	**+63**	**7.696****	**+72**
	HCR	*854 - 1006*		*3.716 – 4.603*		685.0 – 856.2		4.172 – 5.447	
Thymus (mg)	0	32.84		0.157		36.40		0.207	
	30	38.12	+16	0.178	+13	43.88	+21	0.249	+20
	100	29.72	-10	0.138	-12	**48.22****	**+32**	**0.265***	**+28**
	300	40.34	+23	0.181	+15	**49.04****	**+35**	**0.281****	**+36**
	1000	31.52	-4	0.171	+9	**58.88****	**+62**	**0.354****	**+71**
	HCR					39.2 – 55.0		0.224 – 0.315	

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

At gross necropsy, a liver focus was identified in one male at 300 ppm and two males and two females at 1000 ppm. In the control and 1000 ppm groups, histopathology was conducted on the adrenal glands, kidneys, liver (all dose groups), spleen, thyroid glands and when changes at gross necropsy

were found. In the liver, cellular hypertrophy was observed in all treated males (centrilobular) and in all females at 300 and 1000 ppm (diffuse). At 1000 ppm, the hypertrophy was associated with multifocal hepatocellular necrosis, which correlated with foci observed macroscopically on the liver; this was in contrast to the liver focus observed at 300 ppm in one male, which was shown to reflect a peripheral fatty change that was not reported in the high-dose group and is thus regarded by the RMS to be incidental. Oval cell proliferation and bile-duct hyperplasia were reported in some animals at 1000 ppm (see table below). There were no histopathological findings in the adrenals (cortex and medulla), spleen, thymus, and thyroid in any of the animals. The ovaries were not examined by histopathology.

TableB.6.3.1.8. Liver histopathology in 28-day mouse study

	Males					Females				
Dose level [ppm]	**0**	**30**	**100**	**300**	**1000**	**0**	**30**	**100**	**300**	**1000**
No. of animals	5	5	5	5	5	5	5	5	5	5
LIVER										
examined	5	5	5	5	5	5	5	5	5	5
Infiltration, lymphoid, Grade 1	5	5	5	5	5	1	3	5	3	5
Hypertrophy, centrilobular		5	5	5	5					
Grade 1		5	1							
Grade 2			4	1						
Grade 3				4	5					
Hypertrophy, diffuse									5	5
Grade 1									4	
Grade 2									1	5
Necrosis, (multi)focal					4					3
Grade 1					3					2
Grade 2					1					1
Fatty change, peripheral Grade 3				1						
Fatty change, (multi)focal										1
Oval cell proliferation Grade 1					2			2		4
Bile duct hyperplasia Grade 1					1			1		5

Discussion and conclusion

In conclusion, dietary administration of BAS 750 F to mice for 28 days resulted in reduced body weights and body-weight gain, clinical chemistry changes, liver toxicity and increased ovary weight at 1000 ppm. The liver was identified as a target organ. Besides the histopathology (hepatocellular necrosis) at 1000 ppm, there were statistically-significant changes in relative liver weights in males at all doses, although the value at 30 ppm was within the historical-control range. In females, statistically significant increases in relative liver weights occurred from 300 ppm. Apart from hepatocellular hypertrophy, which is a morphological description and not in itself an indication of adversity (see discussion in section B.6.3.5), neither adverse liver histopathology findings nor clinical chemistry changes were observed at doses lower than 1000 ppm, apart from the gross observation of a focus on the liver of one male at 300 ppm. The proposed NOAEL value for males is 300 ppm (47.9 mg/kg bw/d, relative liver-weight increase 22 % at 1000 ppm) and for females is 100 ppm (18.5 mg/kg bw/d, respectively) (relative liver-weight increase at 300 ppm of 33 %).

The co-RMS (FR) considers that the dose of 30ppm (4.8/5.8 mg/kg bw/d) is a LOAEL, based upon the liver weight increase and associated histopathological and clinical chemistry changes at this dose.

The RMS has also undertaken BMD analyses of the increases in relative liver weight, final body weight and the incidences of liver foci and hepatocellular necrosis.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Relative liver weight	15 %	Males	14.5	30.2	2.1
		Females			
Final body weight	10 %	Males	97.5	124.2	1.3
		Females	144.8	179.6	1.2
Hepatocellular necrosis	10 %	Males	36.5	124.2	3.4
		Females			
Liver foci	10 %	Males	20.7	113	5.5
		Females			

The lowest BMDL value of 14.5 mg/kg/d for a 15 % increase in relative liver weight is consistent with the proposed NOAEL. Although this was a range-finding study, with small group sizes, the small values for the BMDU / BMDL ratios indicates that a low degree of uncertainty was associated with the results of the analysed parameters.

B.6.3.1.3. 28-day dog study

In a 28-day dose-range finding study, groups of three male dogs received a daily dose of 300 or 1000 mg/kg bw/d by oral capsule (control groups received empty capsules). After two days, treatment was interrupted for five days because of severe clinical signs in both dose groups (vomitus, impaired general condition, unsteady gait, reduced food intake) and then continued at the lower dose levels of 125 and 250 mg/kg bw/d until sacrifice on study day 35/36. Subsequently, groups of three female dogs received the test substance by daily oral capsule at dose levels of 300 and 500 mg/kg bw. As clinical signs similar to those in males were seen after administration of the first dose, treatment of the females was interrupted for two days and continued on study day 3 at the lower dose levels of 125 and 250 mg/kg bw/d until sacrifice on study day 29/30. For body weight, body-weight gain and food consumption, means and standard deviations of each test group were calculated and a statistical test was used to compare each group with the control group. For clinical pathology (hematology and clinical chemistry) and pathological examinations (organ weights), means and/or medians and standard deviations of each test group were calculated. However, because only three individuals per group and sex were used, statistical analysis was not performed and instead individual values were compared.

No animals died during the study. After the reduction of the dose levels, decreased food consumption occurred in all male and two female animals at 250 mg/kg/d bw/d on individual study days and also one female at 125 mg/kg bw/d. Vomiting was observed in one male at 125 mg/kg bw/d and three males at 250 mg/kg bw/d. The mean body weights of male and female dogs were reduced throughout the period of administration of both doses (see table below). In terms of body-weight gain, male dogs of both test groups lost weight during day 7-14. Thereafter, the dogs gained weight again, but the weight increase was generally lower than that of the control group dogs. Two of three female dogs at 250 mg/kg bw/d lost weight between days 0-14 and regained some of the weight in the second half of the study. The weight of the other female at this dose and of the three females at 125 mg/kg bw/d did not noticeably change throughout the study period.

Table B.6.3.1.9. Mean body weight in 28-day range-finding dog study

Dose level [mg/kg bw/d]	Mean body weight (kg) [% of ctrl]						
MALES	**Day -7**	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**
0	12.1	11.9	12.1	12.4	12.5	12.7	12.9
Test group 1 **300** (Day 0-1) **125** (Day 7-35)	11.7 [96]	11.8 [100]	12.0 [99]	11.6 [94]	11.9 [95]	12.0 [95]	12.1 [94]
Test group 2 **1000** (Day 0-1) **250** (Day 7-35)	11.4 [94]	11.6 [97]	11.6 [96]	11.1 [89]	11.3 [90]	11.4 [90]	**11.3*** **[87]**
FEMALES	**Day -7**	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	
0	10.7	10.8	11.0	11.1	11.4	11.3	
Test group 1 **300** (Day 0) **125** (Day 2-28)	10.2 [96]	10.4 [96]	10.4 [95]	10.2 **[92]**	10.4 **[91]**	10.3 **[91]**	
Test group 2 **500** (Day 0) **250** (Day 2-28)	10.7 [100]	10.7 [98]	10.5 [95]	9.8 **[88]**	10.1 **[89]**	10.0 **[88]**	

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

Table B.6.3.1.10. Body weight development in 28-day range-finding dog study (kg)

	Males			Females		
Test group	**0**	**1**	**2**	**0**	**1**	**2**
Dose level [mg/kg bw/d]	**0**	**300 / 125**	**1000 / 250**	**0**	**300 / 125**	**500 / 250**
Day 0 – 7	0.2	0.2	0.1	0.1	-0.1	**-0.2****
Day 0 – 14	0.5	-0.3*	**-0.5****	0.3	-0.2	**-0.9***
Day 0 – 21	0.6	0.1	-0.3	0.6	-0.1	**-0.5***
Day 0 – 28	0.8	0.2	**-0.2***	0.5	-0.2*	**-0.7****
Day 0 – 35	1.0	0.2	**-0.3***			

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

Six blood samples were taken from each dog on day 23 of the study, at the time points 0, 0.5, 1, 2, 4 and 8 hours post dosing for measurement of plasma concentrations of test substance. The plasma samples from the t=0 sampling time point were taken immediately before the administration of the daily capsule and therefore the corresponding plasma concentrations reflect the residual test substance burden from the previous administration period (day 0-22). Although intra-group variability was high, the mean plasma concentrations of BAS 750 F were very similar between dose groups; male dogs appeared to have slightly higher BAS 750 F plasma levels than females. Plasma concentrations started to increase about 1-2 hours after dosing. By 4-8 hours, the plasma concentration levels had usually reached a plateau or started to decrease (except for two dogs of the high-dose group that still showed clearly increased plasma levels at the last sampling time point of 8 hours post dosing). The overall mean plasma-level increase (= maximum – minimum plasma concentration) was slightly higher in males than in females (10531 vs. 6608 ng/mL).

There were no treatment-related effects on haematology parameters or adverse findings in the urinalysis parameters. Treatment-related changes in clinical-chemistry parameters were a decrease in cholesterol in males and females of both treatment groups and an increase in ALT in the high-dose-

group females, which in one animal was associated with an increase in aspartate aminotransferase (AST) that was outside the historical-control range (see table below).

Table B.6.3.1.11. Clinical chemistry parameters (selected) in 28-day range-finding dog study

	Males (day 30)			Females (day 28)		
Test group	**0**	**1**	**2**	**0**	**1**	**2**
Dose level [mg/kg bw/d]	**0**	**300 / 125**	**1000 / 250**	**0**	**300 / 125**	**500 / 250**
ALT [µkat/L]	0.58	0.78	1.02	1.05	0.99	1.20
AST [µkat/L]	0.58	0.55	0.48	0.57	0.56	0.82
Cholesterol [mmol/L]	4.57	2.59	2.58	4.16	2.40	2.30

At sacrifice, a comprehensive set of organs was weighed and assessed by gross necropsy. The absolute and relative (to body-weight) organ weights are given in the table below.

Table B.6.3.1.12. Organ weights in 28-day range-finding dog study

Sex		**Males (n=3)**				**Females (n=3)**			
Organ weight	**Group**	**Absolute weight**	**%**	**Relative weight [% of bw]**	**%**	**Absolute weight**	**%**	**Relative weight [% of bw]**	**%**
Terminal weight [kg]	0	12.90				11.40			
	1	12.00	-7			10.40	-9		
	2	**11.33**	**-12**			**10.03**	**-12**		
Adrenal glands (g)	0	1.323		0.010		1.150		0.010	
	1	1.307	-1	0.011	+6	1.107	-4	0.011	+5
	2	1.223	-8	0.011	+6	1.257	+9	**0.012**	**+23**
Brain (g)	0	89.34		0.692		81.61		0.717	
	1	87.21	-1	0.727	+5	75.83	-7	0.733	+2
	2	91.47	+2	**0.810**	**+17**	78.64	-4	**0.797**	**+11**
Epididymides (g)	0	2.883		0.022					
	1	2.697	-6	0.022	±0				
	2	**2.360**	**-18**	0.021	-6				
Heart (g)	0	97.7		0.757		87.79		0.771	
	1	98.8	+1	0.820	+8	88.50	+1	0.852	+10
	2	101.6	+3	**0.896**	**+18**	85.13	-3	0.848	+10
Kidneys (g)	0	59.64		0.461		49.39		0.436	
	1	57.01	-4	0.475	+3	46.34	-6	0.449	+3
	2	53.66	-10	0.475	+3	45.26	-8	0.453	+4
Liver (g)	0	388.6		3.014		347.3		3.056	
	1	**503.2**	**+29**	**4.195**	**+39**	**400.4**	**+15**	**3.833**	**+25**
	2	**465.6**	**+20**	**4.098**	**+36**	**403.4**	**+16**	**3.989**	**+31**
Ovaries (g)	0					0.833		0.007	
	1					0.760	-9	0.007	-1
	2					0.820	-2	0.008	+9
Pituitary (g)	0	92.00		0.001		67.33		0.001	
	1	85.67	-7	0.001	±0	63.00	-6	0.001	+1
	2	**79.33**	**-14**	0.001	-1	74.67	+11	**0.001**	**+23**
Prostate (g)	0	2.433		0.019					
	1	1.920	-21	0.016	-16				
	2	2.317	-5	**0.021**	**+12**				
Spleen (g)	0	24.27		0.188		23.82		0.209	
	1	21.88	-10	0.182	-3	23.61	-1	0.223	+7
	2	**20.34**	**-16**	0.179	-5	21.90	-8	0.219	+5
Testes (g)	0	16.89		0.131					
	1	15.81	-6	0.132	+1				

	2	**14.27**	**-16**	0.126	-3				
Thymus (g)	0	13.81		0.106		10.19		0.087	
	1	12.67	-8	0.108	+2	4.753	**-53**	**0.045**	**-49**
	2	**5.257**	**-62**	0.045	**-57**	4.833	**-53**	**0.049**	**-44**
Thyroid (g)	0	0.793		0.006		0.720		0.006	
	1	**1.173**	**+48**	0.010	**+58**	0.837	**+16**	**0.008**	**+29**
	2	**0.990**	**+25**	0.009	**+42**	0.807	**+12**	**0.008**	**+28**
Uterus (g)	0					6.123		0.056	
	1					1.803	**-71**	**0.018**	**-68**
	2					1.077	**-82**	**0.011**	**-81**

The absolute and relative liver weights in both treatment groups and sexes were above the respective historical control values and thus concluded to be treatment related. The absolute decrease of the testes and epididymides weights in males of the high-dose group was due to a single male, which showed lower weights of genital organs, including the prostate. These findings most probably reflected incomplete sexual maturity, as revealed in the histopathological pattern of testes (immature spermatids, slight presence of multinucleated giant cells and luminal debris, associated with slight multifocal degeneration of seminiferous tubules), epididymides (luminal debris and oligospermia) and prostate (immaturity, characterized by small size of acini and abundant interstitial stroma). Moreover, the relative weights of these organs were not changed compared with the control group; thus, they are not regarded as treatment related. The relative prostate weight of males in the high-dose group was increased because of one outlier, with an absolute prostate weight that was almost double the individual prostate weights of control animals. The other two animals in this group showed a decrease in prostate weight that was in line with the decrease in body weight; this is not, therefore, regarded as a treatment-related effect. Both treatment groups showed a marked decrease in absolute and relative uterus weights. This change was accentuated by one control female that had an absolute uterus weight (13.8 g) that was far in excess of the maximum historical control value (9.8 g); the remaining two control females had uterus weights of 2.17 and 2.4 g. Histopathological examination showed that neither controls nor females of the high-dose group had reached the first oestrus (lack of functional or old degraded corpora lutea). Furthermore, mammary glands were immature. In spite of this, a variability in sexual organ development was noted when examining ovaries, vagina, uterus and mammary gland of each female animal. The applicant therefore attributed the decreased uterus weights in females of both treatment groups to sexual immaturity. Notwithstanding, the RMS notes that all animals in the high-dose group and two of three animals in the low-dose group exhibited a decrease in uterus weight, which occurred with a dose-response relationship. The RMS therefore concludes that this was potentially a treatment-related effect, but notes that there were no pathology findings to explain the increase and so it is not a clear adverse effect. No histopathological changes were seen in the thymus, pituitary gland, spleen, thyroid and adrenal glands to explain their weight changes.

Upon gross necropsy, one male of the high-dose group had red foci in the liver (no histopathological correlates) and one female of the same treatment group had focal red discolouration in the jejunum (correlated with focal hyperaemi). Histopathology of the liver was performed for all dogs; the remaining tissues were histopathologically assessed in case of gross necropsy findings and for dogs of the control and high-dose group dogs. The only treatment-related histopathology findings were in the liver. Centrilobular hepatocellular hypertrophy was observed in the liver of all male dogs and in one female dog from each dose group. Additionally, eosinophilic change of minimal or slight severity was diagnosed in the livers of all treatment-group dogs. The eosinophilic change was characterised by a different cytoplasm in the treated animals compared with the more vacuolated appearance of control-animal hepatocytes that are characteristic of the cytoplasmic storage of glycogen.

Table B.6.3.1.13. Histopathology – liver findings in range-finding 28-day dog study

	Males (day 35)			Females (day 29)		
Test group	**0**	**1**	**2**	**0**	**1**	**2**
Dose level [mg/kg bw/d]	**0**	**300 / 125**	**1000 / 250**	**0**	**300 / 125**	**500 / 250**
No. of animals	3	3	3	3	3	3
Liver						
examined	3	3	3	3	3	3
Hypertrophy, hepatocellular	0	3	3	0	1	1
Grade 1 - minimal		3	2		1	1
Grade 2 - slight			1			
Eosinophilic change	0	3	3	0	3	3
Grade 1 - minimal		3	3		2	2
Grade 2 - slight					1	1

Conclusion

In a 28-day dose-range finding study, groups of three male dogs received a daily dose of 300 or 1000 mg/kg bw/d by oral capsule for two days, after which the doses were reduced to 125 and 250 mg/kg bw/d. Groups of three female dogs received the test substance by at dose levels of 300 / 125 and 500 / 250 mg/kg bw. Adverse effects were seen at both dose levels (clinical signs, decreased body weight and body-weight gain, increased relative liver weight with histopathology changes). Because only two dose groups with small numbers of animals were used, this study is not suitable for BMD analysis. A NOAEL was not identified.

B.6.3.2. Oral 90- day studies

B.6.3.2.1. 90-day rat study

BAS 750 F was administered to groups of 10 male and 10 female rats at dietary concentrations of 0, 400, 1200 and 3600 ppm for at least 90 days. The doses corresponded to time-weighted mean intakes of 27, 76 and 256 mg/kg bw/d in males and 30, 91, and 314 mg/kg bw/d in females.

No animals died during the treatment period and there were no clinical signs of toxicity. There were also no adverse findings in the functional observation battery or motor-activity measurements, which were conducted at the end of the treatment period (day 87-90). Mean body weights were consistently lower in high-dose male animals throughout the treatment period and in female animals from study day 35. Overall body-weight gains were reduced in males by 11 % (not statistically significant) and in females by 20 % (statistically significant). No effects on mean body weights or body-weight gains were observed in male and female animals of the low- and mid-dose groups. There were no treatment-related effects on food or water consumption in any group.

Table B.6.3.2.1. Body weight and body-weight gain in 90-day rat study

	Males				Females			
Dose level [ppm]	**0**	**400**	**1200**	**3600**	**0**	**400**	**1200**	**3600**
Body weight [g]								
Day 0	167.4	167.4	167.3	167.4	126.0	130.2	125.9	127.0
Day 91	395.2	409.4	395.1	369.6	233.9	228.9	225.6	**213.0****
% change		3.6	0.0	-6.5		-2.2	-3.6	-8.9
Overall body weight gain (g)	227.8	242.0	227.9	202.2	108.0	98.7	99.7	**86.0****
% change		6.2	0.0	-11.2		-8.6	-7.7	**-20.3**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

There were no treatment-related ophthalmoscopy findings (conducted prior to the start of administration and on day 91) or changes in haematology parameters (samples collected at the end of the treatment period). The only treatment-related clinical chemistry changes that differed from the historical control ranges were at 3600 ppm. These changes consisted of an increase in alkaline phosphatase in males and females and, in females only, an increase in cholesterol and decreases in albumin and total bilirubin. The decrease in total bilirubin was not accompanied by changes in red blood cell parameters and so was likely to have been secondary to increased metabolism via liver-enzyme induction, and hence adaptive rather than adverse. The other aforementioned clinical-chemistry changes were adverse. Other values that were different from controls were within the respective historical control ranges and/or did not show a dose-response relationship (data not shown). Serum samples were collected from non-fasted animals on day 31 with the intention of measuring levels of T3, T4 and TSH in the event of a possible effect on the pituitary-thyroid axis; however, since there were no histopathology findings in the thyroid glands, hormone measurements were not made.

Table B.6.3.2.2. Clinical chemistry parameters (selected) in 90-day rat study

	Males				Females			
Dose level [ppm]	**0**	**400**	**1200**	**3600**	**0**	**400**	**1200**	**3600**
ALP [µkat/l]	1.02	1.15	1.29*	1.76**	0.51	0.63	0.67*	0.78**
	Historical control: 0.91 – 1.45				*Historical control: 0.43 – 0.73*			
Cholesterol [mmol/l]	1.81	1.82	2.12	1.98	1.65	1.44	1.53	2.21*
Albumin [g/l]	38.43	38.25	37.50	38.24	42.88	40.36**	40.51**	38.90**
					Historical control: 39.50 – 43.65			
Total bilirubin [µmol/l]	1.57	1.52	1.43	0.97**	1.93	1.49	1.48	1.67

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)
Historical control data from test facility: 37 studies 90-day treatment of Wistar rats (Jul-2008 to Nov-2012)

Blood samples were collected on study days 24, 45 and 66 from non-fasted animals for the measurement of plasma concentrations of test substance. For a given dose level, a time-dependent decrease in the plasma concentration was observed in both sexes but to a greater extent in male rats. Independent of the sampling time-point, a three-fold increase in dietary concentration / test substance intake (i.e., from 400 to 1200 ppm or from 1200 to 3600 ppm) corresponded to a roughly five- to seven-fold increase of plasma concentration in males and to about an overall seven-fold increase in plasma concentration in females. Urinalysis measurements showed that, in high-dose male rats, incidences of transitional epithelial cells and granulated and epithelial cell casts in the urine sediment were higher compared with the controls, whereas the urine pH value in these individuals was lower (see table below). A reduced pH value of the urine in combination with granulated/epithelial cell casts in the urine sediment is frequently seen in rats suffering from alpha-2-urinary-globulinurea. Specific staining of kidney sections to detect alpha-2-urinary-globulin was performed (see histopathology results below).

Table B.6.3.2.3. Urinalysis findings in 90-day rat study

	Males				Females			
Dose level [ppm]	**0**	**400**	**1200**	**3600**	**0**	**400**	**1200**	**3600**
pH (Day 83)	6.7	6.5	6.5	6.2*	6.4	5.8	6.0	6.2
Transitional epithelial cells	1	1	1	2*	1	1	1	1
Casts	0	0	0	1*	0	0	0	0

Statistics: Wilcoxon test (1-sided), * $p \leq 0.05$; ** $p \leq 0.01$; mean severity: 1=few; 2=many

Statistically significant changes in the following organ weights compared with controls were recorded (see also table below): adrenal gland (absolute weight, males and females), heart (absolute and to some extent relative, males), kidney (absolute and relative, females), liver (relative, males and females), and thyroid (relative, females). The increase in relative liver weights in males from 1200 ppm and in females at 3600 ppm was treatment-related. The increase in relative thyroid weights in high-dose-group females was not accompanied by histopathological findings and was therefore not a clearly treatment-related adverse effect. The same was true of the decreases in absolute adrenal weights, since there was not a histopathological correlate and, additionally, the relative weights were not statistically significantly changed. Since the decreases in heart weight were not dose-dependently altered and did not show histopathological findings, they were unlikely to be treatment-related. The relative kidney weights were increased in all the treatment-group females but did not show a clear dose-response dependency, nor were there clear indications of kidney toxicity at histopathology; this finding therefore did not provide convincing evidence of a treatment-related adverse effect.

The brain, epididymides, ovaries, spleen, testes, thymus and uterus did not show statistically significant absolute or relative weight changes at any dose (data not shown).

Table B.6.3.2. 4. Organ weights (selected) in 90-day rat study

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	%	Relative weight [% of bw]	%	Absolute weight	%	Relative weight [% of bw]	%
Terminal weight [g]	0	372.54				218.68			
	400	386.13	(+4)			216.74	(-1)		
	1200	371.69	(±0)			211.60	(-3)		
	3600	348.14	(-7)			**200.50****	**(-8)**		
Adrenal glands (mg)	0	66.7		0.018		69.1		0.032	
	400	58.9	(-12)	0.015	(-15)	70.7	(+2)	0.033	(+3)
	1200	57.0	(-15)	0.015	(-15)	66.0	(-4)	0.031	(-2)
	3600	**53.5***	**(-20)**	0.015	(-15)	**53.8****	**(-12)**	0.027	(-16)
Heart (g)	0	1.088		0.293		0.716		0.328	
	400	1.017	(-7)	**0.264***	**(-10)**	0.692	(-3)	0.319	(-3)
	1200	1.084	(±0)	0.293	(±0)	0.659	(-8)	0.312	(-5)
	3600	**0.997***	**(-8)**	0.287	(-2)	0.679	(-5)	0.339	(+3)
Kidneys (g)	0	2.327		0.625		1.349		0.618	
	400	2.361	(+1)	0.612	(-2)	1.477	(+9)	**0.682****	**(+10)**
	1200	2.248	(-3)	0.607	(-3)	**1.509***	**(+12)**	**0.714****	**(+16)**
	3600	2.202	(-5)	0.634	(+1)	1.393	(+3)	**0.696****	**(+13)**
Liver (g)	0	8.366		2.248		5.386		2.468	
	400	8.920	(+7)	2.310	(+3)	5.100	(-5)	2.359	(-4)
	1200	8.842	(+6)	**2.376***	**(+6)**	5.369	(±0)	2.541	(+3)
	3600	8.702	(+4)	**2.502****	**(+11)**	5.570	(+3)	**2.779****	**(+13)**
Thyroid (mg)	0	25.2		0.007		19.4		0.009	
	400	26.7	(+6)	0.007	(+3)	21.8	(+12)	0.010	(+13)
	1200	26.3	(+4)	0.007	(+5)	21.7	(+12)	0.010	(+16)
	3600	26.1	(+4)	0.008	(+11)	22.4	(+15)	**0.011***	**(+26)**

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Gross pathology was conducted on animals of all groups. There were no treatment-related gross pathology findings. Histopathology was performed on the adrenals, kidneys, liver, spleen and thyroid / parathyroid of all treatment groups; other organs were examined by histopathology from the control and high-dose groups only. The main histopathology finding was in the liver, where centrilobular hepatocellular hypertrophy (grade 1 – minimal) was observed from 1200 ppm in males and at 3600 ppm in females (see table below). The hypertrophy at 1200 ppm in males was not adverse, since

there were no changes in the clinical chemistry at that dose that indicated liver-cell dysfunction. The only dose-related finding was a small number of mid- and high-dose-group females with basophilic tubules of the kidney, a finding that is classed as a miscellaneous change, i.e., not associated with inflammation, regeneration, degeneration or cell death. It should also be noted that the incidence in the control males was higher than that in the mid- and high-dose females. The study authors also noted that basophilic tubules are a frequent finding in control animals and concluded that the minimal grade and unilateral occurrence in most animals of the current study indicated that they were a spontaneous finding. All other findings occurred either individually or were biologically equally distributed over control and treatment groups and so were incidental. In particular, there were no treatment-related findings in the adrenals, heart or thyroid glands. The special stains performed on kidney sections of male animals to detect tubular protein and alpha-2-urinary-globulin did not reveal differences between control and treated males. There were no findings in the nervous system (peripheral nerves and spinal cord).

Table B.6.3.2.5. Histopathology findings in 90-day rat study

	Males				Females			
Dose level [ppm]	**0**	**400**	**1200**	**3600**	**0**	**400**	**1200**	**3600**
No. of animals	10	10	10	10	10	10	10	10
LIVER								
examined	10	10	10	10	10	10	10	10
Necrosis, (multi)focal								1
Infiltration, lymphoid	10	10	10	10	10	10	10	10
Hypertrophy, hepatocellular			3	8				3
Grade 1			3	8				3
ADRENAL CORTEX								
examined	10	10	10	10	10	9	10	10
Vacuolation, zona fasciculata		1						
Ectopic bone						1		
HEART								
examined	10			10	10			10
Necrosis / fibrosis	3	-	-	4		-	-	
KIDNEYS								
examined	10	10	10	10	10	10	10	10
Tubules, basophilic	4	3	1	1			2	3
Mineralisation, medulla					10	10	10	10
Dilation, renal pelvis		1						
Eosinophilic droplets	10			10				
THYROID GLANDS								
examined	10	10	10	10	10	10	10	10
Hypertrophy / hyperplasia, follicular cell		1		1				
Altered colloid		1		1			1	

Discussion and conclusion

In conclusion, oral administration of BAS 750 F to Wistar rats for 90 days resulted in statistically significantly reduced body-weight gain and final body weight in females of the high-dose group (3600 ppm). Also at this dose, increased relative liver weights in males and females, minimal hepatocellular hypertrophy and changes in clinical-chemistry parameters indicated that there was a slight dysregulation of liver-cell function. There were no adverse findings in the mid- and low-dose groups (400 and 1200 ppm, respectively). The NOAEL was therefore 1200 ppm (76 / 91 mg/kg/d) and the LOAEL was 3600 ppm (256 / 314 mg/kg/d).

To refine the assessment, BMD analysis was performed on the relative liver weight, final body weight and overall body-weight gain.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Relative liver weight	15 %	Males Females	263.5	527.2	2.0
Body weight day 90	10 %	Males Females	241	1144.8	4.75
Overall body-weight gain	10 %	Males Females	91.4	272.6	3.0

The lowest value, a $BMDL_{10}$ for overall body-weight gain of 91.4 mg/kg/ bw/d, was consistent with the proposed NOAEL.

B.6.3.2.2. 90-day mouse study

BAS 750 F was administered to C57BL/6JRj mice at dietary concentrations of 0, 10, 50, 250 and 750 ppm for at least 90 days. The doses corresponded to time-weighted mean intakes of 2, 11, 58 and 174 mg/kg bw/d in males and 3, 15, 67 and 211 mg/kg bw/d in females. The main study comprised 10 animals/sex/group, whilst 5/sex/group were used as satellite animals for the collection of blood samples on days 21, 42 and 63 for plasma analysis of the parent compound. The main purpose of the study was to inform on dose selection for an 18-month mouse carcinogenicity study and thus it did not adhere strictly to the OECD guideline in some minor respects (for example, histopathology was not performed on the satellite animals; there were no urinalysis, ophthalmoscopy, sensory reactivity, grip strength, motor activity or functional observation battery measurements); however, since the study was in excess of the standard data requirements, this does not represent a deficiency or potential data gap.

No animal from any of the main groups died prematurely during the study. However, four satellite females given 750 ppm died on day 21 during isoflurane anesthesia prior to blood sampling. The cause of death could not be ascertained. No clinical signs of toxicity were noted during the observation period.

No statistically significant differences were obtained for absolute body weights of males in any test group. For body weight gain, lower values were obtained throughout treatment at 750 ppm in males, leading to a final body-weight gain that was 32 % lower than the controls. In males at 250 ppm, the overall body-weight gain was lower than the controls, but without statistical significance. In females at 750 ppm, statistically significantly lower absolute body weights were observed in weeks 2 to 5 and week 11, but by the end of the study they were only marginally (not statistically significantly) lower than the controls. No clear changes in food consumption were noted in males. In females of the mid- and high-dose groups, there was a tendency towards lower values for absolute and relative food consumption in the second half of the treatment phase, but without a clear dose-response relationship.

Table B.6.3.2.6. Body weight and body weight gain in 90-day mouse study

	Males					Females				
Dose level [ppm]	**0**	**10**	**50**	**250**	**750**	**0**	**10**	**50**	**250**	**750**
Body weight [g]										
Day 1	21	22	21	21	22	18	17	17	17	17
Day 91	28	28	29	27	27	23	23	23	23	22
% change (compared with control)		±0	+4	-4	-5		±0	±0	±0	-4
Overall body weight gain (%, compared with day 1)	34	28	33	31	**23****	31	29	35	32	31
% change (compared with control)		-18	-3	-9	**-32**		-6	+12	+3	±0

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

Haematology changes were recorded in the following parameters in males: platelet counts, red blood cells (RBC), haemoglobin level (Hb), haematocrit (Ht) and mean corpuscular haemoglobin (MCH); these were considered by the study authors to be related to treatment and adverse. At 750 ppm, samples from only two males were available to measure red blood cell counts, Hb, Ht and MCH, because, in the remaining eight animals, there was clotting of EDTA blood samples, insufficient sample volume or technical errors. The changes in the red blood cell parameters in combination with increased platelet counts at 250 ppm were considered by the study authors to be indicative of haemoconcentration, although there were no signs of haemoconcentration in either the 28-day or 18-month carcinogenicity study at any dose. Haemoconcentration is normally caused by decreased water consumption and/or stress. Water consumption and urine volume were not quantified in this study, and adrenal cortex hypertrophy, which might have indicated a stress-related response, was not observed in the affected animals. In neither this study nor the 18-month mouse carcinogenicity study were there histopathological changes in the bone marrow or in the red pulpa of the spleen to indicate that there was an increased production of red blood cells (polycythaemia); furthermore, haemoconcentration was not observed in the mouse carcinogenicity study.

The study authors considered the effects at 50 ppm (4 % increase of haemoglobin and 5 % increase of haematocrit compared to controls) to be potentially adverse in view of a possible association with haemoconcentration. The applicant considered that 1) since the blood cell counts (red and white blood cells and platelet counts) were not statistically significantly changed at 50 ppm; 2) in the absence of a dose-related increase in any haematological parameter in females; 3) in the absence of polycythemia as an explanation for the observation; and 4) given other more plausible explanations for the observation (decreased water consumption, stress response), the slight increase of Hb and Ht values compared were treatment-related in view of a possible dose-response relationship, but not sufficiently severe or robust to be considered adverse.

In females, there were changes in platelet counts and relative eosinophil counts at 750 ppm. Other changes in haematological parameters in male and female mice did not show a dose-related trend, and therefore these alterations were considered to be unrelated to treatment.

Table B.6.3.2.7. Selected haematology findings in 90-day mouse study

		Males					Females				
Dose level [ppm]		**0**	**10**	**50**	**250**	**750**	**0**	**10**	**50**	**250**	**750**
RBC	[10^{12}/l]	9.78 [6]	9.95 [6]	10.12 [8]	**10.23*** [9]	10.07 [2]	9.68 [9]	**10.13*** [10]	10.05 [10]	**10.48**** [10]	9.72 [6]
Haemoglobin	[mmol/l]	9.1 [6]	9.3 [6]	**9.5**** [8]	**9.8**** [9]	9.2 [2]	9.3 [9]	9.4 [10]	9.3 [10]	**9.7*** [10]	9.0 [6]
Haematocrit	[l/l]	0.455 [6]	0.468 [7]	**0.480**** [8]	**0.484**** [9]	0.471 [2]	0.457 [9]	**0.475*** [10]	0.468 [10]	**0.484**** [10]	0.458 [6]
RDW	[%]	13.4 [5]	13.5 [7]	**12.9*** [8]	13.1 [9]	13.4 [2]	13.8 [9]	13.5 [10]	13.5 [10]	**12.8**** [10]	**13.2**** [6]
Reticulocytes	[% RBC]	1.7 [5]	**2.7**$^{++}$ [7]	**2.4**$^{+}$ [8]	2.1 [9]	2.1 [2]	2.3 [9]	**2.6**$^{+}$ [10]	**2.5**$^{+}$ [10]	2.2 [10]	2.2 [6]
MCH	[fmol]	0.93 [6]	0.93 [6]	0.94 [8]	**0.96**** [9]	0.92 [2]	0.96 [9]	**0.93*** [10]	**0.92**** [10]	**0.93*** [10]	**0.93*** [6]
MCHC	[mmol/l]	19.95 [6]	19.76 [6]	19.77 [8]	20.32 [9]	19.60 [2]	20.28 [9]	**19.74*** [10]	19.85 [10]	20.00 [10]	**19.64**** [6]
MCV	[fl]	46.6 [6]	47.0 [7]	47.4 [8]	47.3 [9]	46.7 [2]	47.2 [9]	46.9 [10]	46.6 [10]	**46.2*** [10]	47.1 [6]
Platelets	[10^{9}/l]	1260 [7]	1234 [7]	1313 [8]	**1511**** [10]	**1579**** [4]	1115 [10]	**1249**** [10]	1205 [10]	1188 [10]	**1257**** [6]
WBC	[10^{9}/l]	4.1 [7]	5.7 [7]	5.5 [8]	**8.3**** [10]	3.7 [4]	3.5 [10]	4.9 [10]	**6.1**** [10]	**8.7**** [10]	5.2 [6]
Eosinophils	[% WBC]	1.4 [7]	1.5 [7]	1.7 [8]	2.3 [10]	2.3 [5]	3.1 [10]	2.4 [10]	2.2 [10]	2.3 [10]	**1.5**$^{+}$ [7]

$^{+}$/$^{++}$ Steel-test significant at 5% (+) or 1% (++) level
*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level
[n] = number of samples that could be evaluated

Statistically significant, treatment-related changes in clinical-chemistry parameters were recorded in alkaline phosphatase in males at 750 ppm, albumin / globulin ratio in males and females at 750 ppm and cholesterol at ≥ 10 ppm. At lower dose levels (< 250 ppm), significant clinical chemistry changes were confined to a reduction of cholesterol in both males and females and hence, in isolation, this finding is not considered to be adverse. The group mean value for albumin/globulin ratio in females at 250 ppm was statistically significantly different from controls but identical to controls. When an average was calculated as a median (1.4 in controls, 1.35 at 250 ppm) or mode (1.4 in controls, 1.3 at 250 ppm), a downwards trend in the ratio was shown. At 750 ppm, increased ALP levels (males only), reduced albumin/globulin ratio and reduced cholesterol indicated possible liver toxicity manifested as impaired liver function in males and females. Albumin values were reduced in all male treatment groups and from 50 ppm in females, but in the absence of a clear dose-response relationship its toxicological relevance is unclear and so the RMS does not regard it as a definite adverse effect. Other statistically significant changes of clinical-chemistry parameters did not show a dose-related trend, and therefore were concluded not to be treatment related.

Table B.6.3.2.8. Clinical chemistry parameters (with statistically significant change, treatment related) in 90-day mouse study

		Males					Females				
Dose level [ppm]		**0**	**10**	**50**	**250**	**750**	**0**	**10**	**50**	**250**	**750**
ALP	[µkat/l]	80	71*	69**	77	**100****	117	111	110	113	104
Cholesterol	[mmol/l]	2.36	2.09**	1.63**	**1.33****	**1.03****	2.03	1.76**	1.63**	**1.01****	**1.01****
Total protein	[g/l]	52.0	49.0**	48.5**	46.8**	49.3**	49.7	48.5	47.1**	45.1**	47.6*
Total globulin	[g/l]	22.5	21.2	21.1	20.7	22.3	20.5	19.6	19.5^{+}	19.1^{+}	21.3
Albumin	[g/l]	29.6	27.8**	27.5**	26.1**	27.0**	29.2	28.9	27.6**	26.0**	26.3**
Alb / Glob ratio		1.3	1.3	1.3	1.3	$\mathbf{1.2}^{+}$	1.4	1.5	1.4	$\mathbf{1.4}^{+}$	$\mathbf{1.2}^{++}$

$^{+}/^{++}$ Steel-test significant at 5% (+) or 1% (++) level
*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

During blood sample collection from the satellite animals for plasma analysis of BAS 750 F, four of the five male animals at 750 ppm died on day 21 during the sampling procedure. Therefore, on this day only two animals could be sampled. Subsequently, a further male died so that on sampling days 42 and 63, blood from only one animal was available for BAS 750 F measurement. For a given dose level, there were no clear differences in the mean plasma concentration at the three sampling time points. Generally, plasma concentrations were marginally higher in males than in females (roughly about 1.5-fold) at a given dietary concentration, but very similar if correcting for test substance intake. Independent of sampling time point, a five-fold increase in dietary concentration / test substance intake (i.e. from 10 to 50 ppm or from 50 to 250 ppm) corresponded to a roughly three- to four-fold increase of plasma concentration in both sexes. The three-fold increase in diet concentration from 250 to 750 ppm corresponded to an approximately 1.5-fold increase in female plasma concentration, possibly indicating saturated absorption in the high-dose group.

Organ-weight measurements revealed statistically-significant alterations of absolute and relative liver weights, which were considered to be treatment related and organ specific (i.e., not secondary to body weight change) in males at doses ≥ 50 ppm and in females at doses ≥ 250 ppm. The absolute and relative adrenal weights of high-dose males were also statistically significantly increased, but in the absence of histopathology findings, the adversity and toxicological relevance of this finding is not clear. Also in males, the kidney weight at 250 ppm and 750 ppm was decreased, but without a dose-related relationship when adjusted for body weight and without histopathological findings; this finding is thus concluded not to be a treatment-related adverse effect. A very slight reduction in absolute brain weight in females at 750 ppm did not translate to a change in relative weight; furthermore, as there were no histopathological changes, the RMS does not regard this isolated finding to be a treatment-related effect. Spleen absolute and relative weights were reduced in females at 250 and 750 ppm but without corresponding histopathological findings, and so do not represent a clear adverse effect.

There were no changes in the weights of testes, epididymides, uterus or ovaries.

Table B.6.3.2.9. Organ weights (selected) in 90-day mouse study

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	%	Relative weight [% of bw]	%	Absolute weight	%	Relative weight [% of bw]	%
Terminal weight [g]	0	26.9				22.6			
	10	26.5	(-1)			22.6	(±0)		
	50	27.2	(+1)			23.2	(+3)		
	250	26.5	(-1)			22.2	(-2)		
	750	25.9	(-4)			21.9	(-3)		
Adrenal glands (mg)	0	4.1		0.0154		100		0.0444	
	10	5.1	(+24)	0.0194	(+26)	98	(-2)	0.0435	(-2)
	50	4.9	(+20)	0.0181	(+18)	110	(+10)	0.0473	(+7)
	250	5.5	(+34)	0.0209	(+36)	98	(-2)	0.0441	(-1)
	750	**6.0***	**(+46)**	**0.0232****	**(+51)**	107	(+7)	0.0489	(+10)
Brain (mg)	0	431		1.604		447		1.977	
	10	432	(±0)	1.633	(+2)	447	(±0)	1.980	(±0)
	50	443	(+3)	1.633	(+2)	442	(-1)	1.917	(-3)
	250	435	(+1)	1.642	(+2)	442	(-1)	1.996	(+1)
	750	434	(+1)	1.677	(+5)	431*	(-4)	1.973	(±0)
Kidneys (mg)	0	358		1.333		275		1.217	
	10	344	(-4)	1.299	(-3)	272	(-1)	1.203	(-1)
	50	361	(+1)	1.327	(±0)	257	(-7)	1.120	(-8)
	250	320*	(-11)	1.208*	(-9)	257	(-7)	1.157	(-5)
	750	317**	(-11)	1.224	(-8)	259	(-6)	1.180	(-3)
Liver (g)	0	1.19		4.45		1.10		4.87	
	10	1.24	(+4)	4.69	(+5)	1.09	(-1)	4.81	(-1)
	50	**1.38****	**(+16)**	**5.09****	**(+14)**	1.16	(+5)	5.03	(+3)
	250	**1.62****	**(+36)**	**6.12****	**(+38)**	**1.36****	**(+24)**	**6.12****	**(+26)**
	750	**2.16****	**(+82)**	**8.34****	**(+87)**	**1.78****	**(+62)**	**8.12****	**(+67)**
Spleen (mg)	0	61		0.228		81		0.359	
	10	62	(+2)	0.235	(+3)	78	(-4)	0.345	(-4)
	50	65	(+7)	0.239	(+5)	77	(-5)	0.334	(-7)
	250	63	(+3)	0.237	(+4)	**66****	(-19)	**0.296****	(-18)
	750	55	(-10)	0.211	(-7)	**62****	(-23)	**0.282****	(-21)

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two sided)

Gross pathology was performed on animals of all groups and did not reveal any treatment-related alterations. Histopathology was performed on the control and high-dose groups; additionally, the liver and thyroid/parathyroid was examined histopathologically in all groups. The liver was identified as the only target organ upon histopathology. Hepatocellular hypertrophy was observed in males at doses of ≥ 50 ppm in a centrilobular pattern and in a diffuse pattern in females at doses of ≥ 250 ppm. The hypertrophy correlated in both sexes with the observed weight increases. At 250 ppm in males and 750 ppm in both sexes, the hypertrophy was associated with hepatocellular necrosis and/or cytoplasmic alteration. The latter was observed in hypertrophied hepatocytes and was characterised by numerous hyaline inclusions and concentric whorls of cell organelles, and was interpreted by the study authors as an early degenerative change. The centrilobular hepatocellular hypertrophy of minimal-grade severity in males at 50 ppm was regarded by the study authors to be an adaptive response.

There were no treatment-related adverse histopathology findings in other organs, including the testes, epididimydes, prostate, uterus, ovaries, brain, kidney, spleen or thyroid / parathyroid.

Table B.6.3.2.10. Histopathology findings in 90-day mouse study

	Males					Females				
Dose level [ppm]	**0**	**10**	**50**	**250**	**750**	**0**	**10**	**50**	**250**	**750**
No. of animals	10	10	10	10	10	10	10	10	10	10
LIVER										
examined	10	10	10	10	10	10	10	10	10	10
Infiltration, lymphocytic	9	9	7	10	8	9	8	10	8	9
Grade 1	7	8	6	8	7	7	7	8	5	4
Grade 2	2	1	1	2	1	2	1	2	2	5
Grade 3									1	
Mean grade/tissue affected	*1.2*	*1.1*	*1.1*	*1.2*	*1.1*	*1.2*	*1.1*	*1.2*	*1.5*	*1.6*
Hypertrophy, centrilobular			8	10	10					
Grade 1			8							
Grade 2				4						
Grade 3				6	8					
Grade 4					2					
Mean grade/tissue affected			*1.0*	*2.6*	*3.2*					
Hypertrophy, diffuse									10	10
Grade 1									9	1
Grade 2									1	9
Mean grade/tissue affected									*1.1*	*1.9*
Cytoplasmic alteration				4	5					2
Grade 1				4	3					1
Grade 2					2					1
Mean grade/tissue affected				*1.0*	*1.4*					*1.5*
Necrosis, single cell				2	8					
Grade 1				2	7					
Grade 2					1					
Mean grade/tissue affected				*1.0*	*1.1*					
Necrosis, (multi)focal										6
Grade 1										2
Grade 2										4
Mean grade/tissue affected										*1.7*
Fatty change									*2*	

Severity grades: 1 = minimal; 2 = slight; 3 = moderate; 4 = marked

Discussion and conclusion

In conclusion, administration of BAS 750 F to C57BL/6JRj mice at the top dose level of 750 ppm caused reductions in body weight gain and clear signs of hepatotoxicity in both sexes, including changes in several clinical chemistry parameters, pronounced liver weight increases with associated hepatocellular hypertrophy, liver cell necrosis and degenerative hepatocellular (cytoplasmic) changes.

At 250 ppm (58 mg/kg bw/d), degenerative liver changes of minimal severity were observed in male mice, together with marked increases in liver weight and increased severity of hepatocellular hypertrophy. In females (67 mg/kg bw/d), liver-weight increases were statistically significant and hepatocellular hypertrophy was evident, albeit of only minimal severity. The toxicological relevance of increased levels of red blood cell parameters (number of red blood cells/litre, haemoglobin, haematocrit), and platelets in male mice at 250 ppm was not clear and may have been caused by decreased water consumption or a result of treatment-related stress. For setting the doses for the mouse carcinogenicity study, the study authors considered that the maximum tolerated dose (MTD) was exceeded at 250 ppm in male mice and was approached at 250 ppm in female mice.

At the dose level of 50 ppm, very slight increases in haemoglobin and haematocrit and a reduction in cholesterol were observed in male mice and were considered to be treatment-related. However, since the haematology changes at this dose were ≤ 5 % and did not occur in females, the RMS does not consider them convincing evidence of an adverse effect. Additional treatment-related findings in male mice at 50 ppm comprised slight but statistically significantly increased liver weights (by 14 %) with associated liver-cell hypertrophy of minimal severity in 8 of 10 males, changes which the applicant considered to be adaptive. In females at 50 ppm, treatment-related effects were confined to slightly reduced cholesterol levels only, which in isolation are not regarded as adverse by the RMS. The dose level of 50 ppm was therefore the NOAEL in males and females, corresponding to intakes of 11 mg/kg bw/d in males and 15 mg/kg bw/d in female mice. At 10 ppm, the only treatment-related finding was a slight decrease in cholesterol in males and females, which in isolation the RMS does not consider to be adverse.

The co-RMS (FR) considers the NOAEL to be 10 ppm (2-3 mg/kg bw/d), based upon increased liver weight in males, hepatocellular hypertrophy and clinical-chemistry parameter variations at 50 ppm.

BMD analysis was performed on several parameters (see table below). As the body-weight change was < 10 % at all doses, the BMR for this parameter has been set at 5 %.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Relative liver weight	15 %	Males	13.7	26.9	2.0
		Females	20.9	40.9	2.0
Body weight day 90	5 %	Males	146.0	913.1	6.3
		Females			
Overall body weight gain ratio (individual)	10 %	Males	178.0	629.1	3.5
		Females	274.1	Infinity	Infinity
Hepatocellular necrosis – single cell	10 %	Males	15.1	90.9	6.0
Hepatocellular necrosis - multifocal		Females	60	228	3.8
Haemoglobin	5 %	Males	-	-	-
		Females	-	-	-
Haematocrit	5 %	Males	-	-	-
		Females	-	-	-

When dose-responses in haemoglobin and haematocrit were analysed, models were not fitted with a response level of 5 %, indicating non-random errors in the data or too-high a response level. This confirms the RMS conclusion above that the findings in these parameters were not appropriate for risk assessment. In this analysis, the most sensitive effect was the change in relative liver weight.

B.6.3.2.3. 90-day dog study

In a guideline-compliant 90-day dog study, BAS 750 F was administered to 5 animals/sex at doses of 0, 15, 90 and 180 mg/kg bw/d by capsule. The capsules were orally administered once daily in the morning, immediately before the feed ration. Control group animals received the same number of (empty) gelatin capsules as the animals of the high dose group.

There were no deaths during the study. In the high-dose group (180 mg/kg bw/d), one male animal showed reduced and retarded food consumption on isolated days (days 3, 6, 8 and 10). This animal also showed vomitus on study day 2. Four females of the high-dose group showed reduced and retarded food consumption on some isolated days throughout the study period; the reduction in food consumption was statistically significant on days 0 and 7 (by up to 17 % on day 7). Two of these females also showed vomitus on study days 1 and 2. At 90 mg/kg bw/d, three female animals showed reduced and retarded food consumption on isolated days in the second half of the study (from day 49

onwards, up to 23 % reduction on day 63); however, the reduction did not show a dose-response relationship and was not statistically significant, hence is not a clear treatment-related adverse effect. At 15 mg/kg bw/d, one female showed reduced and retarded food consumption from day 4 onwards over nearly the whole administration period; however, the same finding was also reported in one of the control females, and hence at the low dose this was not a treatment-related effect. There were no clinical findings in mid- or low-dose males.

At 180 mg/kg bw/d, the body weight of both males and females was reduced over the whole study period but without statistical significance compared with the controls (see table below). The body weight of females at 90 mg/kg bw/d was also slightly reduced over the whole study period, but again without statistical significance. At 180 mg/kg bw/d, body weight gain was reduced statistically significantly in males, with a maximum decrease of 49.4 % over the whole treatment period. In high-dose females, the overall decrease in body-weight gain of 59.6 % was not statistically significant; however, the decrease was statistically significant in the first five weeks of the study (with a maximum decrease of 207 % in the first week) and from study day 0 to 77. The effects on body-weight change in males and females at the high dose were treatment related and adverse.

Table B.6.3.2.11. Mean body weight and body-weight gain in 90-day dog study

	Males				Females			
Dose level [mg/kg bw/d]	0	15	90	180	0	15	90	180
Body weight [kg]								
Day 0	11.8	11.9	12.1	11.8	9.8	9.9	9.8	9.9
Day 91	13.4	13.6	13.8	12.6	10.9	10.7	10.4	10.3
% change (compared with control)		1.0	2.5	-6.4		-1.1	-4.2	-5.3
Body weight gain [kg]	1.6	1.6	1.6	**0.8***	1.0	0.8	0.6	0.4
% change (compared with control)		3.8	2.5	**-49.4**		-19.2	-42.3	-59.6

* $p \leq 0.05$ (Dunnett's test, two sided);

Blood samples for measurement of BAS 750 F concentration in plasma were collected on day 83 before (to reflect residual test substance from the previous administration) and 6 hours (corresponding to the approximate Tmax based on results of the 28-day range finding study) after capsule administration from all (non-fasted) animals. BAS 750 F was detected in all individual samples from the treatment groups and at both time-points, with a dose-dependent increase in plasma concentration. At 6 hours, the mean plasma concentrations in males increased from 1101 ng/mL at the low dose to 13 604 ng/ml at the high dose; in females, similar mean concentrations of 890 / 14 217 ng/mL were obtained at the low / high dose, respectively. The mean plasma concentration at the mid dose was about 1.7-fold higher in males than in females. The BAS 750 F concentrations at 0 hours were about 16-19 % of the 6-hour values at the low dose in both sexes and at the mid dose in the females. At the mid-dose level in males, a concentration ratio of 32 % was obtained, and at the high-dose level in males and females, ratios of 54 % and 40 % were obtained, which appeared to indicate that the excretion at the mid-dose in males and at the high-dose in females was slower than at the low dose.

No substance-related ophthalmoscopy effects were observed.

For haematology and clinical chemistry investigation, blood was collected from fasted animals before capsule administration on days 11, 41 and 85. Females in the mid- and high-dose groups had increased platelet concentrations after 6 and 13 weeks of treatment, although these were within the historical control data (same laboratory, strain and within five years of the evaluated study). The statistical significance of the finding was probably because of the low mean platelet concentrations in the control females, which were outside the relevant historical control range (see table below). After six weeks, activated partial thromboplastin time (PTT) was reduced in females at 90 mg/kg bw/d and relative large unstained cell (LUC) counts were decreased in females at 15 mg/kg bw/d. As neither of these

parameters was dose-dependently changed, they were incidental. Overall, therefore, there were no adverse changes of haematological parameters at either time-point in any dose group.

Table B.6.3.2.12. Haematology parameters (selected) in 90-day dog study

	Week	Males				Females			
Dose level [mg/kg bw/d]		**0**	**15**	**90**	**180**	**0**	**15**	**90**	**180**
Platelets [giga/L]	6	354	325	348	390	250	314	**343****	**367****
	13	336	323	346	318	272	302	**350****	**374****
Historical control (15 studies, sampling 2004-2015) [giga/L]	*6*					*mean: 352; min-max: 289-406*[$]			
	13					*mean: 331; min-max: 294-386*[$]			
LUC [%]	6	0.3	0.4	0.5	0.3	0.6	**0.3***	0.5	0.4
	13	0.3	0.3	0.5	0.3	0.5	0.4	0.5	0.3
PTT [s]	6	11.7	11.6	11.1	11.4	12.3	12.0	**11.4****	11.8
	13	12.2	11.8	11.6	11.6	12.6	11.9	11.7	12.1

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)
[$] minimum and maximum values obtained in studies with sampling performed between 2011-2012

Treatment-related changes in clinical-chemistry parameters were noted in alkaline phosphatase in males and females at 180 mg/kg bw/d and in males at 90 mg/kg bw/d, but it was noted that the control values were below the historical control range. The increased ALP values at 180 mg/kg bw/d were considered to be adverse, but as the slight ALP increase in males at 90 mg/kg bw/d occurred in isolation, without changes in other parameters or adverse liver histopathology findings, the RMS does not consider this adverse. Furthermore, the value measured at 13 weeks in the 90 mg/kg bw/d male group (2.08 µkat/L) was similar to the pre-treatment value (1.95 µkat/L), whilst the concurrent control value was unusually low. Decreases in total protein at 180 mg/kg bw/d were noted in males after 6 weeks of treatment and in females at 6 weeks and three months; these were considered by the study authors to be adverse and possibly related to a slight (about 10 %) reduction in albumin that was not statistically significant. In high-dose females, a decrease in creatinine was adverse, taking into account other findings at this dose that indicated liver-cell dysfunction (ALP, relative weight). A decrease in bilirubin in the same group was attributed by the study authors to increased liver-enzyme induction, resulting in enhanced phase II metabolism and excretion of glucuronidated bilirubin; in support of this, there was no indication of a hypoplastic anemia. This change thus probably reflects an adaptive response to increased metabolic demand.

Table B.6.3.2.13. Clinical chemistry parameters (selected) in 90-day dog study

	Week	Males				Females			
Dose level [mg/kg bw/d]		**0**	**15**	**90**	**180**	**0**	**15**	**90**	**180**
Alkaline phosphatase [µkat/L]	6	1.54	1.48	2.51	2.59	1.47	1.38	1.99	1.97
	13	1.13	1.14	**2.08***	**3.27***	1.18	1.08	1.73	**2.20***
Hist. control (11 studies, sampling 2004-2015) [µkat/L]	*13*	*mean: 1.44; min-max: 1.35-1.50*[$]							
Creatinine [mmol/L]	6	54.8	59.6	58.1	53.5	59.9	60.2	55.5	**52.4***
	13	54.9	61.1	**61.5***	56.0	61.7	60.9	57.5	52.9
Total protein [g/L]	6	55.18	52.93	54.96	**51.78****	55.66	**51.97****	54.15	**52.50***
	13	55.37	53.35	53.80	51.34	54.84	51.66	53.36	**50.95***
Total bilirubin [µmol/L]	6	1.36	1.49	1.53	1.23	1.93	1.60	1.61	**1.04****
	13	0.62	0.79	0.63	0.34	1.22	0.75	0.82	**0.33****

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)
[$] minimum and maximum values obtained in studies with sampling performed in 2013

Urinalysis investigations were performed with fasted animals before the start of test-substance administration and on study days 44/45 and 86/87 in males/females, respectively. No treatment-related changes in urinalysis parameters were observed.

The following alterations of organ weight were considered to be treatment-related and organ-specific (not secondary to body weight change). In males, absolute and relative liver weights were increased at 90 and 180 mg/kg bw/d, the latter being adverse. The increase in absolute liver weight in mid-dose males did not show a clear dose-response relationship, and was not associated with adverse effects on other liver parameters. Absolute and relative liver weights were also increased in females at 180 mg/kg bw/d, but not with statistical significance.

Table B.6.3.2.14. Organ weights (selected) in 90-day dog study

Sex		Males				Females			
Organ weight	Dose [mg/kg bw/d]	Absolute weight	%	Relative weight [% of bw]	%	Absolute weight	%	Relative weight [% of bw]	%
Terminal weight [g]	0	13520				10940			
	15	13640	(+1)			10800	(-1)		
	90	13880	(+3)			10500	(-4)		
	180	12720	(-6)			10280	(-6)		
Liver (g)	0	377.45		2.799		340.95		3.112	
	15	372.38	(-1)	2.739	(-2)	304.89	(-11)	2.825	(-9)
	90	**477.21****	**(+26)**	3.436	(+23)	332.13	(-3)	3.186	(+2)
	180	429.09	(+14)	**3.371****	**(+20)**	378.60	(+11)	3.675	(+18)
Testes / Ovaries [g]	0	17.74		0.131		1.192		0.011	
	15	19.58	(+10)	0.144	(+9)	1.228	(+3)	0.011	(+2)
	90	22.89	(+29)	0.163	(+24)	0.934	(-22)	0.009	(-18)
	180	22.70	(+28)	**0.178****	**(+35)**	0.888	(-26)	0.008	(-22)
Hist. controls,3 studies, same facility, 2005, 2001, 2012		*Range 20.84 - 24.18, mean 22.38g*		*Range 0.156 – 0.16, mean 0.157*					
Epididymides /	0	3.94		0.029		8.784		0.077	
Uterus [g]	15	4.08	(+4)	0.030	(+3)	13.344	(+52)	0.118	(+53)
	90	3.95	(±0)	0.028	(-3)	7.348	(-16)	0.069	(-10)
	180	3.96	(+1)	0.031	(+7)	4.926	(-44)	0.046	(-40)
Kidneys [g]	0	67.73		0.501		46.47		0.424	
	15	59.79	(-12)	0.437	(-13)	43.03	(-7)	0.396	(-7)
	90	59.64	(-12)	0.431	(-14)	42.14	(-9)	0.401	(-5)
	180	56.95	(-16)	0.447	(-11)	49.17	(+6)	**0.479***	**(+13)**
Prostate [g]	0	7.28		0.053					
	15	7.33	(+1)	0.053	(-1)				
	90	4.12	(-43)	0.029	(-46)				
	180	3.71	(-49)	0.029	(-45)				
Spleen [g]	0	29.73		0.220		26.93		0.246	
	15	27.44	(-8)	0.201	(-9)	25.30	(-6)	0.234	(-5)
	90	26.17	(-12)	0.186	(-15)	18.86	(-30)	**0.179****	**(-27)**
	180	28.92	(-3)	0.226	(+3)	21.99	(-18)	0.215	(-12)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis H and Wilcoxon-test, two sided)

In high-dose females, relative kidney weight was statistically significantly increased, but without associated pathology findings. An increase in relative spleen weight in females at 90 mg/kg bw/d did not show a dose-response relationship and no histopathologic correlate and was therefore not treatment related. The ovaries and uterus weights showed variable responses, with increases in the low-dose group but decreases in the mid- and high-dose groups; however, none of these was statistically significant. In males, there was a dose-related increase in relative testes weights, whilst

prostate weights were decreased, albeit without statistical significance. There were no histopathology findings in the testes and the absolute weights were within the historical control range, but the weight decrease in the prostate corresponded to a reduced organ size in some animals (see below).

The applicant attributed the weight deviations observed in the genital organs of males and females to age-related variability in the extent of sexual maturity within the control and test-group animals. Histopathological examination showed that only single animals of control or treated females had already reached the first oestrus (presence of functional or old degraded corpora lutea). Furthermore, mammary glands were immature in most animals. In spite of this, a great range of variability in sexual organ development was noted when examining ovaries, vagina, uterus and mammary gland of each female animal, indicating age-related variations in reaching the first estrus and sexual maturity. Signs of immaturity were also observed in the prostate of some males (see below). To further understand the variability in the weights obtained for these organs, the ranges, means and medians are presented below.

Table B.6.3.2.15. Range and averages of reproductive-organ weight changes in 90-day dog study

Dose mg/kg bw/d	**10**	**15**	**90**	**180**
Prostate absolute				
Range	4.38 – 10.13	4.64 – 14.48	1.46 – 7.43	1.5 – 6.28
Mean	7.28	7.33	4.12	3.71
Median	8.47	6.16	3.55	3.89
Prostate relative				
Range	0.033 – 0.071	0.032 – 0.099	0.012 – 0.05	0.011 – 0.048
Mean	0.053	0.053	0.029	0.029
Median	0.064	0.046	0.027	0.03
Testes absolute				
Range	16.24 – 19.89	16.84 – 22.41	13.88 – 29.29	18.58 – 28.85
Mean	17.74	19.58	22.89	22.70
Median	17.36	19.26	24.53	22.55
Testes relative				
Range	0.116 – 0.139	0.134 – 0.161	0.111 – 0.201	0.16 – 0.214
Mean	0.131	0.144	0.163	0.178
Median	0.134	0.135	0.165	0.171
Uterus absolute				
Range	4.03 – 16.54	4.7 – 32.42	2.94 – 14.9	1.7 – 14.74
Mean	8.78	13.34	7.35	4.93
Median	7.6	4.7	3.56	2.71
Uterus relative				
Range	0.035 – 0.127	0.027 – 0.287	0.032 – 0.125	0.018 – 0.132
Mean	0.077	0.118	0.069	0.046
Median	0.07	0.047	0.035	0.027
Ovary absolute				
Range	0.69 – 1.39	0.76 – 2.28	0.57 – 1.42	0.55 – 1.62
Mean	1.19	1.23	0.93	0.89
Median	1.33	0.87	0.83	0.75
Ovary relative				
Range	0.008 – 0.014	0.007 – 0.019	0.006 – 0.013	0.007 – 0.014
Mean	0.011	0.011	0.009	0.008
Median	0.01	0.009	0.007	0.007

In all cases there was variability in the organ weights in all groups, with overlap of the ranges between the controls and treatment groups. The findings that seemed to show the clearest dose-related changes were those in the prostate and testes, but nevertheless mostly without statistical significance (albeit only five animals per group, thus weakening the statistical analysis). The rather shallow dose-response curves and large variability in the uterus and ovary weights in particular are reflected in the lack of

statistical significance. Overall, the RMS concludes that these weight changes are not evidence of a clear treatment-related effect.

Gross pathology was performed on animals of all groups. The decrease in organ size of the prostate in one male at 90 mg/kg bw/d and in two males at 180 mg/kg bw/day corresponded to the decreased weight in these groups. All other findings were incidental or spontaneous in origin and without any relationship to treatment.

Histopathology was also performed on animals of all groups. Changes were identified only in the liver. Eosinophilic change of minimal severity was diagnosed in the livers of all dogs from the high-dose group (males and females), at the mid-dose level in all male dogs and some females, and at the low-dose in two males. The term eosinophilic change was used to describe the cytoplasmic appearance of centrilobular hepatocytes observed in the treated groups, which differed from the more vacuolated appearance of hepatocytes from the control animals that are characteristic of the cytoplasmic storage of glycogen. The size of the affected hepatocytes was not changed. An increase in eosinophilia is one of the microscopic indicators of hepatocellular hypertrophy. This, together with the absence of additional findings such as inflammation or degeneration of the cells, leads the RMS to conclude that this was not an adverse effect. No other treatment-related findings were observed. In particular, there were no adverse histopathology findings in the testes, prostate, uterus, ovaries or kidneys. Indications of immaturity in the male animals with reduced prostate size were small size of acini and abundant interstitial stroma.

Table B.6.3.2.16. Histopathology (selected) in 90-day dog study

	Males				**Females**			
Test group	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Dose level [mg/kg bw/d]	**0**	**15**	**90**	**180**	**0**	**15**	**90**	**180**
No. of animals	5	5	5	5	5	5	5	5
Centrilobular hepatocytes Eosinophilic change of cytoplasm, grade 1	0	2	5	5	0	0	2	5

Discussion and conclusion

The oral administration of BAS 750 F by capsules to male and female Beagle dogs for three months caused test substance-related, adverse signs of toxicity at a dose level of 180 mg/kg bw/d that comprised reductions in food intake, body weight and body-weight gain, increased alkaline phosphatase, decreased serum protein concentration in both sexes, transiently decreased creatinine in females and increased liver weight in males. At 90 mg/kg bw/d, the only treatment-related clinical pathology change was a slightly increased alkaline phosphatase activity in males, but since at this dose the increased liver weight did not show a dose-response relationship and there were no other indications of hepatotoxicity, the RMS does not consider this to be an adverse effect.

Thus, the only clear target organ was the liver. Although changes in the weights of the testes, prostate, uterus and ovaries were noted in animals exposed to BAS 750 F, these were for the main part without statistical significance, which reflected the shallow dose-response curves and substantial variability in values within all the groups, in addition to the small group sizes. Furthermore, there were no pathology findings to explain these weight changes, with the exception of the prostate, which was reduced in size in 1 / 5 males at 90 mg/kg bw/d and 2 /5 males at 180 mg/kg bw/d. However, this reduction in size did not correlate with any histopathology findings. Overall, the RMS concludes that these organ-weight changes do not provide robust evidence of a treatment-related adverse effect. Likewise, a statistically-significant increase in relative kidney weight in high-dose females only, which was not associated with pathology changes, is not sufficient evidence by itself of adversity.

The NOAEL in this study was 90 mg/kg bw/d in male and female dogs.

The co-RMS (FR) considers the NOAEL to be 15 mg/kg bw/d, based upon increased liver weight with histopathological and clinical chemistry findings.

To refine the assessment, BMD analysis has been performed on individual relative liver weight, mean body weight and individual body-weight gain ratio (day 91 / day 1).

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Relative liver weight	15 %	Males	18	95.1	5.3
		Females	79.9	193	2.4
Body weight day 90	10 %	Males	174.1	667.4	3.8
		Females			
Overall body weight gain ratio	10 %	Males	200.8	1003.6	5.0
		Females			

The lowest BMDL value obtained was for increased relative liver weight. The BMDL obtained was likely to be conservative, given the relatively high BMDU/BMDL ratio, although it is noted that the proposed NOAEL was within the confidence interval.

B.6.3.3. Oral one-year study in dogs

An oral one-year study in dogs has been submitted by the applicant. This study is not a data requirement under Regulation 283/2013, but has been conducted for other regulatory regimes. The RMS has assessed the study because it further explores the prostate effects observed in the 90-day dog study and thus informs on the risk assessment.

In this study, BAS 750 F was administered to groups of dogs (5 / sex / dose) at doses of 0, 10, 30 and 150 mg/kg bw/d in gelatine capsules for 12 months. The animals were distributed into groups according to their weights. The capsules were orally administered once daily, before feeding, on seven days per week. The control animals received empty gelatine capsules.

There were no unscheduled deaths. There were no consistent, treatment-related effects on food consumption or clinical observations.

Seven days prior to the start of dosing, the weight variation of the animals exceeded 20 % of the mean weight (males: -23.0 to + 28.4 %; females: -21.6 to 29.3 %). The study authors did not consider this deviation from the test guideline to affect the validity of the study. During the dosing period, the mean body weight and body-weight change of males was slightly but not statistically significantly decreased at 150 mg/kg bw/d (maximum decrease of 5.8 %); their terminal body weight was, moreover, within the historical control range. This was also the case for females at 30 mg/kg bw/d (maximum decrease 5.7 %, not statistically significant and within historical control range). These changes did not, therefore, indicate a clearly treatment-related effect. In females at 150 mg/kg bw/d, the mean body weight was reduced, with a maximum decrease of 11.6 % towards the end of the study, but without statistical significance. The mean body-weight change of these females was statistically significantly reduced during the first week (study days 0-7, -126.7 % of the control value) and continued to show a decrease thereafter, albeit without statistical significance. The effects on body weights of the high-dose females were treatment-related (although not statistically significant, the terminal weight was outside the historical control range of 11.7 to 15.1 kg from 11 studies conducted at the test facility between 2000 and 2016) and adverse.

Table B.6.3.3.1. Mean body weight and body-weight gain in one-year dog study

	Males				Females			
Dose level [mg/kg bw/d]	0	10	30	150	0	10	30	150
Body weight [kg]								
Day 0	12.3	12.2	12.4	12.4	10.2	10.2	10.3	10.3
Day 364	15.5	15.7	16.0	14.7	12.7	12.8	12.0	11.3
% change (compared with control)		1.4	2.8	-5.4		0.5	-5.7	-11.3
Body weight gain [kg] d 0-364	3.3	3.5	3.5	2.3	2.5	2.5	1.7	0.9
% change (compared with control)		8.6	8.0	-28.8		1.6	-32.8	-63.2

There were no unusual ophthalmology findings in any group.

Clinical pathology measurements were taken at three, six and twelve months. The haematology determinations showed that absolute lymphocyte counts were statistically significantly decreased in males of the high-dose group at three months but not at six or twelve months. However, since the total number of white-blood cells was not changed, the toxicological relevance of this decrease is not clear. The relative lymphocyte counts were decreased in the mid- and high-dose groups, but as a dose-response relationship wasn't shown, this doesn't represent a clear treatment-related effect.

Table B.6.3.3.2. 12-month dog study – white blood cell parameters (selected)

	Day	**Males**				**Females**			
Dose level [mg/kg bw/d]		**0**	**10**	**30**	**150**	**0**	**10**	**30**	**150**
WBC	92	12.03	12.48	12.77	10.10	10.68	11.29	12.26	9.18
[giga/L]	183	10.52	9.78	11.71	8.29	10.44	10.40	10.06	9.01
	361	10.26	9.31	10.83	9.49	10.18	10.26	10.56	8.89
LYMPHA	92	3.95	3.26	3.21	**2.81***	3.69	3.55	3.35	2.67
[giga/L]	183	3.18	2.85	2.88	2.18	3.29	3.22	3.27	2.52
	361	3.17	2.62	2.78	2.44	3.04	3.15	3.17	2.32
LYMPH	92	32.8	27.2	25.3*	27.8*	34.2	31.4	27.5	29.1
[%]	183	30.5	30.4	24.7	25.9	31.7	31.2	32.5	28.2
	361	31.1	28.8	25.7	25.9	30.1	30.8	30.4	25.9

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)

In the high-dose males, the mean corpuscular volume (MCV) and mean corpuscular haemoglobin content (MCH) of males were higher than those of the controls at all time-points; however, the underlying measured red-blood cell parameters of these calculated indices were not changed. The explanation for the higher MCV and MCH values therefore seemed to lie in lower red-blood cell counts, although these values were not statistically significantly different from the controls. Therefore, the slight changes in these indices did not represent clearly treatment-related effects. The prolonged prothrombin time (QT, Quick Test) in females at three months was within the historical control data provided by the applicant from the same test facility (16 studies, range 6.9 to 9.1, mean 7.7, 2004 to 2015) but outside the date-relevant range (6 studies, range 7.1 to 7.7). This finding is of uncertain toxicological significance, since it occurred only at this one time-point and only in females.

Table B.6.3.3.3. 12-month dog study - red blood cell and coagulation parameters (selected)

	Day	Males				Females			
Dose level [mg/kg bw/d]		**0**	**10**	**30**	**150**	**0**	**10**	**30**	**150**
Red blood cells (RBC)	92	7.18	7.04	6.89	6.41	7.01	7.01	7.35	6.71
[tera/L]	183	7.43	7.60	7.36	6.81	7.26	6.57	7.20	7.35
	361	7.39	7.37	7.37	7.21	7.27	6.96	7.12	7.09
Hemoglobin (HGB)	92	9.8	9.7	9.4	9.3	9.8	9.8	10.2	9.5
[mmol/L]	183	10.4	10.5	10.2	10.1	10.2	9.3	10.2	10.5
	361	10.2	10.7	10.4	10.5	10.3	9.8	10.0	10.2
Hematocrit (HCT)	92	0.469	0.460	0.450	0.451	0.468	0.470	0.488	0.451
[L/L]	183	0.485	0.497	0.481	0.477	0.487	0.438	0.482	0.490
	361	0.497	0.518	0.497	0.507	0.501	0.474	0.479	0.492
MCV	92	65.3	65.4	65.3	70.3*	66.8	67.0	66.5	67.1
[fL]	183	65.3	65.4	65.4	70.1*	67.1	66.7	66.9	66.7
	361	67.3	67.9	67.4	71.8**	69.0	68.1	67.4	69.4
MCH	92	1.36	1.37	1.37	1.45*	1.40	1.40	1.38	1.42
[fmol]	183	1.40	1.38	1.39	1.48*	1.41	1.42	1.41	1.42
	361	1.39	1.40	1.41	1.50*	1.42	1.41	1.40	1.44
QT	92	9.2	7.7	7.5	7.7	7.2	7.7	7.5	8.0*
[sec]	183	9.6	8.4	8.2	8.6	7.7	8.3	8.2	8.7
	361	9.3	8.1	8.0	8.1	7.6	8.2	7.6	8.3

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)

In the clinical chemistry measurements, treatment-related increased alkaline phosphatase activities in both sexes and decreased aspartate aminotransferase activities in males at 150 mg/kg bw/d were recorded. The change in aspartate aminotransferase activity was relatively small (25 % decrease) and so not necessarily adverse; it also achieved statistical significance only at three months. At this dose, additional findings comprised an adverse decrease in albumin and total protein levels (males and females) and calcium levels (males and females); also, creatinine (all time-points) and cholesterol (12 months) values were decreased in females. There were no treatment-related adverse urinalysis findings.

Table B.6.3.3.4. 12-month dog study clinical chemistry parameters (selected)

	Month	Males				Females			
Dose level [mg/kg bw/d]		**0**	**10**	**30**	**150**	**0**	**10**	**30**	**150**
Alkaline phosphatase [µkat/L]	3	1.09	1.49*	1.43	**2.49****	1.44	1.18	1.59	**3.25**
	6	0.89	1.31	1.18	**3.44****	1.11	1.10	1.32	**3.79***
	12	0.86	1.23	1.14	**4.01****	1.17	1.09	1.56	**4.19**
Aspartate aminotransferase [µkat/L]	3	0.59	0.56	0.49	**0.44***	0.48	0.50	0.46	0.47
	6	0.53	0.62*	0.45	0.48	0.44	0.43	0.48	0.45
	12	0.68	0.64	0.51	0.52	0.47	0.48	0.48	0.48
Creatinine [mmol/L]	3	67.4	66.3	60.1*	58.4	69.3	66.9	63.0	**53.5***
	6	70.7	70.8	64.5	60.3	65.8	64.0	62.8	**54.1***
	12	75.2	73.0	66.5	66.3	69.4	67.6	60.4	**55.7***
Total protein [g/L]	3	53.47	55.97*	55.46	**51.11****	56.58	54.70	54.85	**52.09**
	6	56.99	58.21	56.89	**54.27**	58.59	56.33	56.50	**53.28**
	12	55.65	58.21	55.72	53.88	57.32	55.55	56.30	**53.58**
Albumin [g/L]	3	31.07	32.16	31.55	**28.32***	33.60	31.67*	32.08	**29.48***
	6	32.84	34.03	33.24	**30.03***	35.52	32.73**	33.45	**31.47****
	12	31.54	33.06	31.90	29.48	33.89	32.04	32.19	**30.02****
Cholesterol [mmol/L]	3	3.97	4.44	4.56	3.26	5.04	4.08	4.46	4.46
	6	3.67	3.89	3.88	3.09	4.71	4.92	4.50	3.77
	12	3.54	3.84	3.72	3.06	4.45	4.53	5.31	**3.45****
Calcium [mmol/L]	3	2.63	2.70*	2.67	**2.57***	2.78	2.72	2.69	**2.62**
	6	2.62	2.61	2.64	2.55	2.70	2.65	2.61	**2.54***
	12	2.56	2.58	2.59	2.51	2.64	2.58	2.60	2.52

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)

The measurement of organ weights showed statistically significant changes in the adrenal glands, kidneys and liver (see table below). The prostate weights are also shown in the table below, to inform on the prostate findings in the 90-day dog study.

Table B.6.3.3.5. Organ-weight changes in one-year dog study

Sex		Males				Females			
Organ weight	Dose [mg/kg bw/d]	Absolute weight	%	Relative weight [% of bw]	%	Absolute weight	%	Relative weight [% of bw]	%
Terminal weight [g]	0	15600				12640			
	10	15740	+1			12740	+1		
	30	16000	+3			11980	-5		
	150	**14700**	**-6**			**11240**	**-11**		
Adrenal glands (g)	0	1.532		0.010		1.524		0.012	
	10	1.596	+4	0.010	+3	1.406	-8	0.011	-10
	30	1.366	-11	0.009*	-13	1.540	+1	0.013	+7
	150	1.410	-8	0.010	-2	1.904	+25	**0.017****	+40
Liver (g)	0	384.732		2.469		344.430		2.733	
	10	424.160	+10	2.689	+9	329.360	-4	2.564	-6
	30	437.062	+14	2.742	+11	387.294	+12	3.237	+18
	150	**484.598***	**+26**	**3.294****	**+33**	403.216	+17	3.583	+31
Kidneys [g]	0	66.412		0.425		49.264		0.391	
	10	64.698	-3	0.412	-3	48.422	-2	0.381	-2
	30	72.744	+10	0.454	+7	53.420	+8	0.446	+14
	150	70.082	+6	0.477*	+12	51.032	+4	0.454*	+16
Prostate [g]	0	9.608		0.061					
	10	10.684	+11	0.068	+11				
	30	10.642	+11	0.067	+9				

Table B.6.3.3.5. Organ-weight changes in one-year dog study

Sex		Males				Females			
Organ weight	Dose [mg/kg bw/d]	Absolute weight	%	Relative weight [% of bw]	%	Absolute weight	%	Relative weight [% of bw]	%
	150	11.646	+21	0.079	+30				

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis H and Wilcoxon-test, two sided)

The increases in liver weight in both sexes at 30 and 150 mg/kg bw/d were treatment-related. The applicant provided historical control data from 11 studies conducted at the test facility between 2000 and 2016; however, only two of these were conducted during a time-period (2014-2015) that was relevant to the present study, and so they were of limited use. The weight increase in the high-dose animals was associated with clinical-chemistry changes indicative of liver impairment and was thus adverse. At the mid-dose level, there were no such clinical-chemistry changes and the increase in liver weight was marginal; therefore, this was likely to have been an adaptive change. The liver-weight increase in males at 10 mg/kg bw/d was likely to be treatment-related but, since it had no histopathology correlates, adaptive rather than adverse.

The increases in absolute and relative adrenal-gland weights in females of the high-dose group were above the historical-control range and are considered by the RMS to be treatment related, although it is noted that there were no histopathology findings. The decreased adrenal-gland weight in males only of the mid-dose group was an incidental finding. The increased relative weights of the kidneys at 150 mg/kg bw/d were related to the decreased terminal body weights; thus they were not adverse.

Absolute and relative prostate-gland weights were increased in males at 150 mg/kg bw/d, but without statistical significance. The increase in the mean value was caused by one prostate having a considerably higher weight (17.5 g) than the remainder of the group (4 animals, mean weight 10.7 g, median 9.9 g). Since only one animal was affected, it is concluded that this was an incidental finding. The RMS also notes that one animal of the control group had a considerably lower prostate-gland weight (5.2 g) than the other animals of the group (4 animals, mean weight 10.7 g, median 10.6 g). Because of this individual, the prostate weights in the low- and mid-dose groups appeared to be marginally increased compared with the controls. There were no morphological findings in the exposed groups to indicate a treatment-related effect. There were no statistically significant, dose-related changes in the weights of the ovaries, uterus, epididymides, pituitary gland or testes.

There were no treatment-related gross lesions. Histopathology revealed treatment-related changes in the livers (all BAS 750 F-exposed groups) and kidneys (females at 150 mg/kg bw/d).

Table B.6.3.3.6. Histopathology findings in the one-year dog study

	Male animals				Female animals			
Dose (mg/kg bw/d)	0	10	30	150	0	10	30	150
No. of animals	5	5	5	5	5	5	5	5
Liver								
Hypertrophy, centrilobular	0	0	3	2	0	0	2	4
• Grade 1			2	1			1	
• Grade 2			1	1			1	4
Hypertrophy, diffuse	0	0	0	3	0	0	0	1
• Grade 1				2				
• Grade 2				1				1
Eosinophilic change	0	3	3	5	0	2	3	5
• Grade 1		3	3	5		2	3	5
Kidneys								
Cytoplasmic vacuolation					4	4	3	3
• Grade 1					1	2	0	2
• Grade 2					1	0	2	1
• Grade 3					2	2	1	0

The observed hepatocellular hypertrophy in the mid- and high-dose groups corresponded with the increased liver weights in these animals. At the high-dose level, the hypertrophy showed a centrilobular or diffuse distribution pattern, whereas only a centrilobular pattern was shown at the mid-dose level. The hypertrophic hepatocytes were minimally to slightly enlarged and showed a pale eosinophilic, finely granular cytoplasm with less-pronounced vacuolation than that of the controls. Some of the animals of the low- and mid-dose groups had an eosinophilic change as described above, but without a change in the cell size. An increase in eosinophilia (in the absence of foci of altered hepatocytes) is an early-stage microscopic indicator of proliferation of the smooth endoplasmic reticulum, which is the cellular response that results in hypertrophy; the observation of eosinophilia is thus part of the finding of hypertrophy, not a separate pathological event. The livers of all males were stained with periodic-acid-Schiff (PAS) to detect glycogen; no difference was detected in the amount of glycogen between the controls and the exposed groups. The less-pronounced vacuolation was thus interpreted by the study authors to be part of the eosinophilic change and the hepatocellular hypertrophy rather than a separate pathological finding.

The kidneys of high-dose females had a slightly decreased number of cytoplasmic vacuoles (lower grade scores) in the cortical tubular epithelial cells of the inner cortex than the other groups. An oil-red-O stain on the kidneys of single animals of each group demonstrated that the vacuoles were lipid droplets. The decreased lipid storage probably occurred as a consequence of lower body-weight gains in this group, and was thus treatment-related but not adverse.

Discussion and conclusion

BAS 750 F was administered via oral capsule to groups of dogs (5/sex/dose) at 0, 10, 30 and 150 mg/kg bw/d for 12 months.

There were no deaths or clinical signs of toxicity. A treatment-related decrease in the body weight of female animals was reported at 150 mg/kg bw/d; the body weights of males were largely unaffected in all groups.

The main treatment-related effect was on the liver. At 150 mg/kg bw/d, the relative liver weight was increased by 33 % in males and 31 % in females. These increases were associated with hepatocellular hypertrophy and clinical-chemistry changes indicative of liver impairment and are thus regarded by the RMS to be adverse at this dose level. The decreased calcium levels might have been explained by the lower albumin levels, so that homeostasis of free calcium was maintained. The lower creatinine

values in females at this dose might have reflected lower muscle activity. Body-weight, particularly of the females, was also affected at 150 mg/kg bw/d, and in males the absolute lymphocyte counts were reduced at three months. Therefore, 150 mg/kg bw/d was a dose that resulted in adverse effects.

At 30 mg/kg bw/d, the increases in liver weight were at the margin of adversity (increases of 11 % in males, 18 % in females) and occurred with hepatocellular hypertrophy in approximately half the animals but without clinical-chemistry changes; therefore, they were potentially adaptive. No changes in other parameters to indicate adversity were recorded at this dose. No adverse effects occurred at 10 mg/kg bw/d.

The RMS identified a NOAEL of 30 mg/kg bw/d from this study.

To refine the assessment, the RMS conducted BMD on the following parameters: body weight, relative liver weight, absolute lymphocytes (3 months) and alkaline phosphatase (12 months).

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Relative liver weight	15 %	Males	11.4	108.1	9.5
		Females			
Terminal body weight	10 %	Males	-	-	-
		Females			
Absolute lymphocytes	10 %	Males	0.13	56.4	433.8
		Females			
Alkaline phosphatase	50 %	Males	17.7	154.1	8.7
		Females			

A BMDL for the terminal body weight could not be calculated, because the change was generally less than the specified BMR and was not statistically significantly changed for either males or females. The BMDL for a 10 % change in absolute lymphocytes was associated with much uncertainty. Considering also that a statistically significant decrease in lymphocytes occurred only in males and only at three months, and that the total number of white-blood cells was unchanged, the RMS proposes to take forwards the BMDL of 11.4 mg/kg bw/d from the analysis of relative-liver weight change as the most sensitive and reliable indicator of toxicity in this study. It is recognised that this value is associated with quite a large uncertainty.

B.6.3.4. Other routes

A 28-day dermal study is available in rats, in which BAS 750 F was applied at doses of 0, 100, 300 and 1000 mg/kg bw/d. The fur of the animals was clipped one day before the first application of the test substance, thereafter when necessary but at least once a week. The test substance was administered to the clipped dorsal skin (at least 10% of the body surface) for five days/week for four weeks (males: 21 applications, females: 22 applications). After application the skin was covered for six hours with a semi-occlusive dressing. After removal of the dressing, the skin was washed with lukewarm water. Vehicle alone was applied to the control animals. All rats were sacrificed after a fasting period of at least 16 hours.

No animal died during the course of the study and there were no signs of systemic or dermal toxicity. Food consumption, water consumption, body weight and body-weight gain were comparable between all groups throughout the study. The functional observation battery and motor activity measurements did not detect any treatment-related findings.

There were no treatment-related changes in haematology or clinical chemistry parameters nor in organ weights.

Gross pathology was performed on animals of all groups; histopathology was performed on animals of the control and high-dose groups; additionally, the liver and any identified gross lesions were investigated histopathologically in all groups. No treatment-related pathology findings were observed.

In conclusion, the dermal application of BAS 750 F over a period of 4 weeks did not result in any signs of local or systemic toxicity in Wistar rats up to a dose level of 1000 mg/kg bw/d. Therefore, under the conditions of this study, the NOAEL was at least 1000 mg/kg bw/d.

B.6.3.5. Summary and conclusion of short-term toxicity

The short-term oral toxicity of BAS 750 F has been investigated in 28-day and 90-day studies in rats, mice and dogs. A one-year study in dogs has also been submitted. Its short-term dermal toxicity has been investigated in a 28-day study in rats. No adverse effects were reported in the dermal study when BAS 750 F was applied at doses up to 1000 mg/kg bw/d; thus the active substance was not locally or systemically toxic by this route of exposure.

Following oral administration from 28 days to one year, the liver was a clear target organ in all species. The applicant has investigated the nature of the hepatotoxicity in rodents and concluded that the likely mode of action is of limited relevance to humans; in contrast, the RMS considers that the effects are potentially of relevance to humans, and so should be taken into account in the risk assessment (see section B.6.8.2). In deciding upon the point of departure from the repeated-dose toxicity studies, the RMS has tried to make a distinction between liver effects that are potentially adverse and those that are more likely to be adaptive. Hepatocellular hypertrophy is typically related to increased functional capacity: the hepatocyte responds to chemical exposure by increasing its metabolic capacity via the induction of metabolising enzymes in order to maintain the organism's homeostasis. A weight-of-evidence approach is needed to determine at which exposure level the normal homeostatic response has been exceeded and thus resulted in an adverse effect. These considerations include: the presence of other histology (necrosis, apoptosis, pigment deposition, hyperplasia); supportive clinical-chemistry changes; transient or sustained changes with progression of the effects; the induction of toxicologically-relevant levels of xenobiotic-metabolising enzymes. In line with reviews by the JMPR (2006[5], 2015[6]) and Chemicals Regulation Directorate (2013), the RMS has concluded that findings of hepatocellular hypertrophy without the above-mentioned associated effects are adaptive and thus not relevant for the setting of reference values.

Based on an assessment of normal biological variation in organ weights, the JMPR (2015) has proposed that a rough threshold for adversity of a change in relative liver weight be 15 % for rats and mice. This is consistent with the general view that increases in liver weight relative to body weight of < 10 % are non-adverse, unless accompanied by indications of hepatotoxicity (Chemicals Regulation Directorate, 2013); whereas there has been no clear guidance on the level of increase that is adverse if the only other liver-related findings are hypertrophy or isolated changes in clinical chemistry. Therefore, in line with the JMPR recommendation, the RMS has applied a response level of 15 % to the BMD analysis of relative liver weight increase. Other liver changes, such as histopathology or clinical chemistry findings, have been analysed as separate effects.

Taking into account the above considerations, adverse effects on the liver comprised treatment-related increases in absolute and relative weights, clinical chemistry alterations and histopathology findings. Impairment of liver function was observed from 256 mg/kg bw/d in rats and 150 mg/kg bw/d in dogs, whilst clear liver toxicity (including liver foci and hepatocellular necrosis) was reported from

[5] FAO/WHO, 2006: Pesticide residues in food - 2006. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues.

[6] WHO, 2015: Pesticide residues in food: WHO Core Assessment Group on Pesticide Residues. Guidance document for WHO monographers and reviewersWHO/HSE/GOS/2015.1, 1-106 pp.

58 mg/kg bw/d in mice. The severity of the liver effects did not markedly increase with an increase in exposure duration from 28 to 90 days, or to one year in dogs. Based upon the dose levels at which liver impairment / toxicity was evident and also the severity of the effects, the mouse was the most sensitive species to liver effects.

Another consistent finding across species was a reduction in body weight and body-weight gain; this wasn't always attributable to a lack of palatability, because it also occurred when BAS 750 F was administered in capsules.

Other findings were not consistent across species and/or studies. Haemoconcentration was noted in the 90-day mouse study, but since there was no clear explanation (for example, no histopathological effects on the adrenal cortex, bone marrow or spleen), the effects were very slight (< 5 % changes in haematology parameters) and they were not replicated in other studies, including the mouse carcinogenicity study (section B.6.5), the RMS concludes that they were not evidence of an adverse event.

Although there were some inconsistent changes in the weights of reproductive organs in the shorter-duration dog studies (without associated histopathology findings), these were not reproduced in the one-year study. Ovary weights were reduced in the 28-day mouse study at 1000 ppm (145 mg/kg bw/d), but there were no effects on mouse ovary weights or pathology in the 90-day study (tested up to 750 ppm = 211 mg/kg bw/d) nor in the carcinogenicity study (tested up to 250 ppm = 61.5 mg/kg bw/d). Overall, the RMS concludes that BAS 750 F does not affect the reproductive organs of rats, mice or dogs.

When the findings from the short-term studies are compared with the criteria for classification for STOT-RE, the only target organ at doses below the guidance cut-off value for category 2 was the liver. Based on the nature of the effects and the doses at which these occurred, the mouse was more sensitive than the rat and dog. This is consistent with the usual perception that mice tend to be particularly susceptible to liver toxicity.

Overall, the RMS concludes that the minimal-to-slight single-cell or multi-focal necrosis and slight increases in the severity of morphological changes in the liver of mice were not sufficiently severe or reproducible to warrant classification for repeated-dose toxicity (please see CLH report).

Table B.6.3.5.1. Summary of BMDL values in short-term toxicity studies

Study	Species	NOAEL mg/kg bw/d	BMDL mg/kg bw/d	Adverse effects
Oral				
28-day	Rat	135	147	↓ body weights, ↓ feed intake, ↑ relative liver weight, impairment of liver function
28-day	Mouse	18.5	15	↓ body weights & body-weight gain, ↑ liver weight, liver toxicity
28-day	Dog	-	-	Clinical signs, ↓ body weights & body-weight gain, ↑ liver weight, liver histopathology
90-day	Rat	76	91	↓ body weights & body-weight gain, ↑ liver weight, impairment of liver function
90-day	Mouse	11	14	↓ body-weight gain, liver toxicity (including histopathology changes)
90-day	Dog	90	18	↓ food intake, body weight & body-weight gain, impairment of liver function
1-year	Dog	30	11	Clinical-chemistry changes, decreased absolute lymphocytes (males, 3 months), lower terminal body weight (females), ↑ liver weight
Dermal				
28-day	Rat	-	-	No adverse effects at any dose

Liver-weight change was the critical effect in both mice and dogs, whereas the critical effects in rats were body-weight effects, liver weight increases and clinical-chemistry changes. Overall, the lowest BMDL obtained from the short-term repeated-dose toxicity studies was 11 mg/kg bw/d from the 90-day mouse study.

B.6.4. GENOTOXICITY

The genotoxic potential of BAS 750 F has been investigated in a series of *in vitro* studies and one *in vivo* investigation.

According to Regulation (EU) 283/2013, photo-mutagenicity testing is not required for substances with a UV/VIS molar extinction/absorption coefficient less than 1000 L x mol^{-1} x cm^{-1}. In the UV/VIS wavelength range of 295-700 nm, the molar absorption coefficient of BAS 750 F is below this value. Moreover, BAS 750 F did not show any evidence of photo-toxicity when tested in an *in-vitro* 3T3 NRU photo-toxicity assay (see section B.6.2.7); in accordance with a tiered approach proposed by the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM/13/S1), photo-mutagenicity testing is not required in the case of a negative *in-vitro* 3T3 NRU photo-toxicity test. The RMS also notes that there is currently no OECD test guideline available for photo-mutagenicity testing and Regulation 283/2013 does not provide any guidance on suitable test methods. It is furthermore noted that the ICH Guideline on Photo-safety Evaluation of Pharmaceuticals S10 (2013) explicitly discourages photo-genotoxicity testing: *"Testing for photo-genotoxicity is not recommended as a part of the standard photo-safety testing program. ... experience ... has indicated that these tests are substantially over-sensitive and even incidences of pseudo-photo-clastogenicity have been reported (Ref. 8). Furthermore, the interpretation of photo-genotoxicity data regarding its meaning for clinically-relevant enhancement of UV-mediated skin cancer is unclear."* The RMS therefore agrees with the applicant that photo-mutagenicity testing of BAS 750 F is not required.

Table B6.4.1. Summary of *in vitro* and *in vivo* genotoxicity studies

Study type	Test system	Dose / concentr. range (purity)	Result
In vitro reverse mutation assay in bacteria (Ames test) OECD 471 (1997); GLP Report CA 5.4.1/1 Woitkowiak, 2014a (2014/1128030) (Stability analysis in CA 5.4.1/2; Becker & Kamp, 2013b; 2015/1040886)	*S. typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; *E. coli* strain WP2 uvrA; plate incorporation and pre-incubation assay With/without S9-mix	1 - 5000 µg/plate in DMSO Tested in triplicate Purity 98.6 %	Negative
In vitro reverse mutation assay in bacteria (Ames test) OECD 471 (1997); GLP Report CA 5.4.1/3 Woitkowiak, 2015a (2015/1116956)	*S. typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; *E. coli* strain WP2 uvrA; plate incorporation and pre-incubation assay With/without S9-mix	3.3 - 5000 µg/plate for plate incorporation and 1.0 to 1000 µg/plate for pre-incubation assays Dissolved in DMSO Tested in triplicate Purity 97.9 %	Negative
In vitro forward mutation assay in mammalian cells (mouse lymphoma assay) OECD 476 (1997); GLP Report CA 5.4.1/4 Wollny, 2015a (2015/1112683)	Mouse lymphoma L5178Y cells With/without S9-mix Thymidine kinase (TK +/-) locus	3.75 - 60 µg/ml in DMSO Tested in duplicate in two independent experiments Purity 98.8 %	Negative
In vitro forward mutation assay in mammalian cells (mouse lymphoma assay) OECD 476 (1997); GLP Report CA 5.4.1/5 Wollny, 2015b (2015/1101908)	Mouse lymphoma L5178Y cells With/without S9-mix Thymidine kinase (TK +/- locus)	3.1 - 62.5 µg/ml in DMSO Tested in duplicate in three independent experiments Purity 97.9 %	Negative
In vitro cytogenicity assay in mammalian cells (micronucleus test) OECD 487 (2010); GLP Report CA 5.4.1/6 Schulz & Landsiedel, 2014a (2013/1375108)	V79 Chinese hamster lung fibroblast cells (clastogenic or aneugenic activity) With/without S9-mix	0.39 – 50 µg/ml in DMSO Two independent experiments ≥ 1000 binucleated cells/culture (2000 cells per test group) Purity 98.8 %	Negative
In vitro cytogenicity assay in mammalian cells (micronucleus test) OECD 487 (2014); GLP Report CA 5.4.1/7 Sokolowski, 2015a	Human lymphocytes With/without S9-mix	2.0 - 8.2 µg/ml in DMSO Two independent experiments 1000 binucleated cells/culture for evaluation of genotoxicity Purity 97.9 %	Negative

(2015/1101907)			
In vivo micronucleus test OECD 474 (1997); GLP Report CA 5.4.2/1 [redacted] 2014a (2014/1043159)	Male NMRI mice, 5/group; single oral (gavage) application	0-375-750-1500 mg/kg bw in DMSO / corn oil (ratio 2:3) 2000 polychromatic erythrocytes evaluated per animal Purity 98.8%	Negative

B.6.4.1. *In vitro* studies

The potential of BAS 750 F to induce gene mutations in bacterial cells, gene mutation/clastogenicity in mammalian cells and clastogenicity/aneuploidy in mammalian cells has been investigated in *in vitro* studies.

B.6.4.1.1. In vitro reverse mutation assays in bacteria (Ames tests)

Two Ames tests have been conducted, both of which used concentrations up to 5000 µg/plate, dependent upon the strain used, and a standard series of test strains. Metabolic activation was provided by a hepatic S9-mix from phenobarbital/ß-naphthoflavone-induced rats. The only notable difference between the two experiments was the batch of test material used.

In the first test (Woitkowiak, 2014a), the stability of the test material in dimethyl sulphoxide (DMSO) was verified analytically. A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ revertants) was observed in the standard plate test dependent upon the strain and test conditions from about 333 µg/plate; because of excessive bacteriotoxicity in the first experiment with strain TA 1537 in the absence of metabolic activation, an additional experiment under these conditions was performed. In the pre-incubation assay, bacteriotoxicity was observed from about 33 µg/plate. Test-substance precipitation was observed from about 1000 µg/plate with and without S9-mix. There was no relevant increase in the number of revertant colonies in any test strain either with or without metabolic activation in several experiments that were performed independently of each other (see tables below). The negative and positive controls gave the expected results and verified the validity of the study.

Table B6.4.1.1. Ames test (plate incorporation) - mean number of revertants (Woitkowiak, 2014a)

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	35	16	57	47	9	12	7	6	60	60
Test substance										
33 µg/plate	24	21	54	30	11	12	7	6	60	45
100 µg/plate	28	19	51	41	12	10	8	5	64	56
333 µg/plate	19	14	37	27	11	8	5 [B]	0.3 [B]	57	47
1000 µg/plate	7 [BP]	6 [BP]	12 [BP]	7 [BP]	7 [P]	9 [BP]	4 [BP]	0 [BP]	61 [P]	54 [P]
2500 µg/plate	0.3 [BP]	1 [BP]	0 [BP]	0 [BP]	2 [P]	2 [BP]	0 [BP]	0 [BP]	58 [P]	54 [P]
5000 µg/plate	0 [BP]	0 [BP]	0 [BP]	0 [BP]	0 [P]	0 [BP]	0 [BP]	0 [BP]	55 [P]	48 [BP]
Pos. control	2497	374	2867	3732	264	5343	258	1022	275	864
Experiment 2^+: Plate incorporation assay										

Strain							TA 1537			
Metabol. activation								-S9		
Neg. control (DMSO)								9		
Test substance										
1.0 µg/plate								7		
3.3 µg/plate								8		
10 µg/plate								8		
33 µg/plate								8		
100 µg/plate								7		
333 µg/plate								2 B		
Pos. control								2127		

+: Data from repeated experiments were included in the table for TA 1537
B = reduced background growth, P = precipitation

Table B6.4.1.2. Ames test (pre-incubation) - mean number of revertants (Woitkowiak, 2014a)

Experiment 3/4+: Pre-incubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	23	17	61	28	9	7	10	5	80	94
Test substance										
3.3 µg/plate	29	11	46	36	10	7	8	6	NA	NA
10 µg/plate	25	14	56	28	10	9	8	7	NA	NA
33 µg/plate	20	13	49	35	9	8	5	6	96	89
100 µg/plate	28	12	54	28	9	5	4	2 B	91	84
333 µg/plate	22	7 B	35	16 B	6	6 B	4 B	0 B	79	60
1000 µg/plate	16 BP	4 BP	9 BP	0 BP	7 BP	4 BP	0 BP	0 BP	80 P	55
2500 µg/plate	NA	NA	NA	NA	NA	NA	NA	NA	62 P	73 P
5000 µg/plate	NA	NA	NA	NA	NA	NA	NA	NA	74 BP	77 BP
Pos. control	1164	397	1527	1936	215	1560	117	1633	208	367

+: Data from experiment 3 with *S. typhimurium* strains and from experiment 4 with *E.coli* strain WP2 uvrA
NA = test concentration not assayed
B = reduced background growth, P = precipitation

In the second Ames test (Woitkowiak, 2015a), a bacteriotoxic effect was observed, dependent upon the strain and test conditions, from about 100 µg/plate with both the plate incorporation and pre-incubation methods. Because of excessive bacteriotoxicity with strain TA 1537 in the first experiment, repeat plate-incorporation (up to 1000 µg/plate) and pre-incubation (up to 333 µg/plate) assays were performed with this strain in the presence and absence of metabolic activation. Test-substance precipitation was observed with and without S9-mix from about 1000 and 333 µg/plate onward in the plate incorporation and pre-incubation assay, respectively. There was no relevant increase in the number of revertant colonies in any test strain either with or without metabolic activation (see tables below). The negative and positive controls gave the expected results.

Table B6.4.1.3. Ames test (plate incorporation) - mean number of revertants (Woitkowiak, 2015a)

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	17	19	95	104	17	17	12	8	21	21
Test substance										
33 µg/plate	20	16	96	91	15	16	8	6	21	17
100 µg/plate	18	13	100	89	11	13	10	6	20	17
333 µg/plate	16	12	85	98	14	14	12	6	22	21
1000 µg/plate	16 BP	6 BP	36 BP	54 BP	10 P	12 P	11 BP	0 BP	16 P	15 P
2500 µg/plate	8 BP	4 BP	16 BP	26 BP	8 BP	8 BP	0 BP	0 BP	13 BP	13 BP
5000 µg/plate	0 BP	0 BP	0 BP	0 BP	5 BP	7 BP	0 BP	0 BP	14 BP	12 BP
Pos. control	2351	443	2819	3192	323	5311	241	1404	149	1495
Experiment 2+: Plate incorporation assay										
Strain							TA 1537			
Metabol. activation							+S9	-S9		
Neg. control (DMSO)							9	7		
Test substance										
3.3 µg/plate							9	6		
10 µg/plate							8	10		
33 µg/plate							7	7		
100 µg/plate							4	6		
333 µg/plate							6	4 B		
1000 µg/plate							6 BP	0 BP		
Pos. control							182	1081		

+: Data from repeated experiments were included in the table for TA 1537
B = reduced background growth, P = precipitation

Table B6.4.1.4. Ames test (pre-incubation) - mean number of revertants (Woitkowiak, 2015a)

Experiment 3: Pre-incubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	25	18	92	80	12	10	15	7	22	20
Test substance										
3.3 µg/plate	22	21	96	97	8	11	9	7	19	23
10 µg/plate	26	12	94	89	7	12	10	8	22	18
33 µg/plate	20	15	89	92	10	14	13	6	26	16
100 µg/plate	26	12	101	74	8	10	14	3	24	17
333 µg/plate	29 BP	10 BP	91 BP	9 BP	11 BP	6 BP	0 BP	0 BP	21 BP	23 BP
1000 µg/plate	12 BP	0 BP	75 BP	0 BP	5 BP	0 BP	0 BP	0 BP	10 BP	12 BP
Pos. control	1682	451	2327	2308	222	2470	167	1006	68	498
Experiment 4+: Pre-incubation assay										
Strain							TA 1537			
Metabol. activation							+S9	-S9		
Neg. control (DMSO)							7	5		
Test substance										
1.0 µg/plate							8	7		
3.3 µg/plate							10	9		
10 µg/plate							9	6		
33 µg/plate							9	7		
100 µg/plate							6 B	6 B		
333 µg/plate							0 BP	0 BP		
Pos. control							144	553		

+: Data from repeated experiments were included in the table for TA 1537
B = reduced background growth, P = precipitation

BAS 750 F therefore showed no potential to induce gene mutations in bacteria in two standard, acceptable studies.

B.6.4.1.2. In vitro *forward mutation assays in mammalian cells (mouse lymphoma assay)*

BAS 750 F was tested in two *in vitro* assays for its ability to induce forward mutations in mammalian cells by assessing the mutation of the TK locus in mouse lymphoma L5178Y cells. Metabolic activation was provided by a hepatic S9-mix from phenobarbital/ß-naphthoflavone-induced rats. The only notable difference between the two experiments was the batch of test material used.

In the first study (Wollny, 2015a), concentrations up to 60 µg/ml were used in the main experiment, based on the results of a preliminary cytotoxicity assay in which concentrations in the range of 31.5 to 4026 µg/ml were tested; excessive cytotoxicity was observed from 62.9 µg/ml in a 4-hour incubation and from 31.5 µg/ml in a 24-hour incubation. In the main test, two independent experiments were performed in two parallel cultures. The treatment intervals for both experiments in the presence and absence of metabolic activation were generally four hours, except in experiment II (in the absence of metabolic activation) where a treatment interval of 24 hours was used. Methylmethanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.

The experiments were begun with more than four concentrations, but following the expression phase of 48 hours the cultures at the highest concentration in experiment I (± S9) and in experiment II (+ S9) were discontinued because of excessive cytotoxicity. In experiment II, the cultures at the lowest concentration without metabolic activation were not continued because the test-guideline condition of at least four analysable test concentrations was met without that concentration being included; i.e., cytotoxicity was not excessive at the highest concentration, and so the cultures at that concentration were continued.

Relevant cytotoxic effects, indicated by a relative total growth of less than 50% of survival in both parallel cultures, were observed in both experiments; thus it is concluded that adequate concentrations were tested. Since the negative and positive control cultures met the acceptance criteria, the study is considered by the RMS to be valid. In the cultures with the test material, no substantial and reproducible dose-dependent increase of the mutation frequency was observed with or without metabolic activation. An isolated increase that exceeded the threshold for a positive result of 126 colonies per 10^6 cells above the corresponding solvent control was noted at 45.0 µg/ml in the second culture of the first experiment with metabolic activation; the statistical analysis indicated a significant dose-dependent trend of the mutation frequency ($p < 0.05$). Excessive cytotoxicity occurred consistently at this concentration. The increase in mutation frequency was not reproduced in the parallel culture under identical experimental conditions nor in the second experiment; neither was a statistically significant trend noted in these cultures. This isolated finding of a trend in one replicate of one experiment, in the presence of excessive cytotoxicity, was therefore judged to be biologically irrelevant.

Table B6.4.1.5. Results of mouse lymphoma assay - experiment I (Wollny, 2015a)

Without metabolic activation, 4-hour exposure period		Rel. total growth	# mutant colonies / 10^6 cells	Threshold ##	Rel. total growth	# mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Neg. ctrl. (DMSO)		100	98	224	100	75	201
BAS 750 F	3.75	83.3	94	224	85.3	62	201
	7.5	99.1	87	224	82.3	91	201
	15	99.7	102	224	91.1	50	201
	30	53.0	43	224	38.7	47	201
	45	2.3	84	224	3.8	93	201
	60	#			#		
Pos. ctrl. (MMS)	19.5	15.8	269	224	24.9	323	201
With metabolic activation, 4-hour exposure period		Rel. total growth	# mutant colonies / 10^6 cells	Threshold	Rel. total growth	# mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2*		
Neg. ctrl. (DMSO)		100	82	208	100	94	220
BAS 750 F	3.75	58.0	124	208	62.6	128	220
	7.5	59.1	134	208	79.9	100	220
	15	76.5	126	208	79.6	93	220
	30	64.8	79	208	55.9	163	220

Table B6.4.1.5. Results of mouse lymphoma assay - experiment I (Wollny, 2015a)

	45	33.9	90	208	21.6	**248**	220
	60	#			#		
Pos. ctrl. (CPA)	3	47.7	267	208	59.7	223	220
	4.5	23.0	387	208	28.5	368	220

#: culture was not continued owing to excessively severe cytotoxic effects *
##: threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126

Table B6.4.1.6. Results of mouse lymphoma assay - experiment II (Wollny, 2015a)

Without metabolic activation, 24-hour exposure period		Rel. total growth	# mutant colonies / 10^6 cells	Threshold ###	Rel. total growth	# mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Neg. ctrl. (DMSO)		100	100	226	100	116	242
BAS 750 F	3.75	##			##		
	7.5	65.8	77	226	54.0	113	242
	15	31.4	126	226	54.0	123	242
	30	25.8	145	226	24.4	110	242
	45	20.1	154	226	16.6	100	242
	60	14.7	123	226	9.8	146	242
Pos. ctrl. (MMS)	19.5	15.0	614	226	23.3	477	242
With metabolic activation, 4-hour exposure period		Rel. total growth	# mutant colonies / 10^6 cells	Threshold	Rel. total growth	# mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Neg. ctrl. (DMSO)		100	77	203	100	76	202
BAS 750 F	3.75	65.8	75	203	76.9	74	202
	7.5	71.2	117	203	132.4	62	202
	15	71.2	106	203	39.4	115	202
	30	31.9	134	203	48.9	94	202
	45	22.7	108	203	22.2	72	202
	60	#			#		
Pos. ctrl. (CPA)	3	25.5	457	203	38.6	263	202
	4.5	10.6	642	203	18.5	468	202

#: 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
###: threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126

In the second study (Wollny, 2015b), the concentrations used (up to 62.5 µg/ml) were based on the findings in the pre-test and main test in the first study (Wollny, 2015a). Three independent experiments were conducted in the presence and absence of metabolic activation, each one in

duplicate. The treatment intervals for all experiments in the presence and absence of metabolic activation were generally 4 hours, except in experiment II (in the absence of metabolic activation), where the treatment interval was 24 hours. The third experiment was performed solely with metabolic activation to verify results that were generated in experiment I with metabolic activation. MMS and CPA served as positive control substances in the experiments without and with metabolic activation, respectively.

The experimental part of experiment I (-S9) was prematurely terminated because of excessive cytotoxicity. This experimental part was repeated with an extended concentration range and the data are reported as experiment I without metabolic activation. In experiment II, cytotoxic effects, indicated by a relative total growth below 50 % in both parallel cultures, occurred at 12.5 µg/ml and above in the absence of metabolic activation (24 hours treatment) and at 50.0 µg/ml in the presence of metabolic activation. The data generated at 62.5 µg/ml (-S9, both cultures) were invalid, as cytotoxicity was excessive. In experiment III, relevant cytoxic effects of less than 50 % relative total growth were noted at 50.0 µg/ml (+S9). The recommended cytotoxic range of approximately 10 – 20 % relative total growth was therefore covered for cultures with and without metabolic activation. The positive and negative control substances gave results that were consistent with the acceptance criteria.

The threshold for a positive response was exceeded exclusively in experiment I in the presence of metabolic activation at 50.0 µg/ml in culture I, and at 12.5 and 25.0 µg/mL in culture II (see table below). However, the increases were not reproduced in the parallel cultures under identical conditions and were, therefore, not considered as reproducible mutagenic effects. Furthermore, the additional, third experiment, which was performed to verify these isolated increases, did not show any increase above the threshold. The RMS therefore considers that the data show BAS 750 F to be non-mutagenic under the conditions of the study.

Table B6.4.1.7. Results of mouse lymphoma assay - experiment I (Wollny, 2015b)

Without metabolic activation, 4-hour exposure period		Rel. total growth	mutant colonies / 10^6 cells	Threshold#	Rel. total growth	mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Solvent ctrl. (DMSO)		100	113	239	100	63	189
BAS 750 F	3.1	127.8	115	239	102.8	97	189
	6.3	225.8	108	239	46.1	126	189
	12.5	178.9	93	239	100.5	95	189
	25.0	184.9	76	239	171.1	97	189
	37.5	66.3	45	239	50.1	72	189
Pos. ctrl. (MMS)	19.5	52.4	900	239	20.5	751	189
With metabolic activation, 4-hour exposure period		Rel. total growth	mutant colonies / 10^6 cells	Threshold	Rel. total growth	mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Solvent ctrl. (DMSO)		100	79	205	100	70	196
BAS 750 F	6.3	67.3	151	205	118.5	175	196
	12.5	52.9	149	205	77.8	**271**	196
	25.0	96.2	57	205	88.4	**304**	196
	50.0	30.4	**238**	205	62.8	149	196
Pos. ctrl. (CPA)	3.0	43.5	728	205	57.3	445	196
	4.5	12.9	1877	205	35.5	495	196

#: threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126

Table B.6.4.1.8. Results of mouse lymphoma assay - experiment II (Wollny, 2015b)

Without metabolic activation, 24-hour exposure period		Rel. total growth	mutant colonies / 10^6 cells	Threshold#	Rel. total growth	mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Solvent ctrl. (DMSO)		100	153	279	100	67	193
BAS 750 F	12.5	40.4	123	279	35.3	54	193
	25.0	34.3	129	279	27.5	90	193
	37.5	23.6	168	279	14.0	54	193
	50.0	9.1	181	279	8.0	56	193
	62.5	3.8	97	279	2.3	60	193
Pos. ctrl. (MMS)	13.0	13.7	1904	279	14.6	482	
With metabolic activation, 4-hour exposure period		Rel. total growth	mutant colonies / 10^6 cells	Threshold	Rel. total growth	mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Solvent ctrl. (DMSO)		100	105	231	100	140	266
BAS 750 F	6.3	101.5	81	231	103.7	131	266
	12.5	87.1	117	231	78.9	167	266
	25.0	56.1	140	231	66.0	168	266
	37.5	52.6	70	231	39.7	205	266
	50.0	19.2	91	231	31.4	101	266
Pos. ctrl. (CPA)	3.0	17.1	1044	231	31.7	668	266
	4.5	10.7	945	231	11.8	952	266

#: threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126

Table B.6.4.1.9. Results of mouse lymphoma assay - experiment III (Wollny, 2015b)

With metabolic activation, 4-hour exposure period		Rel. total growth	mutant colonies / 10^6 cells	Threshold#	Rel. total growth	mutant colonies / 10^6 cells	Threshold
Test item	Conc. [µg/mL]	Culture 1			Culture 2		
Solvent ctrl. (DMSO)		100	64	190	100	149	275
BAS 750 F	6.3	114.3	76	190	100	190	275
	12.5	94.0	79	190	83.8	179	275
	25.0	154.7	46	190	67.5	198	275
	50.0	28.0	53	190	6.9	102	275
Pos. ctrl. (CPA)	3.0	90.8	217	190	51.0	287	275
	4.5	36.8	341	190	38.9	406	275

#: threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126

BAS 750 F therefore showed no potential to induce gene mutations in mammalian (mouse lymphoma) cells in two standard, acceptable studies.

B.6.4.1.3. In vitro cytogenicity assay in mammalian cells (micronucleus test)

BAS 750 F has been tested for its potential to induce clastogenic and aneugenic effects in V79 Chinese hamster lung fibroblasts and in human lymphocytes in two *in vitro* micronucleus tests. In both tests, metabolic activation was provided by a hepatic S9-mixture from phenobarbital/ß-naphthoflavone-induced rats.

In the first test (Schulz & Landsiedel, 2014a), with V79 Chinese hamster lung fibroblasts, the test concentration ranged from 0.39 to 50 µg/ml; this was based on the findings in a preliminary cytotoxicity test, in which the highest concentration was 4000 µg/ml; test-substance precipitation occurred from 15.63 µg/ml and cytotoxicity from 31.25 µg/ml (4-hour exposure) and 7.81 µg/ml (24-hour exposure). In the main test, the vehicle DMSO was used as the negative control, and ethylmethanesulfonate (EMS) and CPA served as the positive control substances in the absence and presence of metabolic activation, respectively. Two independent experiments were performed in which the cells were incubated for four (with and without S9-mixture) or 24 hours (without S9-mixture). Following exposure to the test or control substances, the cell cultures were incubated with cytochalasin B, subsequently fixed and then the DNA and cytoplasm were stained. Cytotoxicity parameters[7] and the number of micronucleated cells were determined in at least 1000 binucleated cells per culture, i.e. 2000 cells for each test group.

Growth inhibition, as indicated by reduced cell counts of below 50 % compared with negative-control cultures, was observed in both the main experiments in the presence and absence of S9-mix at least at the highest applied test substance concentrations (see tables below). Dose-dependent decreases in the proliferation index and replicative index were recorded. Under all experimental conditions, the highest-tested concentrations could not be evaluated for cytogenetic damage because of cytotoxicity.

[7] Relative increase in cell count (RICC) = increase in number of cells in treated cultures relative to increase in negative-control cultures; cytokinesis-block proliferation index (CBPI) = a direct measure of the proliferative activity of the cells, indicating the average number of cell cycles per cell during the period of exposure to the actin polymerisation inhibitor cytochalasin B; replicative index (RI) = an additional parameter for proliferation that indicates the relative number of cells in treated cultures compared with the respective negative-control cultures; cell morphology assessed at the end of the treatment period.

Additionally, cell attachment / cell morphology was adversely affected from 25 µg/ml with S9-mix and from 3.13 µg/ml without S9-mix. It is thus concluded that adequate concentrations were tested.

No biologically-relevant increase in the number of micronucleated cells was observed either with or without S9-mixture (see tables below) in the BAS 750 F cultures. In both experiments, the obtained percentages of micronucleated cells (0.2 - 0.8 %) were close to the concurrent vehicle control values (0.4 - 0.9 % micronucleated cells) and were clearly within the historical negative-control data range (0.1 - 1.8 % micronucleated cells). In experiment I in the absence of S9-mixture, exposure to 300 µg/ml EMS resulted in a micronucleus rate (0.6 %) that was within the laboratory's historical negative-control data range. Therefore, to fulfil the acceptance criteria of the guideline, an additional positive-control test group, treated with 400 µg/ml EMS, was scored. The value of this test group (2.4 % micronucleated cells), together with the values from the other positive control cultures, was clearly above the historical negative control data range and within the historical positive control data range.

Table B.6.4.1.10 Results of *in vitro* micronucleus test in V79 cells – without S9-mix (Schulz & Landsiedel, 2014a)

Without metabolic activation (S9-mix): 4-hour exposure period, harvest at 24 hours						
Experiment I			Cytotoxicity			Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	RICC [%]	CBPI [%]	RI [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO)		n.d.	100	0.0	100	0.7
BAS 750 F	1.56	-	126.1	n.d.	n.d.	n.d.
	3.13	-	97.2	2.5	97.5	0.4
	6.25	-	122.5	4.5	95.5	0.5
	12.5	-	76.3	13.4	86.5	0.3
	25.0	+	-3.3	43.5	56.5	0.2
	50.0	+	-20.8	n.d.	n.d.	n.d.
Pos. ctrl. (EMS)	300.0	n.d.	104.7	3.0	97.0	0.6
	400.0	n.d.	86.9	-1.5	101.5	**2.4***
Without metabolic activation (S9-mix): 24-hour exposure period, harvest at 24 hours						
Experiment II			Cytotoxicity			Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	RICC [%]	CBPI [%]	RI [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO)		n.d.	100.0	0.0	100.0	0.4
BAS 750 F	0.39	-	99.8	12.6	87.4	0.4
	0.78	-	97.3	37.2	62.8	0.2
	1.56	-	124.6	42.4	57.6	0.4
	3.13	-	-41.1	n.d	n.d	n.d
	6.25	-	-63.3	n.s.	n.s.	n.s.
	12.5	-	-66.6	n.s.	n.s.	n.s.
Pos. ctrl. (EMS)	300.0	n.d.	151.8	-1.0	101.0	**2.4***

* statistically significantly increased over corresponding control values

RICC = relative increase in cell count; CBPI = proliferation index; RI = replicative index; n.d. = not determined; n.s. = not scorable because of strong cytotoxicity

Table B.6.4.1.11 Results of *in vitro* micronucleus test in V79 cells – with S9-mix (Schulz & Landsiedel, 2014a)

With metabolic activation (S9-mix): 4-hour exposure period, harvest at 24 hours						
Experiment I			Cytotoxicity			Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	RICC [%]	CBPI [%]	RI [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO)		n.d.	100.0	0.0	100.0	0.9
BAS 750 F	1.56	-	118.7	n.d.	n.d.	0.9
	3.13	-	109.3	n.d.	n.d.	n.d.
	6.25	-	103.5	-0.6	100.6	0.5
	12.5	-	114.3	27.8	72.2	0.3
	25.0	+	61.3	38.6	61.4	0.2
	50.0	+	-48.0	n.d.	n.d.	n.d.
Pos. ctrl. (CPP)	1.0	n.d.	99.7	52.9	47.1	**12.8****
With metabolic activation (S9-mix): 4-hour exposure period, harvest at 44 hours						
Experiment II			Cytotoxicity			Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	RICC [%]	CBPI [%]	RI [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO)		n.d.	100.0	0.0	100.0	0.5
BAS 750 F	1.56	-	138.8	n.d.	n.d.	n.d.
	3.13	-	154.1	n.d.	n.d.	n.d.
	6.25	-	154.5	-0.6	100.6	0.8
	12.5	-	170.4	17.4	82.6	0.5
	25.0	-	94.3	23.0	77.0	0.5
	50.0	+	-10.0	n.d.	n.d.	n.d.
Pos. ctrl. (CPP)	1.0	n.d.	122.0	0.0	100.0	**7.9****

Statistical evaluation: *: p≤0.05; **: p≤0.01 (Fisher's Exact test (1-sided), with Bonferroni-Holm correction); statistically significantly increased over corresponding control values

RICC = relative increase in cell count; CBPI = proliferation index; RI = replicative index; n.d. = not determined

In the second test (Sokolowski, 2015a), two independent experiments were performed in which human peripheral-blood lymphocytes were incubated for 4 (±S9-mix) or 20 hours (-S9-mix) with the test substance at concentrations in the range of 0.3 to 2094 µg/mL; concentrations from 2.0 to 8.2 µg/mL were subsequently evaluated, since excessive cytotoxicity and precipitation of the test substance were observed at higher concentrations. The lymphocytes were obtained from healthy, non-smoking donors: one male for experiment I and one female for experiment II. The vehicle DMSO served as the negative control, mitomycin C (4 hours' incubation) and demecolcin (20 hours' incubation) as positive controls in the absence of metabolic activation and CPA as the positive control in the presence of metabolic activation. Exposure was started after a 48-hour stimulation period with phytohaemagglutinin. Thereafter, cytochalasin B was added and the cultures were fixed and stained after another 20 hours. Cytokinesis-block proliferation index (CBPI) and cytostasis were determined in 500 binucleated cells/culture to measure cytotoxicity and the numbers of micronucleated cells were determined in 1000 binucleated cells/culture for the evaluation of genotoxicity.

No relevant increase in the number of micronucleated cells was observed after treatment with the test item in either experiment, neither in the absence nor presence of S9-mix (see tables below). The positive control substances showed the expected increases in cells with micronuclei, whilst the number

of micronucleated cells induced by the vehicle control was within the range of the historical control data.

Table B6.4.1. 12. Results of *in vitro* micronucleus test in human lymphocytes - without S9-mix (Sokolowski, 2015a)

Without metabolic activation (S9-mix): 4-hour exposure period, harvest at 40 hours					
Experiment I			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipitation	CBPI [%]	Cytostasis [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO) [1]		-	1.95		0.50
BAS 750 F	2.0	-	1.77	18.7	0.40
	4.1	-	1.50	47.8	0.35
	8.2	-	1.26	72.7	0.20
Pos. ctrl. (MMC) [2]	2.0	-	1.41	56.5	**5.60***
Without metabolic activation (S9-mix): 20-hour exposure period, harvest at 40 hours					
Experiment II			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipitation	CBPI [%]	Cytostasis [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO) [1]		-	1.82		0.95
BAS 750 F	2.0	-	1.72	13.0	0.70
	4.1	-	1.63	23.3	0.75
	8.2	-	1.39	52.7	0.75
Pos. ctrl. (DMC) [3]	0.05	-	1.85	n.c.	**3.30***

[1] DMSO = Dimethyl sulfoxide: 0.5 % (v/v); [2] MMC = Mitomycin: 2.0 µg/mL; [3] DMC = Demecolcin: 50 ng/mL
n. c.: not calculated as the CBPI is equal or higher than the solvent control value
Statistical evaluation: *: $p \leq 0.05$ (Chi-square test)

Table 5.4.1-15: Results of *in vitro* micronucleus test in human lymphocytes – with S9-mix

With metabolic activation (S9-mix): 4-hour exposure period, harvest at 40 hours					
Experiment I			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipitation	CBPI [%]	Cytostasis [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO) [1]		-	2.01		0.30
BAS 750 F	2.0	-	1.89	12.2	0.60
	4.1	-	1.63	37.6	0.20
	8.2	-	1.30	70.5	0.55
Pos. ctrl. (CPA) [2]	17.5	-	1.66	34.4	**3.95***
Experiment II			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipitation	CBPI [%]	Cytostasis [%]	Micronucleated cells (%)
Neg. ctrl. (DMSO) [1]		-	2.02		0.75
BAS 750 F	2.0	-	1.97	4.9	0.70
	4.1	-	1.81	20.4	0.80
	8.2	-	1.59	42.0	0.65
Pos. ctrl. (CPA) [2]	15.0	-	1.66	35.9	**5.50***

[1] DMSO = Dimethyl sulfoxide: 0.5 % (v/v); [2] CPA = Cyclophosphamide: 17.5 / 15.0 µg/mL
Statistical evaluation: *: p≤0.05 (Chi square test)

BAS 750 F therefore showed no potential to induce clastogenic or aneugenic changes in Chinese hamster lung fibroblasts or human peripheral-blood lymphocytes in two standard, acceptable studies when tested up to cytotoxic concentrations.

B.6.4.2. *In vivo* studies in somatic cells

A micronucleus test has been conducted to investigate the potential of BAS 750 F to induce chromosomal damage in NMRI mice. A range-finding study was first conducted, in which male and female mice (3/sex/group) were orally administered a single dose of 2000 mg/kg. Based on findings in the range-finding study (severe signs of toxicity), in the main test BAS 750 F was administered in a single oral dose of 375, 750 and 1500 mg/kg in a volume of 20 ml/kg body weight. The vehicle served as the negative and CPA and vincristine as the positive controls. The animals were sacrificed 24 or 48 (additional high-dose group) hours after the administration, with the bone marrow of the two femora being prepared from each animal. For each animal, 2000 polychromatic erythrocytes were evaluated for micronuclei, therefore 10 000 were scored per test group. The normocytes occurring per 2000 polychromatic erythrocytes were also recorded. Blood samples taken immediately after sacrifice were analysed to verify the bioavailability of the test substance.

Administration of BAS 750 F did not lead to any biologically relevant increase in the number of polychromatic erythrocytes that contained micronuclei (see table below). The rate of micronuclei was mostly close to the concurrent negative control and was within the range of the historical control data. The positive-control for clastogenicity, CPA, led to the expected increase in the rate of polychromatic erythrocytes that contained exclusively small micronuclei, whilst vincristine, which is a spindle poison, produced a statistically significant increase in micronuclei, approximately 10 % of which were attributable to large micronuclei.

Table B6.4.2.1 Induction of micronuclei in bone marrow cells in *in vivo* study

Sampling: 24 h post-dosing		PCE				NCE		PCE / NCE ratio
		Scored	With MN			No.	With MN	
			Total [‰]	Small [‰]	Large [‰]		[‰]	
BAS 750 F:	0 mg/kg bw	10 000	0.6	0.6	0.0	5077	0.8	1.97
BAS 750 F:	375 mg/kg bw	10 000	1.2	1.2	0.0	5202	0.6	1.92
BAS 750 F:	750 mg/kg bw	10 000	1.0	1.0	0.0	5097	0.4	1.96
BAS 750 F:	1500 mg/kg bw	10 000	1.6	1.5	0.1	4591	0.7	2.18
CPA:	20 mg/kg bw	10 000	18.9**	18.9**	0.0	4895	0.6	2.04
Vincristine:	0.15 mg/kg bw	10 000	42.0**	31.9**	10.1**	6361	1.4	1.57
Sampling: 48 h post-dosing		PCE				NCE		PCE / NCE ratio
		Scored	With MN			No.	With MN	
			Total [‰]	Small [‰]	Large [‰]		[‰]	
BAS 750 F:	0 mg/kg bw	10 000	1.5	1.5	0.0	4421	1.6	2.26
BAS 750 F:	1500 mg/kg bw	10 000	1.3	1.3	0.0	6799	1.0	1.47

Statistical analysis: ** = $p \leq 0.01$ (Wilcoxon-test, 1-sided); MN = Micronucleated cells;
PCE = Polychromatic erythrocytes; NCE = normochromatic erythrocytes;

The bioavailability of the test substance in blood after oral administration was confirmed by LC/MS analysis in plasma samples from all test animals and also in a biokinetics' study conducted in mice ([redacted], 2014a; section B.6.1). Since the bone-marrow is well perfused, it is expected to be exposed to BAS 750 F or its metabolites. Furthermore, an inhibition of erythropoiesis (decreased PCE/NCE ratio) in the top-dose group at the 48-hour sacrifice indicated bone-marrow toxicity. Clinical signs, which were observed in all dose groups, included piloerection, hunched posture, reduced general condition, lacrimation and irregular respiration. The RMS concludes that a valid negative result was obtained in this study.

Under the experimental conditions of this study, BAS 750 F did not induce cytogenetic damage in the bone marrow cells of NMRI mice *in vivo*.

B.6.4.3. *In vivo* studies in germ cells

The available database on the genotoxicity of BAS 750 F did not give rise to any concern from either the *in vitro* or *in vivo* studies. On this basis, an additional genotoxicity study in germ cells is not required. The absence of this study does not constitute a data gap.

B.6.4.4. Conclusion on genotoxicity

A standard dataset of six *in vitro* studies in bacterial and mammalian cells and an *in vivo* micronucleus study has been submitted. This dataset gave no indication that BAS 750 F has a genotoxic potential. The RMS thus concludes that BAS 750 F is not genotoxic under the conditions of the studies investigated.

B.6.5. LONG-TERM REPEATED-EXPOSURE TOXICITY AND CARCINOGENESIS

The long-term toxicity and carcinogenicity of BAS 750 F have been investigated in a two-year study in rats and in an 18-month study in mice. The NOAELs presented in the table below are those proposed by the RMS. In addition, the RMS has performed BMD analysis to identify a more scientifically robust, transparent reference point.

Table B.6.5.1. Summary of chronic / carcinogenicity studies with BAS 750 F

Study Purity	**Species**	**Doses**	**Chronic NOAEL / BMD (mg/kg bw/d)**	**Main effects**
24-month combined chronic toxicity / carcino-genicity (dietary) OECD 453 GLP Purity 98.8% CA 5.5/1: [redacted] 2016b (2015/1000531) CA 5.5/2 – historical control data: [redacted] 2015a (2015/1261375)	Rat, Wistar 10/sex/dose for chronic phase (12 months) 50/sex/dose for carcino-genicity phase (24 months)	0, 100, 600, 3600 ppm Equivalent intake at 24-months: Males: 0, 4, 25, 163 mg/kg bw/d Females: 0, 6, 38, 302 mg/kg bw/d	**NOAEL** 5 (at 12 months) (100 ppm) **$BMDL_{15}$** 7 (change in urea levels)	**<u>Chronic phase – 12 months</u>** No deaths in any dose group. No overt clinical signs of toxicity. <u>100 ppm:</u> No adverse effects <u>600 ppm:</u> Haematology: ↓ activated partial thromboplastin time in males Altered clinical chemistry parameters in males (↑ ALP & urea) <u>3600 ppm:</u> ↓ mean bw (final bw -8.3%** in males, -13.8%** in females) and bwg (overall -12%** in males, -27.1%** in females) Haematology: ↓ activated partial thromboplastin time in males & females, ↓ platelet counts in males Altered clinical chemistry parameters in males & females (↑ ALP, cholesterol, glucose, urea; ↓ total protein, albumin, creatinine) Increased liver weight (9-22 %, males & females) & centrilobular hypertrophy (minimal / slight) **<u>Carcinogenicity phase – 24 months</u>** Deaths to day 728 (24 months) were: **Dose level (ppm)** / **Mortality (%)** – **Males** / **Females** 0 – 0 – 24 (12/50) 100 – 24 (12/50) – 20 (10/50) 600 – 22 (11/50) – 18 (9/50) 3600 – 6 (3/50) – 10 (5/50) *<u>Non-neoplastic effects</u>* No overt clinical signs of toxicity in any group.

Table B.6.5.1. Summary of chronic / carcinogenicity studies with BAS 750 F

Study Purity	Species	Doses	Chronic NOAEL / BMD (mg/kg bw/d)	Main effects
				100 ppm: No adverse effects 600 ppm: ↑ relative liver wt in females (16 %) 3600 ppm: ↓ mean bw (final bw -11.6%** in males & -21.9%** in females) and bwg (overall bwg -15.7%** in males, -35.1%** in females) ↑ relative liver wt (7 & 23 %, males & females) *Neoplastic findings* Not carcinogenic in rats
18-month carcinogenicity (dietary) OECD 451 GLP Purity 98.8 % CA 5.5/3: [redacted] 2015b (2015/1000532) CA 5.5/4 – historical control data: [redacted] 2015a (2015/1261376)	Mouse (C57BL/6JRj) 50/sex/dose	Males: 0, 20, 50, 200 ppm Equivalent to 0, 3.5, 9.1, 36 mg/kg bw/d Females: 0, 20, 50, 250 ppm Equivalent to 0, 4.9, 12.6, 61.5 mg/kg bw/d	**NOAEL** 3.5 (20 ppm) **$BMDL_{15}$** 5 (relative liver weight)	Survival to termination not affected by administration of test substance. No treatment-related overt clinical signs of toxicity. *Non-neoplastic effects* 20 ppm No adverse effects 50 ppm Relative liver weight ↑ (18 %) with hepatocellular fatty change in males 200 ppm / 250 ppm ↓ body wt & bwg (-14 % in males, -33 % females) Relative liver wt ↑ by 42 % males & 57 % females Increased incidence & severity of fatty change and signs of (pre)degeneration in liver cells (eosinophilic inclusions in males, single cell necrosis in females) ↑ thyroid follicular-cell hyperplasia (74 % of males at 200 ppm compared with 42 % of control males) *Neoplastic findings* Not carcinogenic in mice

bw = body weight; bwg = body-weight gain; * = statistically significant, $p \leq 0.05$; ** = statistically significant, $p \leq 0.01$

B.6.5.1. Chronic / carcinogenicity study in rats

BAS 750 F was administered via the diet to Wistar rats over a period of either 12 or 24 months at dietary concentrations of 0, 100, 600 and 3600 ppm (see table below for corresponding mean intakes at the two time points). Groups of 10 rats/sex were assessed for chronic toxicity after 12 months of exposure and groups of 50 rats/sex were evaluated for carcinogenicity following a 24-month exposure period.

Table B6.5.1.1 Mean test substance intake in rat chronic / carcinogenicity study

	Males			Females		
Dose level [ppm]	**100**	**600**	**3600**	**100**	**600**	**3600**
BAS 750 F (mg/kg bw/d)						
- Satellite groups (Day 0-371)	5	31	191	7	41	300
- Main groups (Day 0-728)	4	25	163	6	38	302

There were no deaths in any of the satellite groups in the chronic phase of the study (12 months' exposure). In the main, carcinogenicity, groups, there was not a treatment-related increase in deaths. Survival exceeded 75 % in all groups. There were no overt clinical signs of toxicity in any group in either the satellite or main cohorts.

Body weights and body-weight gain were reduced in the high-dose male and female groups after both 12 and 24 months of exposure from day 7 and then throughout the duration of the study. There were no statistically significant changes in body weight and body-weight gain in the low- and mid-dose groups. Food consumption and water consumption were unaffected at all doses.

Table B6.5.1.2. Body weight development (main groups) in rat chronic / carcinogenicity study

	Males				Females			
Dose level [ppm]	**0**	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
Body weight [g]								
- Day 0	159.1	159.6	159.2	158.3	128.4	128.1	126.0	127.5
- Day 91	386.3	400.0	386.0	**355.4****	230.8	232.8	230.3	**211.6****
% change from control		+3.5	-0.1	**-8.0**		+0.9	-0.2	**-8.3**
- Day 371	502.0	518.0	502.4	**460.2****	279.4	278.6	270.1	**240.9****
% change from control		+3.2	+0.1	**-8.3**		-0.3	-3.3	**-13.8**
- Day 728	582.1	584.3	572.0	**519.9****	334.1	338.5	317.8	**260.9****
% change from control		+0.4	-1.7	**-11.6**		+1.3	-4.9	**-21.9**
Overall body weight gain (g)								
- Day 91	227.2	240.3	226.7	**197.1****	102.4	104.7	104.3	**84.0****
% change from control		+5.8	-0.2	**-13.3**		+2.2	+1.8	**-18.0**
- Day 371	342.9	358.2	343.2	**301.9****	158.5	155.4	147.9	**115.6****
% change from control		+4.5	+0.1	**-12.0**		-2.0	-6.7	**-27.1**
- Day 728	423.0	424.8	413.3	**356.8****	206.3	210.6	192.5	**133.8**
% change from control		+0.4	-2.3	**-15.7**		+2.1	-6.7	**-35.1**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

At the start of the administration period, ophthalmoscopy was performed on all satellite animals, and then on the eyes of the control and high-dose animals at the end of the administration period (12 months). There were no treatment-related effects.

Blood was collected for haematology and clinical-chemistry parameter investigations from the satellite animals (12 months) at the 3, 6 and 12-month time-points. Treatment-related changes in haematology parameters consisted of slight decreases in the activated partial thromboplastin time (PTT) in males at 600 ppm and both sexes at 3600 ppm, and a decrease in platelet counts in males at 3600 ppm (12 months). Other changes were either not dose-related or were within the relevant historical control ranges (data not shown).

Table B6.5.1.3. Selected haematology parameters (treatment-related) in rat chronic / carcinogenicity study

Dose level [ppm]		Males				Females			
		0	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
Parameter	**Month**								
PTT [s]	3	21.4	21.3	20.1	**18.8**** (-12 %)	19.9	19.7	19.6	**18.5*** (-7 %)
	6	19.7	19.3	**18.8*** (-5 %)	**17.9**** (-9 %)	18.5	18.7	18.4	**17.3** (-6 %)
	12	20.3	19.8	**19.2*** (-5 %)	**18.4**** (-9 %)	19.1	19.6	19.3	**17.9*** (-6 %)
PLT [giga/L]	3	771	727	719	667	819	796	750	781
	6	693	661	663	663	747	724	691	709
	12	724	661	680	**640**** (-12 %)	716	717	666	691

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test two sided)

Several treatment-related changes in clinical-chemistry parameters were recorded at each time-point. These comprised increases in ALP and decreases in ALT, glucose and total bilirubin in males and females. Additionally, urea was increased in males of the mid- and high-dose groups. Creatinine was statistically significantly decreased and cholesterol increased in females at 3600 ppm, but only at the three-month time-point; increases in the latter parameter in males at the three and six-month time-points were within the historical control range and so not clearly attributable to BAS 750 F administration. In high-dose females, albumin and total-protein levels were statistically significantly reduced at three and six months. The changes in ALT levels were relatively small (less than 50 %) and were probably an indication of liver-enzyme induction as a result of an adaptive response rather than adversity, whilst lower glucose levels perhaps reflected increased energy consumption as a result of greater liver-cell metabolism. Likewise, the lower total bilirubin levels probably reflected an increased conjugation rate of bilirubin and a subsequent higher excretion via bile and thus, although treatment-related, were not adverse. Overall, the clinical chemistry investigations indicated that adaptive changes and some alterations to liver-cell metabolism resulted from exposure to BAS 750 F. This was supported by the haematology investigations, since reduced PTT indicated an increased synthesis of coagulation factors in the liver, which led to some consumption of platelets and hence reduced platelet counts by 12 months.

Table B6.5.1.4. Selected clinical chemistry parameters (treatment-related) in rat chronic / carcinogenicity study

Dose [ppm]		Males				Females			
		0	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
Parameter	**Month**								
ALT [µkat/L]	3	0.92	0.68**	0.71*	0.65**	0.53	0.61	0.48	0.55
	HCR 3	*range: 0.53–0.85 µkat/L; mean: 0.70*							
	6	0.72	0.65	0.67	0.61	0.54	0.56	0.49	0.46
	12	0.81	0.71	0.72	0.56*	0.80	0.66	0.73	0.48**
ALP	3	1.08	1.12	**1.38****	**1.59****	0.48	0.61	0.56	**0.88****

Table B6.5.1.4. Selected clinical chemistry parameters (treatment-related) in rat chronic / carcinogenicity study

Dose [ppm]		Males				Females			
		0	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
[µkat/L]				(+27 %)	(+47 %)				(+83 %)
	6	0.92	0.98	**1.24**** (+35 %)	**1.33**** (+45 %)	0.34	0.47**	0.43	**0.63**** (+85 %)
	12	0.92	1.00	**1.28**** (+39 %)	**1.36**** (+48 %)	0.33	0.50*	0.44	**0.72**** (+218 %)
Glucose [mmol/L]	3	6.95	6.54	6.38	**5.49**** (-21 %)	5.46	5.40	5.50	**4.86*** (-11 %)
	6	6.88	6.53	6.29	**5.76**** (-16 %)	5.63	5.26	5.19	5.05
	12	6.59	6.43	6.22	**5.79**** (-12 %)	6.10	5.91	5.64	**4.90**** (-20 %)
Bilirubin, tot. [µmol/L]	3	1.96	1.83	1.76	1.28**	2.00	1.90	1.81	1.23**
	6	1.54	1.69	1.56	1.21	3.08	2.98	2.45*	1.71**
	12	1.33	1.51	1.48	1.15	2.39	2.18	1.96	1.28**
Protein, total [g/L]	3	62.48	62.45	61.74	62.00	67.04	65.49	64.67*	**60.68**** (-9 %)
	HCR 3					*range: 62.13–70.12 g/L; mean: 66.25*			
	6	63.24	63.32	63.58	61.66	66.53	66.25	64.96	**61.77**** (-7 %)
	12	67.26	68.09	68.12	65.99	70.53	71.11	70.31	67.98
Albumin [g/L]	3	34.25	34.41	33.85	34.33	42.52	41.58*	40.76*	**38.30**** (-10 %)
	HCR 3					*range: 37.49–43.65 g/L; mean: 41.07*			
	6	38.50	38.70	38.63	38.02	41.79	41.43	40.63	**38.22**** (-10 %)
	12	39.55	39.82	39.68	38.97	43.15	43.40	42.75	41.12
Globulin [g/L]	3	28.23	28.05	27.89	27.67	24.52	23.91	23.91	22.38*
	HCR 3					*range: 19.54–29.31 g/L; mean: 25.26*			
	6	24.75	24.62	24.95	23.64	24.74	24.81	24.33	23.54
	12	27.71	28.26	28.45	27.02	27.38	27.71	27.56	26.86
Cholesterol [mmol/L]	3	1.63	1.88	1.92*	2.02*	1.39	1.40	1.29	**1.95**** (+40 %)``
	HCR 3	*range: 1.48–2.14 mmol/L; mean: 1.82*							
	6	1.79	2.05	2.12	2.29**	1.60	1.53	1.45	1.96
	HCR 6	*range: 1.84–2.32 mmol/L; mean: 2.09*							
	12	2.33	2.47	2.57	2.77	2.18	2.09	1.97	2.37
Urea [mmol/L]	3	6.79	6.48	7.20	7.05	6.72	7.19	7.26	7.67
	6	5.33	5.67	5.81	**6.27**** (+18 %)	5.86	6.05	5.96	6.78
	12	4.05	4.34	**4.72**** (+17 %)	**5.15**** (+25 %)	6.02	6.01	5.72	6.78

Table B6.5.1.4. Selected clinical chemistry parameters (treatment-related) in rat chronic / carcinogenicity study

Dose [ppm]		Males				Females			
		0	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
Creatinine [µmol/L]	3	55.9	56.1	57.8	55.6	59.2	58.7	60.2	**54.5**** (-8 %)
	6	31.8	31.5	32.6	32.9	36.3	37.2	39.0	36.1
	12	32.1	30.8	33.0	31.8	35.0	35.1	35.6	34.6

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test. two sided)
HCR = Historical control data - 3-month: 48 studies (2008-2013); - 6-month: 11 studies (2004-2014)

There were no treatment-related changes in urinalysis parameters.

Organ weights were measured after 12 and 24 months of exposure to BAS 750 F. At 12 months, increased relative weights of the brain, heart and spleen in females of the high-dose group reflected the decreased terminal body weight of these animals. In these organs, treatment-related histopathological findings were not observed. In males, the terminal body weight was unaffected by BAS 750 F administration and there were no statistically-significant differences in organ weights between test and control groups.

At 24 months, the terminal body weight was statistically significantly decreased in females at 600 ppm and 3600 ppm and in males at 3600 ppm. Consequently, increased relative weights of the brain, epididymides, heart and kidneys were reported in these animals. The increased relative weights of the adrenal glands in females of the mid- and top-dose groups were likewise related to the reduced terminal body weight. There were no histopathological treatment-related findings in any of these organs. Although relative adrenal weights were increased in males of the low- and mid-dose groups, there was no dose-response relationship; therefore the RMS considers this to be an incidental finding. The mean values of the relative ovarian weights of control and high-dose females were identical but showed statistical significance; this was based on the comparison of the median values, i.e., a slightly higher value in the high-dose group. The statistically significant deviation was considered to be of no factual relevance. Furthermore, the large standard deviations (0.058 for the control group and 0.044 for the 3600 ppm group) demonstrated that there was large variation in individual values and overlap between the groups.

The RMS concludes that, since there were histopathological correlates, the increases in relative liver weights in both sexes at both time-points were treatment related. There were no treatment-related changes in the weights of the testes, uterus or thyroid (the latter was determined only at 12 months) at either time-point.

Table B.6.5.1.5. Organ weights (12-month chronic toxicity group) – rat study

Sex		Males				Females			
Organ weight	**Dose [ppm]**	**Absolute weight**	**%**	**Relative weight [% of bw]**	**Δ% #**	**Absolute weight**	**%**	**Relative weight [% of bw]**	**%**
Terminal body	**0**	472.75				263.93			
weight [g]	**100**	461.58	(-2)			264.95	(±0)		
	600	462.84	(-2)			255.29	(-3)		
	3600	442.41	(-4)			**224.89****	**(-15)**		
Adrenal glands	**0**	51.2		0.011		59.2		0.023	
(mg)	**100**	51.6	(+1)	0.011	(+3)	58.5	(-1)	0.022	(-1)
	600	47.9	(-6)	0.010	(-5)	62.2	(+10)	0.026	(+14)
	3600	47.8	(-7)	0.011	(±0)	**51.6***	**(-13)**	0.023	(+3)
Brain	**0**	2.195		0.466		2.028		0.774	
(g)	**100**	2.233	(+2)	0.488	(+5)	2.024	(±0)	0.769	(-1)
	600	2.210	(+1)	0.480	(+3)	2.088	(+3)	0.823	(+6)
	3600	2.185	(±0)	0.499	(+7)	1.965	(-3)	**0.880***	**(+14)**
Epididymides (g)	**0**	1.165		0.247		79.6		0.030	
	100	1.183	(+2)	0.258	(+5)	75.5	(-5)	0.029	(-4)
Ovaries (mg)	**600**	1.130	(-3)	0.247	(±0)	88.7	(+11)	0.035	(+14)
	3600	1.086	(-7)	0.248	(±0)	105.7	(+33)	0.047	(+56)
Heart	**0**	1.128		0.239		0.795		0.303	
(g)	**100**	1.112	(-1)	0.241	(+1)	0.827	(+4)	0.314	(+3)
	600	1.106	(-2)	0.239	(±0)	0.801	(+1)	0.314	(+3)
	3600	1.071	(-5)	0.243	(+1)	0.779	(-2)	**0.349****	**(+15)**
Kidneys	**0**	2.326		0.492		1.727		0.657	
(g)	**100**	2.437	(+5)	0.530	(+8)	1.651	(-4)	0.625	(-5)
	600	2.384	(+2)	0.516	(+5)	1.664	(-4)	0.653	(-1)
	3600	2.303	(-1)	0.521	(+6)	**1.465****	**(-15)**	0.654	(-1)
Liver	**0**	9.58		2.031		5.537		2.103	
(g)	**100**	9.746	(+2)	2.114	(+4)	5.951	(+7)	2.239	(+6)
	600	9.581	(±0)	2.068	(+2)	5.777	(+4)	2.270	(+8)
	3600	9.797	(+2)	**2.212***	**(+9)**	5.734	(+4)	**2.559****	**(+22)**
Spleen	**0**	0.667		0.141		0.507		0.192	
(g)	**100**	0.695	(+4)	0.151	(+7)	0.462	(-9)	0.175	(-9)
	600	0.704	(+6)	0.152	(+8)	0.500	(-1)	0.197	(+3)
	3600	0.686	(+3)	0.155	(+10)	0.498	(-2)	**0.222***	**(+16)**
Testes (g)	**0**	4.019		0.855		1.193		0.462	
	100	4.004	(±0)	0.871	(+2)	0.888	(-26)	0.336	(-27)
Uterus (g)	**600**	3.893	(-3)	0.846	(-1)	0.883	(-26)	0.350	(-24)
	3600	3.808	(-5)	0.866	(+1)	0.793	(-34)	0.363	(-21)
Thyroid	**0**	25.0		0.005		18.4		0.007	
(mg)	**100**	23.6	(-4)	0.005	(-4)	18.6	(+1)	0.007	(+1)
	600	23.0	(-8)	0.005	(-6)	18.0	(-2)	0.007	(±0)
	3600	23.1	(-8)	0.005	(-1)	16.5	(-10)	0.007	(+5)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

Table B.6.5.1.6. Organ weights (24-month carcinogenicity group) – rat study

Sex		Males				Females			
Organ weight	**Dose [ppm]**	**Absolute weight**	**%**	**Relative weight [% of bw]**	**%**	**Absolute weight**	**%**	**Relative weight [% of bw]**	**%**
Terminal body	**0**	559.634				320.505			
Weight [g]	**100**	557.305	(±0)			319.898	(±0)		
	600	548.531	(-2)			**296.361****	(-8)		
	3600	**492.340****	**(-12)**			**245.609****	**(-23)**		
Adrenal glands	**0**	57.260		0.010		60.514		0.019	
(mg)	**100**	**61.158***	**(+7)**	**0.011***	**(+7)**	63.675	(+5)	0.021	(+6)
	600	61.231	(+7)	**0.011***	**(+8)**	62.439	(+3)	**0.022***	**(+11)**
	3600	**52.532****	**(-8)**	0.011	(+3)	**55.933***	**(-8)**	**0.023****	**(+18)**
Brain	**0**	2.254		0.412		2.048		0.652	
(g)	**100**	2.265	(±0)	0.414	(±0)	2.085	(+2)	0.670	(+3)
	600	2.272	(+1)	0.423	(+3)	2.087	(+2)	**0.717****	**(+10)**
	3600	2.214	(-2)	**0.453****	**(+10)**	2.047	(±0)	**0.839****	**(+29)**
Epididymides	**0**	1.261		0.229					
(g)	**100**	1.149	(-9)	0.210	(-8)				
	600	1.459	(+16)	0.271	(+18)				
	3600	1.143	(-9)	**0.233****	**(+2)**				
Heart	**0**	1.307		0.235		0.952		0.301	
(g)	**100**	1.276	(-2)	0.230	(-2)	0.946	(-1)	0.302	(±0)
	600	1.287	(-1)	0.237	(+1)	0.942	(-1)	**0.322***	**(+7)**
	3600	**1.217****	**(-7)**	**0.248****	**(+5)**	**0.876****	**(-8)**	**0.358****	**(+19)**
Kidneys	**0**	2.935		0.529		2.002		0.634	
(g)	**100**	2.971	(+1)	0.538	(+2)	2.018	(+1)	0.643	(+1)
	600	2.918	(-1)	0.536	(+1)	1.995	(±0)	**0.682***	**(+8)**
	3600	**2.743***	**(-7)**	**0.559****	**(+6)**	**1.776****	**(-11)**	**0.724****	**(+14)**
Liver	**0**	11.655		2.078		6.674		2.094	
(g)	**100**	11.394	(-2)	2.036	(-2)	6.987	(+5)	2.201	(+5)
	600	12.266	(+5)	2.214	(+7)	7.100	(+6)	**2.435****	**(+16)**
	3600	10.940	(-6)	**2.224****	**(+7)**	**6.337***	**(-5)**	**2.580****	**(+23)**
Ovaries	**0**					142.838		0.047	
(mg)	**100**					117.150	(-18)	0.036	(-23)
	600					113.805	(-20)	0.039	(-15)
	3600					112.224	(-21)	**0.047****	**(±0)**
Spleen	**0**	0.958		0.171		0.649		0.204	
(g)	**100**	0.940	(-2)	0.169	(-1)	0.721	(+11)	0.231	(+13)
	600	1.933	(+102)[i]	0.310	(+81)	0.664	(+2)	0.229	(+13)
	3600	0.913	(-5)	0.185	(+8)	**0.600****	**(-8)**	**0.242**	**(+19)**
Testes	**0**	4.358		0.779					
(g)	**100**	4.137	(-5)	0.747	(-4)				
	600	4.042	(-7)	0.752	(-3)				
	3600	4.229	(-3)	0.863	(+11)				
Uterus	**0**					1.072		0.342	
(g)	**100**					1.158	(+8)	0.388	(+14)
	600					1.323	(+23)	0.462	(+35)
	3600					1.081	(+1)	0.442	(+29)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

[i] high mean spleen wt in males at 600 ppm were due to 3 males (#112: 24.12 g, #135: 13.58 g, #145: 3.7 g); and arose from a malignant lymphoma (#112) or from infiltration of a histiocytic sarcoma with severe extramedullary hematopoiesis (#135) or from massive extramedullary hematopoiesis(#145)

There were no treatment-related findings upon gross necropsy at the 12-month interim sacrifice. At 24 months, foci were noted in the testes of some high-dose males and in the adrenal cortex of all the female treatment groups. Neither of these findings showed a clear dose-response relationship nor a histopathological correlate (see further analysis below). Consequently, the RMS does not consider that they provide conclusive evidence of a treatment-related effect.

Table B.6.5.1.7. Gross necropsy findings in rats administered BAS 750 F for two years

	Males				Females			
Dose level [ppm]	**0**	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
No. of animals	50	50	50	50	50	50	50	50
ADRENAL GLAND								
Foci in adrenal cortex		2	1	1	4	12	9	11
TESTES								
Foci	2	5	4	10	—			

Histopathology at 12 months did not reveal any treatment-related neoplasms in males or females. A minimal or slight treatment-related hepatocellular centrilobular hypertrophy was observed at 3600 ppm. In a small number of females of the high-dose group, a focal hyperplasia was observed in the *pars distalis* of the pituitary gland. Since the number of adenomas and of focal hyperplasia in the *pars distalis* of the pituitary gland was comparable between control and high-dose females after two years of treatment, the RMS concludes that this finding was likely to be incidental.

Table B.6.5.1.8. Incidence of histopathology findings in rats administered BAS 750 F for 1 year

	Males				Females			
Dose level [ppm]	**0**	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
No. of animals	10	10	10	10	10	10	10	10
LIVER								
examined	10	10	10	10	10	9	10	10
Hypertrophy, centrilobular				6				5
Grade 1				4				5
Grade 2				2				
PITUITARY GLAND								
examined	10			10	10			10
Hyperplasia, pars distalis	1							3
Cyst(s), pars distalis	3							
Cyst(s), pars intermedia	1				2			

Histopathology at 24 months revealed statistically-significant increases in the incidences of non-neoplastic changes in the liver, lungs and the thyroid gland. Given the increases in relative weights, the findings in the liver were concluded by the RMS to be treatment-related. An increased number of males with congestion of the lungs was observed in the mid-dose group. Congestion (presence of blood in alveolar capillaries) was only observed in animals that died prematurely (passive congestion), independently of the test group, and thus was not a treatment-related adverse effect. Because all control males survived until scheduled sacrifice, this finding was not seen in that group. In the absence of a dose-response relationship, the RMS considers the increase in altered colloid of the thyroid gland of low- and mid-dose males to be incidental.

Table B.6.5.1.9. Incidence of selected non-neoplastic findings in rats administered BAS 750 F for 2 years

	Males				Females			
Dose level [ppm]	**0**	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
No. of animals	50	50	50	50	50	50	50	50
LIVER								
examined	50	50	50	50	50	50	50	50
Hypertrophy, centrilobular				**15****				**7****
Grade 1				15				7
LUNGS								
examined	50	50	50	50	50	17	18	50
Congestion		2	**6***	1	2	2		1
PITUITARY GLAND								
examined	50	49	50	50	50	23	26	50
Hyperplasia, pars distalis (m)f	31	12	14	20	12	3	3	13
Hyperpl. p. intermedia, (m) f.	3	1	2	4		1		1
Hyperpl. p. intermedia, diff.	1							
Cyst(s), pars distalis	5	6	4	3	2	1	1	3
Cyst(s), pars intermedia	17	9	11	12	10	6	4	14
THYROID GLAND								
examined	50	50	50	50				
Altered colloid	4	**15****	**18****	7	4	10	9	4

Statistical analysis: *: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact test, 1-sided)

Extensive histopathological assessment of the gross necropsy findings (foci in the testes of males and the adrenal cortex of females) was undertaken. The macroscopically-diagnosed foci in the testes mostly represented Leydig cell adenomas, Leydig cell hyperplasia, multifocal tubular degeneration, or tubular mineralization, without any relationship to treatment.

Table B.6.5.1. 10. Incidence of findings in the testes (histopathology) – 24 months

	Males			
Dose level [ppm]	**0**	**100**	**600**	**3600**
No. of animals	50	50	50	50
TESTES				
examined	50	50	50	50
Adenoma, Leydig cell	1	2	1	3
Hyperplasia, Leydig cell, (multi)focal	8	2	5	8
Hyperplasia, Leydig cell, diffuse		2	3	1
Infiltrates, lymphoma		1		
Arteritis, mutifocal	0	1	0	0
Metastasis	2	1	3	
Tubular degeneration, (multi)focal	12	9	8	14
Tubular degeneration, diffuse	2		4	4
Dilation, tubular, diffuse		2		
Dilation, rete testis				1
Edema	5	3	3	6
Fibrosis, rete testis	1			
Hemorrhage, (multi)focal	1			
Infiltration, lymphoid, (multi)focal	1			

Tubular mineralization	6	2	1	8
Vascular mineralization	1			1
Pigment storage	1			

The macroscopically observed foci in the adrenal cortex in females correlated with a variety of histopathologic findings, including cortical adenoma, multifocal cortical hyperplasia, accessory cortical tissue, cystic degeneration, multifocal fatty change and multifocal hypertrophy. None of these findings showed statistically significant differences between control and high-dose animals or dose-response relationships and thus are concluded by the RMS not to be treatment-related.

Table B.6.5.1.11. Incidence of findings in the adrenal cortex (histopathology) – 24 months

	Females			
Dose level [ppm]	**0**	**100**	**600**	**3600**
No. of animals	50	50	50	50
ADRENAL GLAND CORTEX				
examined	50	19	13	50
Adenoma, cortical	1	1	1	2
Hemangioma				1
Hyperplasia, cortical, (multi)focal	25	6	4	29
Hyperplasia, subcapsular c.				1
Infiltrates, lymphoma			1	
Metastasis		1		
Accessory cortical tissue	3	4	3	4
Cyst(s)	1			
Degeneration, cystic	45	18	11	37
Fatty change, (multi)focal	4	2	2	
Hematopoiesis, extramedullar	1		1	1
Hypertrophy, (multi)focal	15	4	4	17
Hypertrophy, subcaps., (multi)focal	36	11	6	41
Necrosis, (multi)focal		1		

In terms of neoplastic findings at 24 months, there were slight increases in the incidence of malignant lymphoma (haemolymphoreticular system) in males and of adenocarcinoma in the uterus of females in the high-dose groups compared with the controls. Neither of these was statistically significantly changed from the controls in any treatment group. There were no treatment-related tumour findings in the pituitary gland. The total numbers of primary, benign, and malignant neoplasms were comparable between control and high-dose females. In males, the total numbers of primary neoplasms (67 versus 42), benign neoplasms (53 versus 35) and malignant neoplasms (14 versus 7) was higher in the control group than in the high-dose group. The total numbers of systemic and metastasised neoplasms were comparable between control and high dose males and females (not shown).

Table B.6.5.1.12. Incidence of neoplastic findings in rats administered BAS 750 F for 2 years

	Males				Females			
Dose level [ppm]	**0**	**100**	**600**	**3600**	**0**	**100**	**600**	**3600**
No. of animals	50	50	50	50	50	50	50	50
HEMOLYMPHRET SYSTEMexam.	50	50	50	50	50	10	10	50
Lymphoma, malignant		2 (4%)	2 (4%)	3 (6%)			1 (2%)	
Sarcoma, histiocytic	1	1	2				1	
PITUITARY GLAND exam.	50	49	50	50	50	23	26	50
Adenoma, pars distalis	14	22	18	11	23	14	22	22
Adenoma, pars intermed.						1		
Carcinoma, pars distalis					1	1		1
UTERUS exam.	—				50	38	37	50
Adenocarcinoma, endometrial					1 (2%)	7 (18%)	3 (8%)	5 (10%)
Schwannoma, malignant						1	1	1
Adenoma, endometrial					1	1		2

Statistical analysis: *: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact test, 1-sided)

HISTORICAL CONTROL DATA (24-month data from 12 studies in Wistar rats, started: Jan-2003 to Feb-2013)
(Survival rate: males 74 – 100%, females 58 – 100%)

♂ Malignant lymphoma:	Mean: 2.5% (15/600);	min.: 0% (0/50);	max. 6% (3/50)
♀ Uterus, Adenocarcinoma, endometrial	Mean: 16.2% (97/600);	min.: 2% (1/50);	max: 30% (15/50)

The applicant has provided historical control data for these tumour types from the same test facility and conducted in the same strain of rat (see table above; [redacted] 2015) but spanning a period of ten years before the conduct of the BAS 750 F study. The RMS has narrowed the time-frame of these data to studies that were conducted within five years of the BAS 750 F study (dosing period during 2013 to 2015) (see below).

The three male animals in the high-dose group in which malignant lymphoma was diagnosed were all decedents. The incidence in this group (6 %) was at the uppermost boundary of the provided historical control range but slightly above that of the most relevant range, correlating to one additional animal in the group of 50 presenting with this tumour. A clear dose-response relationship in the BAS 750 F study was not evident. Survival to termination of the study in the high-dose group males was high (47/50) and was comparable between the low- and mid-dose groups, so the lack of a clear dose-response relationship is not explained by early deaths of animals in the high-dose group.

Table B.6.5.1.13. Summary of relevant historical control data – tumours of the haemolympho-recticular system in male Wistar rats

Study duration	**Application**	**Animal numbers**	**Lymphoma**		**Histiocytic sarcoma**		**Lymphoma**		**Survival rate**
			No.	**%**	**No.**	**%**	**Surv**	**Dec**	
2009-2011	Drinking	50	2	4	0	0	0	2	80 %
2006-2008	Feeding	50	2	4	0	0	1	1	76 %
2007-2009	Feeding	50	2	4	0	0	0	2	88 %
2009-2011	Feeding	50	2	4	0	0	0	2	76 %
2013-2015	Feeding	50	0	0	1	2	0	0	68 %
Sum		250	8						
Mean				3.2					

Surv = survivors. Dec = decendents

One of the high-dose females in which uterine adenocarcinoma was diagnosed was a decedent. The incidence of this tumour in all the exposed groups (10 % in the high-dose group) was within the historical control range of the most relevant studies. Moreover, the incidence did not show a dose-response relationship; the RMS thus concludes that this was an incidental finding in the treatment groups.

Table B.6.5.1.14. Summary of relevant historical control data – uterine tumours in female Wistar rats

Study duration	Application	Animal numbers	Adenoma		Total Adenocarcinoma		Adenocarcinoma		Survival rate
			No.	%	No.	%	Surv	Dec	
2009-2011	Drinking	50	0	0	9	18	8	1	88 %
2006-2008	Feeding	50	1	2	14	28	9	5	74 %
2007-2009	Feeding	50	0	0	8	16	6	2	74 %
2009-2011	Feeding	50	0	0	10	20	7	3	72 %
2013-2015	Feeding	50	0	0	6	12	4	2	84 %
Sum		250			47				
Mean						18.8			

Surv = survivors. Dec = decendents

Discussion and conclusion

When BAS 750 F was administered orally to rats at dietary concentrations of 0, 100, 600 and 3600 ppm for 12 or 24 months, the main effects were on body weight and body-weight gain (males and females at 3600 ppm) and on the liver from 600 ppm (increased relative liver weight, hypertrophy, associated haematology and clinical-chemistry findings).

The liver effects after 12 months of administration comprised increased relative weights at 3600 ppm, which were associated with minimal-to-slight hypertrophy, clinical chemistry changes (changes in levels of glucose, ALP, total protein, urea, cholesterol) and haematology changes (reduced PTT and platelet counts, resulting from altered liver-cell metabolism) at the same dose. Indications of treatment-related toxicity were also reported at 600 ppm in males, comprising increases in ALP and urea and decreases in PTT.

There were no indications that any other organs were targeted by BAS 750 F. Histopathology findings in the pituitary gland of a small number of high-dose females at 12 months did not translate to pituitary tumours or hyperplasia at 24 months and hence are regarded by the RMS to be incidental.

Increases in tumour incidences compared with the concurrent controls were noted in the haemolymphoreticular system in males (malignant lymphoma) and the uterus of females (adenocarcinoma), although neither of these was statistically significant. A clear dose-response relationship was not evident in the malignant-lymphoma incidences, and the high-dose group incidence (6 %) was within the wider historical control range, exceeding the more recent historical control range (upper range 4 %, mean 3.2 %) by just one animal. Furthermore, the haemolymphoreticular system was not a target of BAS 750 F in any of the repeated-dose toxicity studies. The RMS thus concludes that the occurrence of malignant lymphoma in one additional animal in the high-dose group did not provide evidence of a carcinogenic potential for BAS 750 F. The incidence of uterine adenocarcinoma did not show a dose-response relationship, with the incidence in the low-dose group (18 %) being higher than that in the high-dose group (10 %). The incidences in all the treatment groups were also well within the relevant historical control range (12 – 28 %, mean 18.8 %). The increases in the BAS 750 F study above the concurrent controls were thus regarded by the RMS to be incidental. Moreover, the total number of primary, benign and malignant neoplasms was higher in the control males than in the animals exposed to 3600 ppm and comparable between

control and high-dose females. Therefore, BAS 750 F did not induce tumours in rats at doses that resulted in systemic toxicity (11 to 22 % decreases in body weight, and liver effects).

The RMS identified a NOAEL for chronic toxicity (12 months) of 100 ppm (5 mg/kg bw/d at this time-point), based on the changes in haematology and clinical chemistry parameters at 600 ppm.

To refine the assessment, the RMS has performed BMD analyses on relative liver weight, body weight, body-weight gain and clinical chemistry parameters at the 12-months' time-point. Increases in urea of 9 % in males and up to 16 % in females were not statistically significant. Since an increase in 17 % in males at 600 ppm was statistically significant, the BMR for increases in urea has been set at 15 %.

Parameter	**Response level**	**Covariate**	**Lowest BMDL (mg/kg/d)**	**Highest BMDU (mg/kg/d)**	**BMDU / BMDL ratio**
Relative liver weight	15 %	Males	461.4	91415	198.1
		Females	28.3	174.7	6.17
Body weight	10 %	Males	159.4	248.7	1.6
		Females			
Overall body-weight gain	10 %	Males	90.4	180.3	2.0
		Females	46.4	157.1	3.4
ALP	50 %	Males	46.1	284.5	6.2
		Females			
Urea	15 %	Males	6.9	131.7	19.1
		Females	74.7	142730	1910

The lowest value (6.9 mg/kg bw/d), based on a 15 % change in urea levels in males, is consistent with the proposed NOAEL, although it is associated with a greater level of uncertainty than some of the other analysed parameters. The RMS recognises that this is a precautionary estimate, since some of the other BMDL values were far higher, and clinical-chemistry parameters tend to be subject to much variability. It is therefore likely that the NOAEL was also precautionary.

B.6.5.2. Carcinogenicity study in mice

Groups of 50 male and 50 female C57BL/6JRj mice were exposed via the diet to BAS 750 F for at least 18 months at dose levels of 0, 20 and 50 ppm (both sexes), 200 ppm (males) and 250 ppm (females). The dietary concentration corresponded to mean intakes of 3.5, 9.1 and 36 mg/kg bw/d in males and 4.9, 12.6 and 61.5 mg/kg bw/d in females. Additional satellite animals (7 / sex/ group, same doses as the main study) were assigned for the determination of the plasma concentration of the parent substance.

There were no overt clinical signs of toxicity in any test group. Survival to the end of the study was not affected by exposure to BAS 750 F and exceeded 80 % in all groups. Necropsy of decendents did not show any evidence of a treatment-related cause of death.

Table B6.5.2.1. Unscheduled deaths in mouse carcinogenicity study

BAS 750 F Dose level [ppm]	Spontaneous death	Killed *in extremis*	Other cause of death	Mortality total	Mortality Corrected [a]
MALES					
0		1		1 / 50 (2%)	1 / 50 (2%)
20		2		2 / 50 (4%)	2 / 50 (4%)
50				0 / 50 (0%)	0 / 50 (0%)
200				0 / 50 (0%)	0 / 50 (0%)
FEMALES					
0		3	1 [b]	4 / 50 (8%)	3 / 49 (6%)
20	1	1	2 [b]	4 / 50 (8%)	2 / 48 (4%)
50		2	7 [b]	9 / 50 (18%)	2 / 43 (5%)
250	1	4	3 [b]	8 / 50 (16%)	5 / 47 (11%)

[a] "Other causes of death" were disregarded for the calculation of corrected mortality
[b] Animals were sacrificed for humane reasons, owing to skin lesions (a common spontaneous occurrence in this strain)

No statistically significant changes were noted in total or corrected mortality vs. control (Fisher's Exact test)

Treatment-related reductions in body weight and body-weight gain occurred in males at 200 ppm from week 11 onwards and in females at 250 ppm from week 7 onwards; at the mid-dose of 50 ppm, body weights and body-weight gains of females were reduced from week 34. Food consumption was unaffected in males at all doses, whilst in females at 250 ppm, food consumption was decreased in weeks 6 – 26, followed by normal food intake for the remainder of the study. At doses of 50 ppm and below, food consumption was not affected in females.

Table B.6.5.2.2. Body-weight development in mouse carcinogenicity study

	Males				Females			
Dose level [ppm]	**0**	**20**	**50**	**200**	**0**	**20**	**50**	**250**
Body weight [g]								
- Week 0	23.0	22.9	22.7	22.7	18.9	18.6	18.8	18.9
- Week 13	29.5	28.9	28.6*	28.1**	23.6	23.4	23.3	**22.8****
% change from control		-2	-3	-5				**-3**
- Week 26	34.2	34.2	33.7	32.6*	27.3	27.5	26.9	**24.5****
% change from control		±0	-1	-5		+1	-1	**-10**
- Week 54	37.4	37.0	36.0	35.0**	33.9	33.1	**31.5***	**29.1***
% change from control		-1	-4	-6		-2	**-7**	**-14**
- Week 78	39.0	37.2	37.7	36.7	36.7	36.0	34.1	**32.4****
% change from control		-5	-3	-4		-2	-7	**-12**
Overall body weight gain (%)								
- Week 13	28	26	26	**24****	25	25	24	**21***
% change from control		-7	-7	**-14**		±0	-4	**-16**
- Week 26	49	50	48	44	45	48	43	**30****
% change from control		+2	-2	-10		+7	-4	**-33**
- Week 54	63	61	58	**55***	79	77	**68***	**54****
% change from control		-3	-8	**-13**		-3	**-13**	**-32**
- Week 78	70	63	66	62	94	93	82	**71****
% change from control		-10	-6	-11		-1	-13	**-24**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

The plasma concentration of BAS 750 F in the satellite animals was determined on days 7, 28, 85 (week 13), 176 (week 26) and 357 (week 52). An approximately dose-dependent increase in the mean plasma concentrations was measured in both sexes.

Haematology parameters were determined at 12 and 18 months. Blood smears were fixed and stained for all animals, but only examined for control and high-dose groups and for inter-currently sacrificed animals. There were no treatment-related changes in haematology parameters.

Gross necropsy did not reveal any treatment-related changes. Statistically significant changes in organ weights are presented in the table below. Relative liver weights were increased at all doses in males and at the high-dose level in females. In the high-dose-group females and the mid- and high-dose males, the increases in liver weight were adverse, since increased incidences and severity of fatty change were noted at the same doses. A dose-related decrease in absolute kidney weights was reported only in males; when this was adjusted for body weight, only the change at 200 ppm was statistically significant. However, histopathology changes (decreased incidence of tubular vacuolation) were observed in the kidneys of males of the mid- and high-dose groups; hence the RMS considers the reduced absolute kidney weight at 50 ppm to be a real and not artefactual effect. Adrenal-gland weights were increased in the high-dose males (absolute and relative) and females (relative). In males, there were no associated macroscopic or microscopic findings. However, in females, there were histopathology changes (increased incidence of eosinophilic cytoplasmic change and increase in size of individual eosinophilic cells); hence, the weight increase in high-dose females was potentially adverse. Since the increase in relative heart weight in males occurred only in the low-dose group, the RMS concludes that this finding was unrelated to treatment. The relative uterus weight was increased in high-dose females. Since absolute uterus weights were not statistically significantly changed and there were no gross or histopathology findings, the RMS concludes that the increased relative weight

was secondary to the body weight reduction in the high-dose group females and thus an unspecific finding.

Table B.6.5.2.3. Mean absolute and relative organ weights of mice administered BAS 750 F for 18 months (percent change compared with control)

Sex	Males			Females		
Dose level [ppm]	**20**	**50**	**200**	**20**	**50**	**250**
Animals examined	48	50	50	46	41	42
TERMINAL BODY WT	-5%	-4%	**-7%***	-3%	-7%	**-13%****
LIVER						
Absolute	+6%	**+14%****	**+33%****	+4%	+22% [+7%]&	**+41%****
Relative to body weight	**+12%****	**+18%****	**+42%****	+5%	**+31%*** [+13%]&	**+57%****
KIDNEYS						
Absolute	**-5%***	**-8%****	**-13%****	0%	+32% [-1%]@	-5%
Relative to body weight	+1%	-4%	**-7%***	+2%	+40% [+6%]@	+8%
ADRENAL GLAND						
Absolute	-7%	+10%	**+27%****	-15%	-6%	+8%
Relative to body weight	+1%	+16%	**+39%****	-14%	+1%	**+22%***
HEART						
Absolute	+3%	+1%	-1%	-1%	-1%	**-7%***
Relative to body weight	**+10%****	+6%	+6%	+1%	+6%	+5%
UTERUS						
Absolute				+8%	+7%	+11%
Relative to body weight				+13%	+19%	**+32%***

Statistical analysis: Dunnett's-test, * : p≤0.05; ** : p≤0.01; & The high absolute and relative mean liver weight in 50 ppm females was caused by female 622 having a massive infiltration of a histiocytic sarcoma. When this female was removed from calculations, the difference from the control group was much less (values in brackets)

@ The high absolute and relative mean kidney weights in 50 ppm females are caused by female #609 having a very large cyst. When this female was removed from calculations, no differences from the control group were observed (see values in brackets).

Histopathology was performed on animals of the control and high-dose groups, except for the liver, thyroid and gross lesions / tissue masses, for which all groups were investigated. All unscheduled-sacrificed animals were also subjected to complete necropsy and full histopathologic evaluation.

Non-neoplastic microscopic changes were noted in the liver and thyroid glands (males and females), kidneys (males) and in the adrenal glands (females).

In the liver, a diffuse fatty change of hepatocytes was observed in most animals and was characterised by a microvesicular cytoplasmic change. Although there was no difference in total incidence of this change compared with the control animals, the severity was slightly increased in males treated at 50 and 200 ppm and in females at 250 ppm. In the same dose groups, the incidence and severity of macrovesicular fatty change was increased; this finding was characterised by the presence of large vesicles within the hepatocytes. The livers of two animals per group per sex were stained with Oil-Red-O to confirm that the vacuoles in the liver represented fat (fatty change); all were stained, indicating a positive result. The increased incidence and severity of fatty changes might have

represented a slight exacerbation of age-related pathology, since the incidence in the controls was also high; the absence of fatty change in any group in the shorter-duration studies would support this view. In addition to the fatty changes, eosinophilic cytoplasmic inclusions in hepatocytes (centrilobular distribution) were recorded in the majority of the males at 200 ppm and hepatocellular single cell necrosis (minimal severity) occurred in 20 % of the high-dose females.

Table B.6.5.2.4. Incidence of non-neoplastic liver findings in mouse carcinogenicity study

	Males				**Females**			
Dose level [ppm]	**0**	**20**	**50**	**200**	**0**	**20**	**50**	**250**
No. of animals	50	50	50	50	50	50	50	50
LIVER exam	50	50	50	50	50	50	50	50
Fatty change, diffuse (microvesic.)	48	46	47	48	40	44	38	43
Minimal (gr. 1)	7	5	2	1	10	11	5	3
Slight (gr. 2)	30	15	3	3	29	32	21	6
Moderate (gr. 3)	10	24	33	35	1	1	11	8
Marked (gr. 4)	1	2	9	9			1	26
Mean severity grade	*<2.0>*	*<2.3>*	*<2.9>*	*<3.0>*	*<1.4>*	*<1.6>*	*<1.7>*	*<2.9>*
Fatty change, macrovesicular	23	16	**35***	**46****	21	16	22	**39****
Minimal (gr. 1)	20	14	8	13	14	13	7	3
Slight (gr. 2)	3	2	14	16	7	3	12	2
Moderate (gr. 3)			11	13			3	11
Marked (gr. 4)			2	4				23
Mean severity grade	*<0.5>*	*<0.4>*	*<1.5>*	*<2.0>*	*<0.6>*	*<0.4>*	*<0.8>*	*<2.6>*
Eosinophilic inclusions, centrilob.				**38****				
Single cell necrosis, increased							1	**10***
Minimal							1	10

Statistical analysis: Fisher's Exact Test (1-sided); * : p≤0.05; ** : p≤0.01

In the thyroid, the incidence of follicular-cell hyperplasia of males at 200 ppm (statistically significant) and of females at 250 ppm (not statistically significantly different from control females) was increased. One of the criteria for the diagnosis of follicular cell hyperplasia was piling up of the epithelium into the lumen with the presence of (a) stromal component(s). The follicular cell hyperplasia incidences observed in the male and female high-dose groups were above the date-relevant historical control range of the test facility, although it is noted that this was also the case in the female control group. Follicular-cell hyperplasia also did not show a clear dose-response relationship, particularly in females.

Table B.6.5.2.5. Non-neoplastic thyroid-gland findings in mouse carcinogenicity study

	Males				**Females**			
Dose level [ppm]	**0**	**20**	**50**	**200**	**0**	**20**	**50**	**250**
No. of animals	50	50	50	50	50	50	50	50
THYROID exam.	50	50	50	50	50	50	50	50
Hyperplasia, follicular cell, (multi)focal	21 (42 %)	16 (32 %)	17 (34 %)	**37**** (74 %)	19 38 %	14 28 %	**8*** 16 %	26 52 %

Statistical analysis: *: p ≤ 0.05, **: p ≤ 0.01 (Fisher's Exact test, 1-sided)

HISTORICAL CONTROL DATA (four 18-month studies in C57BL/6JRj mice, started: Jul-2013 to Mar-2014)
Males: mean: 31% (52/148); min.: 18% (9/50); max. 45% (22/49)
Females: mean: 18% (31/146); min.: 6% (3/50); max: 28% (13/47)

In the kidney, a dose-dependent decrease in the incidence of tubular vacuolation in males was reported, which correlated with the decreased kidney and body weights at 50 and 200 ppm. This finding was considered by the study authors to be a subtle change that was not indicative of a

degenerative process, but more likely an expression of increased (energy-consuming) excretion activity rather than an adverse effect.

Table B.6.5.2.6. Incidence of non-neoplastic kidney findings in mouse carcinogenicity study

	Males				Females			
Dose level [ppm]	**0**	**20**	**50**	**200**	**0**	**20**	**50**	**250**
No. of animals	50	50	50	50	50	50	50	50
KIDNEY exam	50	50	50	50	50	5	10	50
Tubular vacuolation	42	42	**34***	**6****	0	0	0	0

Statistical analysis: Fisher's Exact Test (1-sided); * : p≤0.05; ** : p≤0.01

In the adrenal glands, a statistically significant increase in the incidence of eosinophilic cytoplasmic change was observed in 250 ppm females. This finding was characterised by diffuse eosinophilic appearance of the cortical-cell cytoplasm of all three zones, together with a minimal to slight diffuse size increase of the individual cells. No signs of degenerative processes were observed in conjunction with this finding. The incidence of cortical hypertrophy without cytoplasmic changes in exposed animals was low and comparable with the control females.

Table B.6.5.2.7. Incidence of selected non-neoplastic adrenal-gland findings

	Males				Females			
Dose level [ppm]	**0**	**20**	**50**	**200**	**0**	**20**	**50**	**250**
No. of animals	50	50	50	50	50	50	50	50
ADRENAL GLAND exam.	50			50	50	50	50	50
Cytoplasmic change, eosinophilic	0			0	2	6	3	**20****
Hypertrophy, cortex, diffuse	1			0	6	6	**0***	4
Minimal					3	3		2
Slight	1				3	3		2

Statistical analysis: Fisher's Exact Test (1-sided); * : p≤0.05; ** : p≤0.01

In terms of neoplastic findings, there were very low incidences of follicular-cell adenomas of the thyroid gland and hepatocellular adenomas and carcinomas of the liver (see table below).

Table B6.5.2.8. Incidence of selected neoplastic findings in mouse carcinogenicity study

	Males				Females			
Dose level [ppm]	**0**	**20**	**50**	**200**	**0**	**20**	**50**	**250**
No. of animals	50	50	50	50	50	50	50	50
THYROID exam.	50	50	50	50	50	50	50	50
Adenoma, follicular cell				2 (4 %)	1 (2 %)		1 (2 %)	3 (6 %)
LIVER exam.	50	50	50	50	50	50	50	50
Adenoma, hepatocellular	1 (2 %)	2 (4 %)	3 (6 %)					
Carcinoma, hepatocellular	1	1	1		1			

HISTORICAL CONTROL DATA (Test facility, four 18-month studies in C57BL/6JRj mice, started: 2013 - 2014)

Males: thyroid, follicular cell adenoma: Mean: 1.5% (3/198); min.: 0% (0/50); max. 2% (1/50)
Females: thyroid, follicular cell adenoma Mean: 2.6% (5/196); min.: 0% (0/50); max: 6% (3/47)

HISTORICAL CONTROL DATA (BASF, five 18-month studies in C57BL/6JRj mice, started: 1998 - 2007)

Males: thyroid, follicular cell adenoma: Mean: 1.2% (3/250); min.: 0% (0/50); max. 6% (3/50)
Females: thyroid, follicular cell adenoma Mean: 3.6% (9/250); min.: 0% (0/50); max: 8% (4/50)

The adenomas and carcinomas in the liver of male mice of the low- and mid-dose groups were not treatment-related, since there were no such lesions in the 200 ppm group. All animals in the male high-dose group survived to termination of the study (one unscheduled death in the control males), and so the absence of tumours in the high-dose males is not because of early deaths of those animals. There were no hepatocellular adenomas in female mice; the sole incidence of hepatocellular carcinoma was in a control female. There was therefore no evidence that BAS 750 F induced liver tumours in mice.

The incidence of adenomas in the thyroid glands was slightly increased in the 200/250 ppm treated animals compared with controls. The incidence was not statistically significantly changed compared with the control groups and was comparable to the background incidences in this strain of mice. The applicant provided two sets of historical control data. When compared with that of BASF (same strain and feed), the incidence in males and females exposed to BAS 750 F was within the upper range; when compared with date-relevant data from the test facility that conducted the BAS 750 F study (dosing period 2013 – 2015), the incidence in males exposed to 200 ppm exceeded the upper range by one animal. The incidence in females was within the historical control range. The relevant historical control data are presented below.

Table B.6.5.2.9. Summary of relevant historical control data – thyroid-gland findings in C57BL mice (18 months; same test facility as the BAS 750 F study)

In-life period	**2013-2015**		**2014-2015**		**2014-2015**		**2014-2015**		**BAS 750 F 200/250 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Survival rate (%)	*96*	*88*	*92*	*86*	*86*	*86*	*82*	*86*	*100*	*89*
THYROID GLANDS[a], follicular cell	50	50	49	47	49	50	50	49	50	50
Hyperplasia	9 18 %	3 6 %	22 45 %	13 28 %	16 33 %	12 24 %	14 28 %	6 12 %	37 74 %	26 52 %
Adenoma	1 2 %	0	1 2 %	3 6.4 %	0	0	1 2 %	2 4.1 %	2 4 %	3 6 %
Adenocarcinoma	0	0	0	2 4.3 %	0	0	0	0	0	0
Number of tumour-bearing animals	1	0	1	5	0	0	1	2	2	3

[a] = Number of tissues examined from each group

No thyroid follicular cell adenocarcinomas were detected in either sex. The two cases of thyroid follicular cell adenomas in high-dose males occurred in animals that survived to termination of the study (there were no unscheduled deaths in this group). In females, 1, 0, 1, 3 adenomas were present in animals at 0, 20, 50, 250 ppm that survived to termination; therefore adenomas in the high-dose group did not result in early deaths. In all affected high-dose animals, adenomas occurred singly. Furthermore, in one of two males and two of three females, they were reported in the absence of thyroid follicular-cell hyperplasia. There was therefore no evidence that the adenomas occurred as a continuum of treatment-related pathological consequences, with a progression from hyperplasia. Moreover, the total number of neoplasms in all groups was comparable, with equal numbers in the control and high-dose males (13 in each group, in 12 animals each) and fewer in the high-dose females (20 in 18 animals) than in the controls (27 in 21 animals). No animal in any treatment group had metastasis, whereas one was reported in a control female.

Discussion and conclusion

When BAS 750 F was administered to mice at dietary concentrations of 0, 20, 50 and 200 / 250 ppm for 18 months, the main effects were on body weight, liver weight and pathology, kidney weight, adrenal glands and thyroid.

Body weights and body-weight gains were reduced in males at 200 ppm and in females from 50 ppm. At the latter dose, the reduction in body-weight gain exceeded 10 % in females from week 34, although it was not statistically significant by week 78. Relative liver weights were increased with statistical significance at all doses in males and at 250 ppm in females. Liver histopathology was reported at 50 and 200 ppm in males (increased incidence and severity of fatty changes, probably representing a treatment-related exacerbation of age-related pathology; centrilobular eosinophilic inclusions) and 250 ppm in females (increased incidence and severity of fatty changes; single-cell necrosis, minimal).

The treatment-related kidney effects comprised a weight increase and a decreased incidence of tubular vacuolation in males from 50 ppm. Since there were no degenerative renal changes and the tubular vacuolation and consequent weight increase probably resulted from increased excretion activity, the RMS concludes this was a treatment-related but not adverse effect. Relative adrenal-gland weights were increased in males and females at 200 / 250 ppm. There were no adrenal-gland pathology changes in males, but in high-dose females the incidence of eosinophilic cytoplasmic change was increased; degenerative changes were not observed. Mild changes in adrenal-gland weights and histology are consistent with mild repetitive stress of the animals (Everds *et al*., 2013)[8].

There was a very slight increase in the incidence of thyroid follicular-cell adenomas which, nevertheless, was within the historical control range for females and above the historical control range for males by only one animal. A dose-response relationship in the incidence of adenoma and follicular-cell hyperplasia was not evident in the females. In males, there was a statistically significant increase in the incidence of hyperplasia in the high-dose males (200 ppm), although again without a dose-response relationship. Notwithstanding, the RMS considers that the increase in hyperplasia in the high-dose males was treatment-related and perhaps reflected an exacerbation of age-related thyroid changes. The increased incidence of hyperplasia was not associated with thyroid follicular-cell tumours in either the BAS 750 F-exposed groups or the historical control data. The total number of neoplasms and animals affected was identical for the high-dose and control males and lower in the high-dose females than the controls. Overall, the RMS concludes that all tumours arose spontaneously, not as a result of exposure to BAS 750 F.

The RMS has identified a NOAEL of 20 ppm (3.5 mg/kg bw/d) based on the liver histopathology findings at 50 ppm (9.1 mg/kg bw/d). *The applicant supported a NOAEL of 50 ppm (9.1 / 12.6 mg/kg bw/d in males and females, respectively), based upon reduced body weight gain and adverse liver changes in males at 36 mg/kg bw/d (200 ppm).*

The RMS performed BMD analysis on individual body weight, individual body weight gain ratio, individual relative liver weight, incidence of hepatocellular necrosis (females only; no cases in males) and incidence and severity of liver histology changes. An ordinal data analysis was used to evaluate the dose relationship of the incidence and severity of macrovesicular fatty change.

[8] Everds, N.E., Snyder, P.W., Bailey, K.L., Bolon, B., Creasy, D.M., Foley, G.L., Rosol, T.J., Sellers, T. (2013). Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. *Toxicologic Pathology*, **41**: 560-614.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Relative liver weight	15 %	Males	5.2	11.4	2.2
		Females			
Body weight	10 %	Males	35	838.4	24
		Females			
Overall body-weight gain ratio	10 %	Males	72.8	Inf	Inf
		Females	18.9	121	6.4
Hepatocellular single-cell necrosis	10 %	Females only	23.4	59.1	2.5
Macrovesicular fatty change	10 %	Grade 1	6.7	13.3	2.0
		Grade 2	27	35.1	1.3
		Grade 3	44.6	56.4	1.3
		Grade 4	64.5	81.1	1.3

The lowest BMDL of 5.2 mg/kg bw/d, based on a 15 % change in relative liver weight, is consistent with the proposed NOAEL and is associated with a relatively low level of uncertainty.

B.6.5.3. Summary and conclusion of chronic toxicity and carcinogenicity

The chronic toxicity and carcinogenic potential of BAS 750 F have been investigated in rats and mice in two guideline-compliant studies.

Long-term exposure to BAS 750 F resulted in decreased body weight and body-weight gain in both species. The liver was the only target organ in rats and mice. Treatment-related hepatic effects comprised liver-weight increases, hepatocellular hypertrophy, clinical-chemistry and haematology changes. In mice, histopathology investigations recorded increased incidence and severity of fatty changes and single-cell necrosis. Despite the longer exposure, the effects did not increase in severity in either species compared with the shorter-duration studies. Moreover, the hepatic effects in the short-term studies did not translate to liver carcinogenicity in rats or mice.

In the mouse study, organ-weight changes and some histopathology observations in the kidneys and adrenal glands were attributed by the RMS to BAS 750 F exposure but were concluded to be non-adverse.

There was no evidence of a treatment-related increase in tumours in either species. It is proposed that BAS 750 F should not be classified for carcinogenicity (please see CLH report).

Table B6.5.2.10. Summary of NOAEL values from chronic / carcinogenicity studies

Study	Species	NOAEL mg/kg bw/d	BMDL mg/kg bw/d	Adverse effects
Oral				
12-month	Rat	5.0	7	↑ liver weight, ↓ body weight & body-weight gain, clinical-chemistry changes
18-month	Mouse	3.5	5	↓ body weight & body-weight gain, liver histopathology, ↑ liver weight

B.6.6. REPRODUCTIVE TOXICITY

The reproductive toxicity of BAS 750 F has been investigated in a two-generation study in rats and developmental toxicity studies in rats and rabbits. The NOAELs presented are those proposed by the RMS. In addition, the RMS has performed BMD analysis to identify a more scientifically robust, transparent reference point.

B.6.6.1. Generational studies

A guideline-compliant two-generation study is available in rats.

Table B.6.6.1.1 Summary of two-generation study

Study **Species,** **Dose levels** **Reference**	**NOAEL / BMDL (mg/kg bw/d)**	**LOAEL (mg/kg bw/d)**	**Critical effects**
Rat (Wistar, Crl:WI(Han) Dietary administration with dose adjustment 0, 25, 75, 200 mg/kg bw/d 25 / sex / group OECD 416 GLP Purity 98.8% Report CA 5.6.1/1 [redacted] 2015c (2014/1170754)	**Parental toxicity**		
	NOAEL **25** **$BMDL_{50}$** **34** (change in ALP levels)	**LOAEL** **75**	F_0 generation <u>≥ 75 mg/kg bw/d</u>: ↑ ALP (males & females), cholesterol (males), liver wt (males & females) <u>200 mg/kg bw/d:</u> ↓ food consumption, body weight and body-weight gain (males & females) ↑ liver cell hypertrophy (males) 1 dam with total litter loss by PND 2; one pup of this litter showed signs of insufficient nursing (no milk in stomach); no findings in 5 other pups; remainder cannibalised.
			F_1 generation <u>200 mg/kg bw/d:</u> ↓ food consumption, body weight and body-weight gain (males & females) ↑ ALP (males & females), urea & inorganic phosphate (males), triglycerides (females) ↑ liver weight (males & females), liver cell hypertrophy (15/25 males, minimal) 1 dam with total litter loss by PND 3; pups showed signs of insufficient nursing (reduced nutritional condition, no milk in stomachs) 1 dam with only stillborn pups; this dam showed poor general state and piloerection
	Fertility		
	NOAEL **200**	–	F_0- & F_1 generation No treatment-related adverse effect

Table B.6.6.1.1 Summary of two-generation study

Study **Species,** **Dose levels** **Reference**	**NOAEL / BMDL (mg/kg bw/d)**	**LOAEL (mg/kg bw/d)**	**Critical effects**
	Offspring toxicity		
	NOAEL **75** **$BMDL_{10}$** **163** (body-weight gain)	**200**	F_1 pups (200 mg/kg bw/d) ↓ live pups (PND 4) owing to 1 total litter loss ↓ pup weight and weight gain during lactation (& secondary organ-weight effects)
			F_2 pups (200 mg/kg bw/d) ↓ live pups (PND 0 and PND 4) owing to 1 dam with only stillborn pups and 1 dam with total litter loss ↓ pup weight and body-weight gain during lactation (& secondary organ-weight effects)

In a guideline-compliant two-generation study in rats, BAS 750 F was administered to groups of 25 / sex / dose in the food; the dietary concentrations were adjusted to obtain target dose levels of 0, 25, 75 and 200 mg/kg bw/d. At least 75 days after the beginning of treatment, F_0 animals were mated to produce an F_1 generation. Mating pairs (one male to one female) were from the same dose group and F_1 animals selected for breeding were continued in the same dose group as their parents. The same group sizes and doses were repeated to produce the F_2 generation. Test diets that contained BAS 750 F were offered continuously throughout the study.

Parental toxicity

There were no treatment-related deaths in any group. One female of the 75 mg/kg bw/d group was sacrificed during the pre-mating period for reasons unrelated to BAS 750 F administration. There were also no treatment-related overt clinical signs in any F_0 or F_1 parental animal during the pre-mating or mating phases. Dam health during gestation and lactation is reported below.

Body-weight gain was impaired at 200 mg/kg bw/d in parental F_0 and F_1 animals. In F_0 males at this dose, body weights were statistically significantly below the concurrent control values from pre-mating day 13 onwards and remained so until the end of the study (up to 11 % reduction). The body-weight gain of these males was statistically significantly below the control values during major parts of the pre-mating period (up to 33 %) and consistently lower than the control during other study periods (mating, post-mating), albeit without statistical significance. In the high-dose F_0 females, body weights were consistently below concurrent controls throughout the study, and gained statistical significance during pre-mating days 48–55 (up to 5 % reduction), during the entire gestation period (up to 9 %) and during lactation days 1–14 (up to 13 % decrease). The body-weight gain of these females was statistically significantly below the control values during major parts of the gestation period (up to 24 %), although they generally gained more weight during lactation (23.7g, 26.6g, 34.5g. 44.9g at 0, 25, 75, 200 mg/kg bw/d, gain over lactation day 1 to 21). The F_0 females showed no clinical signs during gestation and lactation, apart from one high-dose dam (number 200) that had complete litter loss on post-natal day 2.

The body weights of the F_1 males at 200 mg/kg bw/d were statistically significantly below the control values from the beginning of pre-mating onwards and remained so until the end of the study (up to 12 % decrease). The body-weight change of these males was statistically significantly below the control values during major parts of the pre-mating period (up to 32 % lower) and during post-mating

days 0 - 7 (a reduction of about 58 %). Also at 200 mg/kg bw/d, the body weights of the F_1 females were statistically significantly below the concurrent control values during the entire pre-mating, gestation and lactation periods (decreases of up to 11 %, 16 % and 17 %, respectively). The body-weight change of these females was below the control values during most of the pre-mating period (days 0 – 69, reduced by about 11%) and throughout gestation (up to 32 % lower). In contrast, these females generally gained more weight during lactation (28.2, 26.4, 29.9 and 36.5 g at 0, 25, 75 and 200 mg/kg bw/d, gain over lactation day 1 to 21). One high-dose F_1 female (number 379) showed severe poor general condition, piloerection and an inability to deliver on gestation day 23, followed by delivery of a still-born litter on gestation day 24; otherwise, there were no clinical signs during gestation and lactation.

The body weights and body-weight gains of the F_0 and F_1 parental animals at 25 and 75 mg/kg bw/d were comparable to the concurrent-control groups throughout the study.

Food consumption of the F_0 males at 200 mg/kg bw/d was statistically significantly below the control values during the entire pre-mating period (up to 11 %), during mating days 9 - 14 (about 10 %) and throughout the post-mating period (up to 10 %). In F_0 females at 200 mg/kg bw/d it was also consistently below the control values throughout the study, although the difference gained statistical significance only during pre-mating days 63 - 69 (about 6 %), during the entire gestation period (up to 15 %) and during lactation days 7 - 14 (about 12 %). The high-dose F_1 males had consistently and statistically significantly reduced food consumption during the entire pre-mating (up to 9 %) and post-mating (up to 10%) periods. Food consumption in both sexes and generations was unaffected at 25 and 75 mg/kg bw/d.

Oestrous cycle determinations were evaluated by daily analysis of vaginal smears for all F_0 and F_1 female parental rats for a minimum of 3 weeks prior to mating and were continued throughout the mating period until the female exhibited evidence of copulation. Additionally, the stage of the oestrous cycle for each female was determined at scheduled sacrifice. The investigations revealed the occurrence of regular cycles in all groups of both generations. In the F_0 females, the mean oestrus-cycle duration was 4.1, 4.2, 4.1 and 4.2 days at 0, 25, 75 and 200 mg/kg bw/d, respectively. In the F_1 females, the mean duration was 4.1, 4.0, 4.1 and 4.6 days at 0, 25, 75 and 200 mg/kg bw/d, respectively. The value for the F_1 high-dose females was statistically significantly ($p < 0.05$) above the control value, by half a day, and the number of cycles within the observation period was accordingly lower (3.48 versus 4.32 in controls, $p < 0.01$). The duration of cycles was within the historical control range of the test facility [6 studies (2010-2014): 3.99 – 4.8 days, mean 4.33]. Furthermore, neither time to pairing nor pairing success were affected. Therefore, the RMS does not consider this apparent slight lengthening of the oestrus cycle in the high-dose group to be evidence of an adverse effect.

The notable clinical chemistry findings in adults are reported in the table below. ALP was increased in both sexes in both generations and was adverse, as was the increase in cholesterol in F_0 males, urea in F_1 males and triglyceride in females. The decrease in total bilirubin in males and females, in the absence of signs of anaemia, was likely to be a reflection of increased phase-II hepatic metabolism with a consequent increased biliary excretion of bilirubin, and hence an adaptive change.

Table B.6.6.1.2. Clinical-chemistry findings (selected) in rat 2-generation study

		Males				**Females**			
Dose level [mg/kg bw/d]		**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
F_0 generation									
ALP[µkat/l]	F_0-gen	1.23	1.45	**2.03****	**2.26****	0.77	0.74	**0.96***	**1.15****
	F_1-gen	1.24	1.55	**1.95****	**2.36****	0.89	0.86	0.84	**1.26****
INP [mmol/L]	F_0-gen	1.64	1.69	1.77	1.74	1.52	1.37	1.32	1.77
	F_1-gen	1.56	1.70	1.69*	**1.81****	1.25	1.21	1.25	1.16
		Historical control: 1.49 – 1.79							
Triglycerides	F_0-gen	0.95	0.81	0.76	0.80	0.99	0.88	1.12	1.47

	F_1-gen	1.19	0.87*	0.89*	0.81*	0.72	0.94	**0.98***	**1.09****
[mmol/l]		*Historical control: 0.61 – 1.29*							
Cholesterol	F_0-gen	1.87	2.11	**2.30***	**2.56****	2.05	2.10	1.95	2.21
[mmol/l]	F_1-gen	2.13	2.35	2.21	2.45	1.99	2.11	1.86	2.10
Urea [mmol/l]	F_0-gen	7.22	7.30	7.81	7.70	9.81	8.53*	8.62*	10.09
	F_1-gen	6.02	6.62	6.40	**7.91****	7.10	7.62	7.00	7.33
Total bilirubin	F_0-gen	2.11	2.02	1.58**	1.58**	3.04	3.16	3.25	3.38
[µmol/l]	F_1-gen	1.52	1.37	1.21**	1.11**	1.69	1.55	1.44	1.24*

Statistical evaluation: * p≤0.05; ** p≤0.01 (Kruskal-Wallis + Wilcoxon test (2-sided)
Historical control data: 46-48 3-month studies, 18-week Wistar rats (16-h fasting), sampling between Sep-2009 and Jun-2014

The majority of noted organ weight changes in adults were a reflection of the decreased terminal body weights. For example, the absolute adrenal, spleen (males), testes, cauda epididymis, ovaries, kidney (females) and pituitary gland (females) were decreased, but when adjusted for body weight showed no difference from controls. In both sexes, the relative brain weight was increased in the same magnitude as the decrease in body weight; this and the other mentioned weight changes were thus not regarded by the RMS to be organ-specific effects. Treatment-related increases in absolute and relative liver weights were recorded in males and females at 75 and 200 mg/kg bw/d in both generations. Neither absolute nor relative weights of the prostate (males), thyroid (either sex), spleen (females) and uterus were statistically significantly changed in either generation.

Table B.6.6.1.3. Terminal body weight and organ weights (selected) - males

Generation			F_0 Males				F_1 Males			
		Dose [mg/kg]	Absolute weight	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [mg]	Δ%[&]	Relative weight [% of bw]	Δ%
Terminal weight	[g]	0	397.676		0.016		383.764			
		25	384.532	(-3)	0.016		384.600	(±0)		
		75	385.384	(-3)	0.016		386.144	(+1)		
		200	**354.112****	(-11)	0.016		**339.328****	**(-12)**		
Adrenal gland	[mg]	0	63.28		0.016		66.88		0.017	
		25	60.68	(-4)	0.016	(-1)	63.60	(-5)	0.017	(-5)
		75	60.12	(-5)	0.016	(-2)	61.88	(-3)	0.016	(-8)
		200	**55.60****	**(-12)**	0.016	(-1)	**58.24****	**(-13)**	0.017	(-1)
Epididymides	[g]	0	1.186		0.299		1.193		0.312	
		25	1.158	(-2)	0.303	(+1)	**1.136***	**(-5)**	0.297	(-5)
		75	1.200	(+1)	**0.312***	**(+4)**	1.187	(±0)	0.308	(-1)
		200	**1.115****	**(-6)**	**0.316***	**(+6)**	**1.102****	**(-8)**	0.326	(+4)
Kidneys	[g]	0	2.501		0.630		2.415		0.631	
		25	2.445	(-2)	0.636	(+1)	2.514	(+4)	0.654	(+4)
		75	2.491	(±0)	0.647	(+3)	**2.556****	**(+6)**	**0.663***	**(+5)**
		200	**2.329****	**(-7)**	0.659	(+5)	2.323	(-4)	**0.686****	**(+9)**
Liver	[g]	0	9.170		2.303		9.178		2.387	
		25	9.012	(-2)	2.342	(+2)	9.478	(+3)	2.460	(+3)
		75	9.324	(+2)	**2.417****	**(+5)**	9.737	(+6)	**2.521****	**(+6)**
		200	9.094	(-1)	**2.567****	**(+11)**	9.132	(-1)	**2.689****	**(+13)**
Seminal vesicle	[g]	0	1.314		0.331		1.299		0.340	
		25	1.395	(+6)	**0.364***	**(+10)**	1.350	(+4)	0.353	(+4)
		75	1.368	(+4)	0.355	(+7)	1.346	(+4)	0.350	(+3)
		200	1.321	(+1)	**0.371****	**(+12)**	1.242	(-4)	0.367	(+8)

Statistical analysis: * p ≤ 0.05, ** p ≤ 0.01 [Kruskal-Wallis and Wilcoxon-test (two-sided)]

Table B6.6.1.4. Terminal body weight and organ weights (selected) - females

Generation		Dose [mg/kg]	F_0 Females Absolute weight	Δ%	Relative weight [% of bw]	Δ%	F_1 Females Absolute weight [mg]	Δ%	Relative weight [% of bw]	Δ%
Terminal weight	[g]	0	277.456				228.616			
		25	224.664	(-1)			219.321	(-4)		
		75	220.546	(-3)			219.104	(-4)		
		200	**213.160****	**(-6)**			**202.472****	**(-11)**		
Adrenal gland	[mg]	0	82.640		0.036		81.240		0.036	
		25	78.800	(-5)	0.035	(-3)	76.625	(-6)	0.035	(-2)
		75	78.333	(-5)	0.036	(-1)	79.800	(-2)	0.037	(+3)
		200	77.800	(-6)	0.037	(+1)	**71.840****	**(-12)**	0.035	(±0)
Liver	[g]	0	7.285		3.198		7.596		3.317	
		25	7.183	(-1)	3.197	(±0)	7.253	(-5)	3.303	(±0)
		75	**7.843***	**(+8)**	**3.595****	**(+12)**	7.686	(+1)	**3.509***	**(+6)**
		200	**8.033***	**(+10)**	**3.772****	**(+18)**	7.393	(-3)	**3.633***	**(+10)**

Statistical analysis: * $p \leq 0.05$, ** $p \leq 0.01$ [Kruskal-Wallis and Wilcoxon-test (two-sided)]

There were no treatment-related gross pathology findings in the F_0 and F_1 parental animals. Histopathology revealed a treatment-related, minimal centrilobular hypertrophy in 15 / 25 F_0 males and 15 / 25 F_1 males at 200 mg/kg bw/d. There were no other treatment-related microscopic findings. A differential ovarian follicle count on the F_1 females showed that there were no biologically or statistically significant differences in the numbers of primordial, growing and combined incidence of follicles between the controls and the high-dose group.

Male reproductive parameters

The male mating index[9] was 100 % in all test groups in both generations. The male fertility index[10] for the F_0 parents was 100 % in all test groups. For the F_1 parental males, two high-dose males (numbers 283 and 293) did not generate F1 pups, so that the male fertility index ranged between 92 % and 100 %. The applicant provided extensive historical control data from the test facility (33 studies), dating from 2008 to 2013, to argue that this finding reflected the normal range of biological variation inherent in the strain of rats used (range 80-100, mean = 91.8); the index in the high-dose group was thus identical to the mean of the historical-control data. The RMS notes that the BAS 750 F study was conducted in 2013, and so the date ranges of the historical control data are appropriate. The two males in question did not show gross necropsy, histopathological findings or changes in sperm quality that would suggest infertility. The RMS thus considers the finding in 2 / 25 males, only in the F_1 generation and within the historical-control range, to be incidental.

[9] $\text{Male mating index}[\%] = \frac{\text{number of males with confirmed mating*}}{\text{number of males placed with females}} \times 100$

* defined as females with vaginal sperm or with implants in utero

[10] $\text{Male fertility index}[\%] = \frac{\text{number of males proving their fertility*}}{\text{number of males placed with females}} \times 100$

* defined as females with implants *in utero*

Table B.6.6.1.5. Reproduction parameters of male rats treated with BAS 750 F

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
- # animals per group	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
- # males placed with females	25	25	24	25	25	25	25	25
- # males mated	25	25	24	25	25	25	25	25
- Male mating index [%]	100	100	100	100	100	100	100	100
- # mated females pregnant	25	25	24	25	25	25	25	23
- Male fertility index [%]	100	100	100	100	100	100	100	92

Statistical analysis: Fisher's Exact test (1-sided -); *: p< 0.05; **: p<0.01

Historical control data [33 studies run 2008-2013 at test facility with Wistar rats (supplier: Charles River)]

- Male fertility index [%]: 80-100, mean = 91.8

Sperm analyses, which investigated sperm motility and determined the incidence of sperm head counts in the testis as well as the percentage of abnormal sperm in the testis and cauda epididymis, did not indicate any effects of treatment in F_0 or F_1 males.

Table B.6.6.1.6. Sperm parameters of male rats treated with BAS 750 F

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
- # animals per group	25	25	25	25	25	25	25	25
Sperm count [10^6 / g]								
- testis	110	N.A.	N.A.	117	102	N.A.	N.A.	103
- cauda epididymis	740	N.A.	N.A.	824	740	N.A.	N.A.	730
Sperm motility [%]	88	87	88	88	89	87	88	87
Abnormal sperm [%]	6.0	N.A.	N.A.	6.0	6.0	N.A.	N.A.	6.0

Statistical analysis: Wilcoxon with Bonferoni-Holm (1-sided -); *: p< 0.05; **: p<0.01

Female reproductive parameters

BAS 750 F did not adversely affect reproduction or delivery of the F_0-generation parental females. The female mating index[11] was 100 % in all groups. All female F_0 rats delivered pups or had implants *in utero*. The fertility index[12] was thus 100 % in all test groups. The mean duration of gestation was slightly increased in the high-dose group (22.4 compared with 22.1 in controls [$p \leq 0.05$]), although this represented a difference from the control group of less than half a day; furthermore, all values were within the historical control range of the test facility (31 studies, 2008 to 2013: 21.9 to 22.5, mean 22.13). Parturition appeared to proceed normally in all animals of the high-dose group; clinical signs

[11] $$\text{Female mating index}[\%] = \frac{\text{number of females mated*}}{\text{number of females placed with males}} \times 100$$

* defined as the number of females with vaginal sperm or with implants in utero

[12] $$\text{Female fertility index}[\%] = \frac{\text{number of females pregnant*}}{\text{number of females mated**}} \times 100$$

* defined as the number of females with implants in utero

** defined as the number of females with vaginal sperm or with implants in utero

were not reported in the dams, and the live-birth index[13] of the high-dose group was comparable with the controls, as was the number of still-born pups. Generally, the post-natal pup survival of the high-dose group was also comparable with the controls (see section on offspring toxicity), apart from one dam (number 200), which delivered eleven live pups on gestation day 23 (none dead), but had total litter loss on post-natal day 2. This dam had markedly lower food consumption on post-natal days 1 – 4 (16.5 g) than the group means (32.0, 30.8, 32.6 and 28.8 g at 0, 25, 75 and 200 mg/kg bw/d, respectively). The gestation index[14] was 100 % in all groups.

The mean number of implantation sites in the F_0 parental females was comparable between all groups. Furthermore, there were no statistically significant differences in post-implantation loss between the groups nor the litter size in the mid- and high-dose groups (the RMS considers a slightly lower value only in the low-dose group to be incidental).

The female mating index of the F_1 parental animals was 100 % in all groups. All female F_1 rats delivered pups or had implants *in utero* except for high-dose female number 383 (mated with male number 293) and high-dose female number 393 (mated with male number 283); these females were sperm-positive but did not become pregnant. There were no corroborative gross or histopathological findings in the sexual organs of these two females and the time-to-mating was also unremarkable. The fertility index in the high-dose group was thus 92.0 %, compared with 100 % in the other groups. This value for the high-dose group was within the range of the date-relevant and extensive historical control data of the test facility in the same strain (32 studies, 2008 to 2013: range 80 to 100, mean 97 %). The mean duration of gestation was similar in all test groups (i.e., between 22.0 and 22.2 days). The gestation index of the high-dose group (91.3 %) was slightly below that of the other groups (100 %) owing to two females (number 379: 6 still-born pups; number 383: 1 implantation site, no pups present) that were pregnant but did not deliver any live pups. This value was within the historical control range of the test facility over an appropriate time-frame and in the same strain (31 studies 2008-2013: range 90 to 100 %, mean = 99 %). Within this historical-control data, two females had entirely still-born litters; when additional studies from 2014 and 2015 (same test facility) are included, two more dams with only still-born pups are reported. Therefore, it is not clear that BAS 750 F was responsible for the still-born litter in the present study.

The mean number of implantation sites per dam was slightly but statistically significantly lower in the high-dose-group F_1 parents (mean 10.0, range 1 to 14, compared with mean 12.0, range 9 to 16 in the controls). The mean of the high-dose group was affected by one female (number 388) that contained only one implant. Notwithstanding, the mean value of the high-dose group was within the historical-control range of the test facility (31 studies, 2008-2013: range 9.4 to 14.0, mean 12.1). A dose-related change was also not seen, since the mean number of implantation sites in the mid-dose group was higher than that in the control group. Overall, therefore, the RMS considers that the slightly lower value in the high-dose group reflected biological variation and was not a treatment-related effect. Although there were slight increases in post-implantation loss in the mid- (7.1 %) and high-dose (8.9 %) groups compared with the controls (2.4 %), these were also most likely to be a reflection of normal biological variation and were well within the historical control range (31 studies, 2008-2013: range 0.9 to 17.7, mean 6.7). Also, the value of the high-dose group (which was not statistically significantly different from the control value) was strongly influenced by the female (number 388) with only one implant; this implant was resorbed, which resulted in a post-implantation loss of 100 % for this animal. Exclusion of this animal from the calculation would lead to a group mean of 4.8 %.

[13] $\text{Live birth index}[\%] = \frac{\text{number of live born pups at birth}}{\text{total number of pups born}} \times 100$

[14] $\text{Female gestation index}[\%] = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant*}} \times 100$

* defined as the number of females with implants in utero

As a consequence of the fewer implants, the mean number of F_2 pups delivered per dam (excluding those that were not pregnant or did not deliver) was statistically significantly decreased in the high-dose group (9.9 compared with 11.9 in the controls) but without a clear dose-response relationship. Furthermore, since the mean value was within the historical control range of the test facility (9.2 – 13.4, mean 11.4), there was no evidence that BAS 750 F exposure affected the number of pups born per dam. The live-birth index was also unaffected and within the historical control range of the test facility at all doses, with six of the nine still-born pups at 200 mg/kg bw/d being from one litter, the dam of which was obviously unwell.

Table B.6.6.1.7. Reproduction parameters of female rats exposed to BAS 750 F

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
- # animals per group	25	25	25	25	25	25	25	25
- # females placed with males	25	25	24	25	25	25	25	25
- # females mated	25	25	24	25	25	25	25	25
- Female mating index [%]	100	100	100	100	100	100	100	100
- # females pregnant	25	25	24	25	25	25	25	23
- Female fertility index [%]	100	100	100	100	100	100	100	92
Pre-coital interval [mean days]	2.8	2.4	3.0	2.8	3.0	3.0	2.5	2.8
Duration of gestation [mean days]	22.1	22.2	22.2	**22.4***	22.2	22.0	22.0	22.2
Implantation sites, total	307	284	288	295	300	285	308	229
- per dam	12.3	11.4	12.0	11.8	12.0	11.4	12.3	**10.0***
Post-implantation loss [mean %]	3.9	5.5	1.3	5.2	2.4	5.0	**7.1****	8.9
Females with live-born	25	25	24	25	25	25	25	21
- with still-born pups	1	1	0	1	1	3	2	3
- with all still-born	0	0	0	0	0	0	0	1
- Gestation index [%]	100	100	100	100	100	100	100	91.3
Pups delivered	297	267	284	277	298	269	285	217
- per dam	11.9	**10.7***	11.8	11.1	11.9	10.8	11.4	**9.9****
- live-born	296	266	284	274	295	263	283	208
- still-born	1	1	0	3	3	6	2	9
- Live-birth index [%]	99.7	99.6	100	98.9	99.0	97.8	99.3	95.9

Statistical analysis: *: p< 0.05; **: p<0.01

Historical control data [33 studies run 2008-2015 at test facility with Wistar rats (supplier: Charles River)]
- Duration of gestation: 21.8 – 22.9 days
- Gestation index: 87.5 – 100 %
- Implantation sites/dam: 9.4 – 14.0
- Post-implantation loss [mean %]: 0.9 – 17.7
- Pups delivered/dam: 9.2 – 13.4
- Live-birth index [mean %]: 92.1 – 100

Historical control data [31 studies run 2008-2013 at test facility with Wistar rats (supplier: Charles River); narrowed to date-relevant studies by RMS]
- Duration of gestation: 21.9 – 22.5 days
- Gestation index: 90 – 100 %
- Implantation sites/dam: 9.4 – 14.0
- Post-implantation loss [mean %]: 0.9 – 17.7
- Pups delivered/dam: 9.2 – 13.4
- Live-birth index [mean %]: 95.8 – 100

Offspring toxicity

The viability index of F_1 pups during early lactation (PND 0 - 4) was similar across the groups, without statistically significant differences. The slightly lower value in the high-dose group (93.5 %, compared with 99.1 % in the controls), resulting from more dead pups (18 vs. 3 in the controls), was a consequence of one litter (dam number 200) in which 11 pups died or were subject to cannibalism; the

one pup that could be examined had indications of improper nursing (empty stomach). The remaining seven dead pups (two found dead, five cannibalised) at 200 mg/kg bw/d originated from the litters of six additional F_0 dams. The viability indices in the study were just outside the range of historical controls (31 studies conducted 2008-2013: 95.3 % – 100 %). However, the RMS notes that in control data from an additional study that was conducted in 2014 in the same test facility, the viability index was 89.4 %. Since the slightly reduced viability index at 200 mg/kg bw/d was heavily influenced by one litter, at a dose at which maternal toxicity was evident (mean body weight reduced by up to 13 %), the RMS does not consider that this constitutes evidence of a specific effect on post-natal survival. The lactation index indicated that pup survival between PND 4 and 21 was high in all the groups.

The viability index of the F_2 pups indicated that pup survival during early lactation was similar in all the groups, with no statistically significant differences. The slightly lower value at 200 mg/kg bw/d was explained by a higher number of pups dying during PND 1 – 4 (15 vs. 1 in the controls). Eleven of the 15 dead pups came from one litter (dam number 397), and were not properly nourished during PND 1 – 3, as became evident by a severely reduced nutritional condition and the absence of milk in the stomach of the investigated pups. Consequently, this animal lost its entire litter within the first three days after birth. The food consumption of this dam during post-natal days 1 – 4 (13.3 g) was notably lower than the group means (33.5, 30.2, 33.3 and 27.2 g at 0, 25, 75 and 200 mg/kg bw/d). The remaining four dead pups, which were subject to cannibalism, originated individually from the litters of four F_1 dams. The lactation index indicated that pup survival between PND 4 and 21 was high in all the groups.

There was no evidence of a substance-related effect on the sex ratio in either generation.

Table B.6.6.1.8. Pup survival and sex ratio

Parental generation	**F_0**				**F_1**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
Number of litters	25	25	24	25	25	25	25	22
- with live-born pups	25	25	24	25	25	25	25	21
- with still-born pups	1	1		1				1
Pups live-born	296	266	284	274	295	263	283	208
Pups found dead (day 1-4)	1	2		10			1	2
Pups cannibalized (day 1-4)	2			8	1	1	2	13
Pups PND 4 (pre-cull)	293	264	284	256	294	262	280	193
- Viability index [%]	99.1	99.4	100	93.5	99.7	99.6	99.0	93.6
Pups culled day 4	99	73	92	69	95	66	81	38
Pups PND 4 (post-cull)	194	191	192	187	199	196	199	155
Pups found dead (day 5-21)								
Pups cannibalized (day 5-21)			1			1		1
Pups PND 21	194	191	191	187	199	195	199	154
- Lactation index [%]	100	100	99.0	100	100	99.5	100	99.4
Sex ratio [% live males], PND 0	47.7	49.5	51.3	49.2	49.4	44.4	52.8	50.2
Sex ratio [% live males], PND 21	47.6	50.7	47.8	50.2	48.9	48.0	50.8	48.7

Statistical analysis, viability and lactation indices: Wilcoxon with Bonferoni-Holm (1-sided -), sex ratio: Wilcoxon test (2-sided); * $p \leq 0.05$, ** $p \leq 0.01$

No substance-related clinical observations were apparent in the F_1 pups. Of the F_2 pups, six from a dam dosed with 200 mg/kg bw/d BAS 750 F (number 397) showed evidence of a reduced nutritional

condition and an absence of milk in the stomach on PND 1 and 2. There were no clinical signs of toxicity in any of the other F_2 pups.

Male pups were investigated for the presence of nipples and areolae on PNDs 12 and 20. At PND 12, there was no dose-related effect on the apparent number and percentage of male F_1 or F_2 pups having areolae. By PND 20, no areolae were detected in any of the male pups of either generation.

Anogenital distance was measured on PND 1. There were no dose-related effects on anogenital distance or index[15] in the male or female F_1 pups. The slightly higher index in the high-dose group was likely to be an incidental finding, since a dose-related change in the index was not evident and the distance was very slightly less than that of the controls. In the F_2 male pups, the index was apparently higher than the controls in the high-dose group; this was a consequence of the lower body weight, since the actual distance was identical to that of the controls. The anogenital distance of the F_2 female pups was slightly below the control in all BAS 750 F-exposed groups. However, as the index was unchanged and very close to the controls in all the exposed groups, these minimal differences were unlikely to be related to treatment.

Table B.6.6.1.9. Anogenital distance and anogenital index

Pup generation	**F_1 pups**				**F_2 pups**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
MALES (No. examined)	1.43	1.32	148	127	145	115	148	103
- Anogenital distance [mm] (day 1)	3.02	3.08*	3.01	2.97	3.05	3.04	3.05	3.05
[Δ% control]		2.0	-0.3	-1.7		-0.3	±0.0	±0.0
- Anogenital index [---] (day 1)	1.61	1.62	1.62	1.63	1.62	1.62	1.63	1.66**
[Δ% control]		0.6	0.6	1.2		±0	+0.6	+2.5
FEMALES (No. examined)	150	132	136	132	149	147	132	96
- Anogenital distance [mm] (day 1)	1.50	1.47	1.50	1.49	1.58	1.55*	1.53**	1.52**
[Δ% control]		-2.0	±0.0	-0.7		-1.9	-3.2	-3.8
- Anogenital index [---] (day 1)	0.81	0.79*	0.82	0.84**	0.85	0.84	0.84*	0.84
[Δ% control]		-2.5	1.2	3.7		-1.2	-1.2	-1.2

Statistical analysis: * $p < 0.05$; ** $p < 0.01$ (Dunnett-test, 2-sided)

At 200 mg/kg bw/d, lower F_1 pup body weights were noted on PND 1 (about 9 % below controls). Pup body weights remained lower until weaning (about 10 % below controls on PND 21). Accordingly, mean body-weight change was below the controls in the high-dose F_1 pups (by up to 15 %) throughout lactation. There were no treatment-related changes in F_1 pup body weights/body-weight change at 75 and 25 mg/kg bw/d.

In the F_2 pups at 200 mg/kg bw/d, lower pup body weights were noted on PND 1 (about 7 % below controls). Pup body weights remained lower until weaning (about 14 % below controls on PND 21). Overall, the mean body-weight change was below the concurrent controls in the high-dose F_2 pups (up to 19 % lower) throughout lactation.

Pup organ weights (brain, spleen and thymus) were measured from one male and one female in each litter on PND 21 (weaning). The body weights on PND 21 were used to calculate relative organ weights. The mean absolute brain (-2.9 %), spleen (-12.7 %) and thymus (-12.6 %) weights of male and female F_1 pups from the high-dose group were decreased. The relative brain weights of the high-dose male and female F1 pups were increased by 9.1 %; there were no significant differences in the

[15] $\text{Anogenital index} = \frac{\text{anogenital distance [mm]}}{\text{cubic root of pup weight [g]}} \times 100$

relative thymus or spleen weights between control and test groups. In the high-dose F_2 pups, absolute brain (-3.5 %), thymus (-16.2 %) and spleen (-17.6 %) weights were statistically significantly decreased. The relative brain weights of F_2 pups were increased by 13.1 % at 200 mg/kg bw/d; the relative spleen and thymus weights were not statistically significantly different from the controls at any dose. The RMS considers the observed organ weight changes in both generations to be secondary to the lower pup body weights in the high-dose group.

Upon necropsy of the pups, one F_1 pup of litter 200, which was entirely lost, had an empty stomach; nothing abnormal was detected in five other pups of this litter. The remaining five pups of the litter were subject to cannibalism before they could be examined. In the F_2 pups, dilated renal pelves were found in 23 pups at 200 mg/kg bw/d (6 males, 17 females from 9 litters) compared with three affected female pups from three litters in the control group. The finding was treatment related but probably secondary to the general delay in development of the high-dose pups (up to 19% decrease in body-weight gain, statistically significant); the study authors assumed this to be a largely reversible effect. All other findings in both generations occurred spontaneously across all the groups.

Table B.6.6.1.10. Incidence of dilated renal pelvis in F_1 and F_2 pups

	Male pups				**Female pups**			
Dose [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
F_1 pups								
Animals examined	119	107	121	109	127	107	110	117
Renal pelvis, dilated	3 (3)	2 (2)	5 (4)	2 (2)	1	3 (3)	3 (3)	3 (3)
F_2 pups								
Animals examined	145	120	148	112	152	149	132	105
Renal pelvis, dilated		1	1	6 (5)	3 (3)	2 (2)	1	17 (9)

( ) values in brackets give litter incidence

Sexual maturation was determined in each male and female F_1 pup that was selected to become an F_1 parent. The mean age at which females reached sexual maturity was slightly delayed in the high-dose pups (31.8 days compared with 30.0 days in controls), although there wasn't a clear dose-response relationship, with there being a statistically significant delay also in the low-dose but not the mid-dose group. The days to opening were within the relevant historical control range in all the groups (15 studies, range 29.5 to 31.9, mean 30.5). The control value also seemed to have an unusually low standard deviation: the days to vaginal opening in this group ranged from 29 to 31, whereas additional historical control data provided by the applicant showed that the day of vaginal opening usually has a wider distribution (study 1: 28 to 37; study 2: 27 to 33; study 3: 28 to 34; study 4: 28 to 36). Although the female pup weight in the high-dose group was equivalent to the control group when vaginal opening was determined, it was reduced throughout lactation; any treatment-related effect was thus a developmental delay that was secondary to maternal toxicity. Therefore, the RMS does not regard this as evidence of a specific adverse effect. The age of preputial separation in male pups was not affected even in the high-dose group.

Table B.6.6.1.11. Sexual maturation of F_1 pups

Parental generation	**Females / Vaginal opening**				**Males / Preputial separation**			
Dose level [mg/kg bw/d]	**0**	**25**	**75**	**200**	**0**	**25**	**75**	**200**
# animals examined	25	25	25	25	25	25	25	25
- Days to criterion	30.0	30.9*	30.2	31.8**	43.1	42.7	43.2	43.5
- Body weight at criterion [g]	91.6	93.5	90.8	91.9	182.3	177.3	183.6	167.9**

Statistical analysis: * $p < 0.05$; ** $p < 0.01$ (Dunnett-test, 2-sided)
Historical control data: 17 studies (2010-2015) from the test facility with Wistar rats (supplier: Charles River)
Day of vaginal opening: 29.5 – 31.9 days (body wt at criterion: 83.1 – 100.7 g)
Day of preputial separation: 40.5 – 45.2 days (body wt at criterion: 168.1 – 195.3 g)

Historical control data: 15 studies (2010-2013) from the test facility with Wistar rats (supplier: Charles River)	
Day of vaginal opening:	29.5 – 31.9 days (body wt at criterion: 83.1 – 100.7 g)
Day of preputial separation:	40.5 – 45.2 days (body wt at criterion: 168.1 – 195.3 g)

Discussion and conclusion

The potential of BAS 750 F to adversely affect fertility, pregnancy outcome and post-natal offspring survival has been investigated in a guideline-compliant two-generation reproduction study in Wistar rats.

Parental toxicity was evident in the high-dose group (200 mg/kg bw/d) as consistent reductions in food consumption, body weights and changed clinical-chemistry parameters that, together with the increased liver weights, indicated some disruption of normal liver function. Some evidence of liver dysfunction was also evident in the mid-dose group (75 mg/kg bw/d).

In terms of fertility, the male and female mating index was 100 % in all groups in both generations, as was the fertility index in the F_0 parents. Two F_1 male/female pairs at 200 mg/kg bw/d failed to result in implants, but as there was no pathological explanation and the resultant fertility indices were within the historical control range, the RMS concludes that this does not provide evidence of a treatment-related effect on fertility. In the F_1 generation there were some additional reductions in reproduction parameters in the high-dose group (implantation sites per dam, with a consequent reduction in pups delivered per dam) but, again, these were within the historical control ranges and were likely to be a reflection of normal biological variation, possibly compounded to some extent by the maternal toxicity at this dose. Two high-dose females of the F_1 generation did not deliver any live pups; one of them (number 379) delivered six still-born pups, whilst the other (number 383) showed evidence of just one implantation site but no pups. These two females were responsible for the slight reduction in the gestation index at this dose that was, nevertheless, not statistically significant and was within the historical control range. Overall, there was no evidence of intra-uterine embryo- or foetal-lethality, since the post-implantation loss in the high-dose group in both generations was similar to the concurrent and historical controls.

The duration of gestation was very slightly but statistically significantly increased in the F_0 parent females; the increase represented only half a day and might have been an artefact of the monitoring of the animals. Within the F_0 generation, delivery appeared to proceed normally; there were no consequences on the number of live births or post-natal survival, apart from one dam that delivered on gestation day 23 and had total litter loss on post-natal day 2. However, there is no information to link the litter loss to the delay in parturition, and this occurrence only in one dam does not provide evidence of a treatment-related effect on pregnancy outcome. The mean duration of gestation was not affected in F_1 dams. It is noted, however, that one of the F_1 dams (number 379) appeared to show normal condition on gestation day 22, but on gestation day 23 had severe general condition, piloerection and was unable to deliver, with the eventual delivery of only still-born pups on gestation day 24. Notwithstanding, this was an isolated occurrence and the RMS thus concludes that BAS 750 F did not adversely affect gestation duration or parturition.

There was no evidence that BAS 750 F had a specific adverse effect on pup survival from post-natal days 1 to 4 or 4 to 21. Slight reductions in the viability index in both generations at 200 mg/kg bw/d, neither of which was statistically significant, were a consequence of single whole-litter losses; these appeared to be the result of inadequate nursing, arising from much reduced food intake of the respective dams. The overall lower food consumption and lower body weights early in lactation of the dams in the high-dose group affected development of the pups: in each generation, pup body weights of the 200 mg/kg bw/d group were reduced compared with the controls from post-natal day 1 and throughout lactation. An increased incidence of dilated renal pelves only in the high-dose-group F_2 generation was likely to be treatment related but not indicative of specific developmental toxicity; rather, it was a variation that was a secondary effect of delayed development, resulting from the lower

body weights at this dose. This was also the most likely explanation for minor changes in some markers of development and sexual maturation.

Overall, the RMS concludes that a specific effect on fertility, reproduction and pregnancy outcome was not demonstrated by this study. Slight changes in some of the reproduction parameters and offspring toxicity were evident only at a dose that also resulted in parental toxicity (decreased food consumption and body weights) and an apparent lack of maternal care. Therefore, BAS 750 F did not show evidence of specific reproductive toxicity in this study.

The NOAEL for general, systemic toxicity was 25 mg/kg bw/d, based on evidence of liver toxicity at the LOAEL of 75 mg/kg bw/d. The NOAEL for fertility and reproductive performance was 200 mg/kg bw/d, whilst that for offspring toxicity was 75 mg/kg bw/d, based upon the decreases in pre-weaning pup body weights and body-weight gains at 200 mg/kg bw/d.

To refine the assessment, the RMS has calculated BMD values for systemic parental toxicity and developmental toxicity. The NOAEL for fertility will be used, since there were no adverse effects on fertility on which to base a BMD analysis.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Parental systemic toxicity					
Relative liver weight	15 %	F_0 Males	217.2	311.6	1.4
		F_0 Females			
		F_1 Males			
		F_1 Females			
Body weight (gestation)	10 %	F_0 Females	199.3	314.1	1.6
		F_1 Females	116.7	195.3	1.7
Overall body-weight gain (gestation)	10 %	F_0 Females	145.3	225.7	1.6
		F_1 Females	81.8	187.2	2.3
Alkaline phosphatase	50 %	F_0 Males	42.3	70.7	1.7
		F_0 Females	90.3	248.3	2.7
		F_1 Males	34.1	96.0	2.8
		F_1 Females	181.9	314.7	1.7
Offspring toxicity					
Body weight (PND 7)	10 %	F_1 Males	167.5	217.3	1.3
		F_1 Females			
		F_2 Males			
		F_2 Females			
Body-weight gain (lactation day 1 to 21)	10 %	F_1 Males	163.2	202.9	1.2
		F_1 Females			
		F_2 Males			
		F_2 Females			

The lowest BMDL for systemic toxicity was thus 34.1 mg/kg bw/d for a 50 % increase in alkaline phosphatase activity, whilst the lowest BMDL for offspring toxicity was 163.2 mg/kg bw/d, which was based upon a 10 % decrease in pup body-weight gain. These were consistent with the proposed NOAELs, taking into account the dose spacing.

B.6.6.2. Developmental toxicity studies

Two developmental toxicity studies have been submitted, one of which was in rats and one in rabbits.

Table B.6.6.2.1. Summary of developmental toxicity studies

Study **Species,** **Dose levels** **(batch / purity)**	**NOAEL / BMDL (mg/kg bw/d)**	**LOAEL (mg/kg bw/d)**	**Critical effects**
Rat oral prenatal developmental toxicity			
Rat (Wistar, Crl:WI(Han)) 0, 50, 150, 400 mg/kg bw/d, gavage GD 6-19 25 females / group Vehicle: 1 % CMC OECD 414 GLP Purity 97.7 % CA 5.6.2/1: [redacted]., 2015a (2014/1170755)	**Maternal toxicity**		
	NOAEL **150** **$BMDL_{10}$** **40.2** (corrected body-weight gain)	**400**	At 400 mg/kg bw/d: ↓ food consumption (-8 %), body weight (-7 %) and body-weight gain (-34 % corrected weight) No adverse effects at other doses
	Developmental toxicity		
	400	---	No treatment-related, adverse effects; all findings within historical control ranges.
Rabbit oral prenatal developmental toxicity			
Rabbit (New Zealand White) 0, 5, 15, 25 mg/kg bw/d, gavage GD 6-28 30-33 females / group Vehicle: 1 % CMC OECD 414 GLP Purity 95.5 % CA 5.6.2/2: [redacted]., 2015b (2014/1170757); CA 5.6.2/3: [redacted], 2016 (2016/1321110)	**Maternal toxicity**		
	NOAEL **25**	---	No treatment-related adverse effects (Dose selected on basis of range-finding studies, in which 2/3 non-pregnant rabbits at 50 mg/kg bw/d and 1/3 at 25 mg/kg bw/d were sacrificed in a moribund condition.)
	Developmental toxicity		
	NOAEL **25**	---	No treatment-related, adverse effects

B.6.6.2.1. Developmental toxicity in rats

BAS 750 F was administered to groups of 25 presumed-pregnant rats by gavage at dose levels of 0, 50, 150 and 400 mg/kg bw/d during days 6 to 19 of gestation. At terminal sacrifice on gestation day (GD) 20, 24 to 25 females per group had implantation sites.

Only pregnant dams were used for the calculations of mean maternal food consumption, body weight and body-weight change. Only pregnant dams with scheduled sacrifice on GD 20 were taken for the calculation of mean gravid uterine weights, body-weight change corrected for uterine weight and summary of reproduction data. Therefore, the following females were excluded from the above-

mentioned calculations: control female number 22 (not pregnant), mid-dose female number 70 (not pregnant) and high-dose female number 92 (not pregnant).

There were no deaths or clinical signs of toxicity in any group. The mean food consumption of the dams treated with 400 mg/kg bw/d was substantially reduced from GD 8 onwards until scheduled sacrifice on GD 20. The average reduction of food consumption in the high-dose dams compared with the controls during the entire treatment period (GD 6-19) was 8 %. The mean food consumption of the dams from the low- and mid-dose group was comparable to the controls.

Table B.6.6.2.2. Food consumption

Dose [mg/kg bw/d]	Day of Gestation									
	0–6	**6–19**	**0–20**	**6–8**	**8–10**	**10–13**	**13–15**	**15–17**	**17–19**	**19–20**
0 [g/animal]	16.4	21.5	20.1	18.4	20.4	20.8	21.9	23.4	24.1	22.4
50 [g/animal]	16.2	21.3	19.8	18.8	19.9	21.0	21.8	23.1	23.1	22.0
[% control]	99%	99%	99%	102%	98%	101%	100%	99%	96%	98%
150 [g/animal]	16.5	21.4	20.0	18.8	19.8	21.1	22.4	23.4	23.1	21.4
[% control]	101%	100%	100%	102%	97%	101%	102%	100%	96%	96%
400 [g/animal]	16.0	19.8	18.6	17.9	**17.9****	19.5	20.6	21.9	**21.0****	**19.3****
[% control]	98%	92%	93%	97%	**88%**	94%	94%	94%	**87%**	**86%**

* $p < 0.05$, ** $p < 0.01$ (Dunnett test, two-sided)

A treatment-related effect on body weight occurred at 400 mg/kg bw/d from GD 15 onwards. At this dose the mean body-weight gain was statistically significantly reduced during GD 10-15 (up to 27 % below the concurrent control value). When calculated for the entire treatment phase (GD 6-19), the mean body-weight gain was about 17 % below the controls. Body weights and body-weight gains were unaffected at 50 and 150 mg/kg bw/d.

Table B6.6.2.3. Body weight

Dose	Day of Gestation								
	0	**6**	**8**	**10**	**13**	**15**	**18**	**19**	**20**
0 mg/kg bw/d Mean bw [g]	163.9	198.2	206.9	216.5	233.3	243.8	261.7	282.6	294.9
50 mg/kg bw/d Mean bw [g]	161.0	196.3	203.8	213.1	228.9	238.0	255.0	274.3	285.7
[% control]	98%	99%	99%	98%	98%	98%	97%	97%	97%
150 mg/kg bw/d Mean bw [g]	167.8	202.7	211.2	220.5	234.9	246.6	264.0	283.7	297.0
[% control]	102%	102%	102%	102%	101%	101%	101%	100%	101%
400 mg/kg bw/d Mean bw [g]	164.1	197.5	204.5	212.0	224.3	**232.1***	**248.0***	**266.8***	**278.6***
[% control]	100%	100%	99%	98%	96%	**95%**	**95%**	**94%**	**94%**

* $p < 0.05$, ** $p < 0.01$ (Dunnett test, two-sided)

At sacrifice, the uterus weight, carcass weight and corrected body-weight gain were determined. The mean weights of the unopened uteri were comparable between groups. The corrected body weight gain (terminal body weight on GD 20 minus weight of the unopened uterus minus body weight on GD 6) was statistically significantly lower at 400 mg/kg bw/d (about 34 % below the concurrent control value), as was the carcass weight of the dams of this group (about 7 % below controls). Statistically significant changes were not recorded at 50 and 150 mg/kg bw/d.

Table B.6.6.2.4. Uterus weight, carcass weight and corrected (net) body weight gain

Parameter (g)	BAS 750 F dose level (mg/kg bw/d)			
	0	**50**	**150**	**400**
Gravid uterus	51.7 ± 10.0	49.3 ± 14.9	54.3 ± 9.3	51.8 ± 12.2
[% control]		95%	105%	100%
Carcass	243.2 ± 15.0	236.4 ± 17.2	242.7 ± 20.1	**226.8** ± **10.5**
[% control]		97%	100%	**93%****
Net weight change from GD 6	44.4 ± 5.6	40.1 ± 9.3	40.1 ± 9.5	**29.3** ± **5.6**
[% control]		90%	90%	**66%****

* p < 0.05, ** p < 0.01 (Dunnett test, two-sided)
Carcass weight = terminal body weight minus uterine weight
Net weight change from GD 6 = carcass weight minus GD 6 body weight

There were no treatment-related gross necropsy findings in the dams.

The caesarean section data showed that 24, 25, 24 and 24 rats were pregnant at 0, 50, 150 and 400 mg/kg bw/d. None of the pregnant dams aborted or gave birth prematurely. One dam of the low-dose group had only resorptions with no live foetuses; otherwise, all dams had viable foetuses. There were no dose-related differences between control and test groups in the mean number of pre- and post-implantation losses, the number of resorptions or viable foetuses. Furthermore, all values were within the range of the historical control data performed at the same test facility with the same rat strain (see table below). Exposure to BAS 750 F did not result in changes in the sex ratio.

Table B.6.6.2.5. Caesarean section data

BAS 750 F (mg/kg bw/d)		**0**	**50**	**150**	**400**
Corpora lutea	[N]	10.2 ± 1.50	10.8 ± 2.42	11.1 ± 1.48	11.3 ± 1.52
total number	[N]	245	270	267	272
Implantation sites	[N]	9.7 ± 2.01	9.3 ± 2.65	10.4 ± 1.88	9.9 ± 2.10
total number	[%]	232	232	249	238
Pre-implantation loss	[%]	6.0 ± 11.3	14.7 ± 19.5	7.1 ± 8.7	12.1 ± 17.8
Post-implantation loss	[%]	3.7 ± 6.2	8.2 ± 20.5	7.2 ± 8.0	6.8 ± 9.1
Resorptions	[N]	0.4 ± 0.65	0.4 ± 0.71	0.8 ± 0.88	0.7 ± 0.92
total number	[N]	9	11	19	16
Early resorptions	[N]	0.4 ± 0.65	0.4 ± 0.71	0.8 ± 0.90	0.5 ± 0.59
total number	[N]	9	11	18	11
Late resorptions	[N]	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.20	0.2 ± 0.59
total number	[N]	0	0	1	5
Dead fetuses	[N]	0	0	0	0
Live fetuses	[N]	9.3 ± 1.99	9.2 ± 2.06	9.6 ± 1.74	9.3 ± 2.29
total number	[N]	223	221	230	222
males	[N]	112	109	125	108
females	[%]	111	112	105	114
male / female ratio		50.2 / 49.8	49.3 / 50.7	54.3 / 45.7	48.6 / 51.4
Placental weight	[g]	0.44 ± 0.06	0.44 ± 0.05	0.46 ± 0.05	**0.50** ± **0.07**
males	[g]	0.46 ± 0.07	0.46 ± 0.06	0.47 ± 0.05	**0.51*** ± **0.08**
females	[g]	0.43 ± 0.06	0.42 ± 0.05	0.45 ± 0.06	**0.49**** ± **0.06**
Foetal weight	[g]	3.6 ± 0.35	3.7 ± 0.13	3.7 ± 0.22	3.5 ± 0.21
males	[g]	3.7 ± 0.36	3.8 ± 0.17	3.8 ± 0.23	3.6 ± 0.22
females	[g]	3.6 ± 0.39	3.5 ± 0.20	3.6 ± 0.22	**3.4*** ± **0.24**

* p < 0.05, ** p < 0.01 (Dunnett test, two-sided)

The mean placenta weights were slightly but statistically significantly increased at 400 mg/kg bw/d (approximately 113 % of the control group value). This change was not associated with impaired foetal development and was, moreover, well within the historical control range and thus is considered by the RMS to be non-adverse. The mean foetal weights were not affected by exposure to BAS 750 F at any dose, with the exception of a marginal decrease in the females at 400 mg/kg bw/d (3.4 vs. 3.6 g, 94% of control value). However, as the group mean exactly matches the mean of the historical control (3.4 g) and as there is no effect in the corresponding male foetuses, the RMS concludes that this does not represent a treatment-related effect.

Table B.6.6.2.6. Historical control data

Historical control data	Mean	±	SD	Range (per study)		95% spread
				Minimum	Maximum	2.5% – 97.5%
Corpora lutea	11.8	±	1.96	10.0	16.0	n.d.
Implantation sites	11.0	±	2.26	9.1	15.3	n.d.
Pre-implantation loss [mean %]	6.9	±	13.09	1.4	17.5	n.d.
Post-implantation loss [mean %]	7.3	±	11.19	3.5	18.1	n.d.
Resorptions [N]	0.8	±	1.00	0.3	1.5	n.d.
Live litter size [N]	10.2	±	2.34	8.3	14.8	n.d.
Placenta weights [g]	0.46			0.33	1.16	0.32 – 0.60
Males	0.47			0.30	1.16	0.33 – 0.61
Females	0.45			0.28	0.99	0.32 – 0.58
Foetal weights [g]	3.5			2.3	5.1	2.2 – 4.1
Males	3.6			2.4	5.4	3.0 – 4.2
Females	3.4			2.2	4.9	2.9 – 4.1

76 studies performed at the test facility between 2009–2014 with Wistar rats (Charles River)
(1518 pregnant dams; 1502 litters with 15402 viable fetuses)

External malformations were recorded in 0, 0, 1 (mandibular micrognathia with severely malformed skull bones) and 1 (multiple malformations: cleft palate, microphthalmia, malformed mandible) foetuses at 0, 50, 150 and 400 mg/kg bw/d. The two foetuses with multiple skull-bone malformations showed a different spectrum of bone alterations. Moreover, historical control data from the same laboratory and rat strain showed that multiple malformations of the skull bones appeared fairly frequently and uniformly between 2009 and 2016 (76 studies between 2009 and 2014: 10 cases, range of foetal incidences 0 to 2 %; 79 studies between 2011 and 2016: 11 cases, range of foetal incidences 0 to 2 %). External variations were recorded in 0, 1 (limb hyperflexion), 0 and 0 foetuses at 0, 50, 150 and 400 mg/kg bw/d. There was therefore no association of external malformations and variations with exposure to BAS 750 F.

Upon visceral examination, two soft tissue variations were noted in both the control and exposed groups but without statistical significance. Dilated renal pelvis was noted in 2 (1.9 %), 1 (0.9 %), 3 (2.7 %) and 8 (7.6 %) foetuses at 0, 50, 150 and 400 mg/kg bw/d. The litter incidence for this variation was 2 (8.3 %), 1 (4.2 %), 3 (13 %) and 5 (21 %), respectively. In historical control data from 75 studies performed at the test facility between 2009 and 2014 with Wistar rats, the range for the foetal incidence was 0-11.8 % (mean 2.5 %), whilst the range for the litter incidence was 0-57.1 % (mean 11.9 %) (1453 litters with 7096 viable foetuses examined). Dilated ureter was observed in one foetus from each of the control and high-dose groups. Therefore, there was no treatment-related effect on the incidence of visceral malformations or variations in this study.

Skeletal malformations were noted in 2, 0, 1 and 2 foetuses at 0, 50, 150 and 400 mg/kg bw/d. These consisted of misshapen cervical vertebra (control), severely malformed sternum (control), severely malformed skull bones (mid-dose group), malpositioned and bipartite sternebra (high-dose group) and multiple skeletal malformations (high-dose group; also described in section on external findings). There was therefore no relationship of these findings to exposure to BAS 750 F.

Statistically significant increases in the incidence of two skeletal variations were recorded, comprising an increase in supra-occipital holes at 150 and 400 mg/kg bw/d (but without a clear dose-response relationship) and an increase in misshapen sacral vertebrae at 400mg/kg bw/d. The historical control data provided by the applicant, from the same test facility and appropriate time-span and in Wistar rats, is presented below. The foetal incidences, litter incidences and mean affected foetuses / litter were within the historical control ranges. A dose-related increase in the number of foetuses with dumb-bell ossification of the thoracic centrum with dumb-bell-shaped cartilage of the centrum was noted, but none of the increases was statistically significant at any dose and all the incidences were within the historical control data, with the foetal incidence in the high-dose group being almost the same as the historical control mean; this finding cannot, therefore, be positively attributed to BAS 750 F.

Table B.6.6.2.7. Skeletal variations (selected)

BAS 750 F (mg/kg bw/d)		**0**	**50**	**150**	**400**
Litters evaluated		24	24	24	24
Foetuses evaluated		117	115	120	117
Live		117	115	120	117
Dead		0	0	0	0
Supra-occipital hole(s)					
Foetal incidence	# (%)	8 (6.8)	6 (5.2)	21 (18)	17 (15)
Litter incidence	# (%)	6 (25)	5 (21)	**15 (63)****	**13 (54)***
Affected foetuses / litter	%	6.5 ± 12.18	5.1 ± 10.81	**18.4 ± 19.24****	**17.9 ± 23.44***
Misshapen sacral vertebra					
Foetal incidence	# (%)	3 (2.6)	5 (4.3)	7 (5.8)	12 (10)
Litter incidence	# (%)	3 (15)	3 (13)	5 (21)	**9 (38)***
Affected foetuses / litter	%	2.7 ± 7.37	3.9 ± 10.79	5.8 ± 12.55	**10.9 ± 18.06***
Dumb-bell ossification of thoracic centrum with dumb-bell-shaped cartilage of centrum					
Foetal incidence	# (%)	7 (6.0)	8 (7.0)	9 (7.5)	18 (15)
Litter incidence	# (%)	6 (25)	7 (29)	7 (29)	10 (42)
Affected foetuses / litter	%	5.6 ± 10.83	7.1 ± 11.90	7.8 ± 14.82	14.2 ± 20.63

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* : $p \leq 0.05$; ** : $p \leq 0.01$

Table B.6.6.2.8. Historical control data – fetal skeletal variations (rats)

	Foetuses (7684)			**Litters (1431)**			**Affected foetuses / litter**	
	No.	%	Range %	No.	%	Range %	% Mean	Range %
Supra-occipital holes	954	12.4	0.0 – 52.3	521	36.4	0.0 – 100.0	12.2	0.0 – 50.8
Misshapen sacral vertebra	250	3.3	0.0 – 10.2	212	14.8	0.0 – 41.7	3.4	0.0 – 10.9
Dumb-bell ossification of thoracic centrum with dumb-bell-shaped cartilage of centrum	318	4.1	0.0 – 16.2	249	17.4	0.0 – 56.0	4.3	0.0 – 19.4

76 studies performed at the test facility (2009–2014) with Wistar rats (Charles River) (1431 litters with 7684 viable fetuses examined)

The laboratory use of the nomenclature 'misshapen sacral vertebra' refers to a minor change in the direction (from ventral to cranial, i.e. either to the left or right) of one of the sacral vertebral arches (generally the first one), thus giving the first sacral vertebra a more 'lumbar-like' appearance. Cartilage was also reported to be present. In the study authors' experience, this is a minor anatomic variant that is neither permanent nor detrimental to post-natal survival or health. It is thus appropriately classified as a small anatomical variation. Dumb-bell shaped ossification of the vertebral centrum is also classified as a variation of low concern. Supra-occipital holes are very small, discrete

areas with no ossification or bone precursor and, as shown by the historical control data, are very common spontaneous findings. These represent a slight developmental delay without any developmental effect and do not provide evidence of developmental toxicity.

Overall, there was no relationship to BAS 750 F exposure in the incidence of total external, visceral and skeletal observations (see table below).

Table B.6.6.2.9. Total malformations and variations – rat study

BAS 750 F (mg/kg bw/d)		0	50	150	400
Litters evaluated		24	24	24	24
Foetuses evaluated		223	221	230	222
Live		223	221	230	222
Dead		0	0	0	0
Total malformations					
Foetal incidence	# (%)	3 (1.3)	0 (0.0)	1 (0.4)	2 (0.9)
Litter incidence	# (%)	3 (13)	0 (0.0)	1 (4.2)	2 (8.3)
Affected foetuses / litter	%	1.3 ± 3.41	0.0 ± 0.00	0.5 ± 2.27	1.0 ± 3.68
Total variations					
Foetal incidence	# (%)	119 (53)	116 (52)	123 (53)	124 (56)
Litter incidence	# (%)	24 (100)	24 (100)	24 (100)	24 (100)
Affected foetuses / litter	%	53.5 ± 4.49	52.7 ± 3.52	53.5 ± 3.66	55.7 ± 8.13

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* $p < 0.05$, ** $p < 0.01$

Discussion and conclusion

A guideline-compliant developmental toxicity study has been conducted in rats at doses of BAS 750 F up to 400 mg/kg bw/d, administered daily from implantation to one day prior to the expected day of parturition (gestation days 6-19). At this dose, maternal toxicity was evident as reduced food consumption, body weight and body-weight gain. There were no indications of maternal toxicity at the low- and mid-doses of 50 and 150 mg/kg bw/d.

The reproduction data were comparable between all the groups; hence, there was no evidence that BAS 750 F resulted in the deaths of embryos or foetuses. There was also no consistent evidence that the test substance affected foetal weights. Although placental weights were increased in the high-dose group, the RMS considers this to be a non-adverse change, since it was not associated with impaired foetal development and the values were within the historical control range.

Two foetuses had multiple malformations, one in the mid-dose group and one in the high-dose group. The malformations in each foetus were different and thus a relationship to BAS 750 F exposure was not established. Other malformations were distributed equally across the groups and so, likewise, were not associated with the test substance.

There was no treatment-related effect on the incidence of visceral malformations or variations in this study, nor on skeletal malformations. Slight, statistically significant, increases above the concurrent controls were noted in the incidence of two skeletal variations, supra-occipital hole and misshapen sacral vertebrae. Both findings were, nevertheless, within the historical control ranges, which showed them to be common findings; additionally, in the case of the former, the incidence was not dose related. There was, therefore, not a clear relationship between these findings and exposure to BAS 750 F.

Overall, the RMS concludes that BAS 750 F was not a developmental toxicant in rats under the conditions of this study.

The RMS proposes a NOAEL of 150 mg/kg bw/d for maternal toxicity and a NOAEL of 400 mg/kg bw/d for developmental toxicity.

The co-RMS (FR) proposes a NOAEL for developmental toxicity of 50 mg/kg bw/d, based upon increased incidences of variations (dilated renal pelvis and supra-occipital hole(s)) at 150 and 400 mg/kg bw/d.

To refine the assessment, the RMS has calculated maternal BMDL values for body weight (carcass weight) and corrected body-weight gain.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Parental systemic toxicity					
Carcass weight	10 %	Females	409.1	1015.6	2.5
Corrected body-weight gain	10 %	Females	40.2	329.6	8.2

The lowest BMDL, for corrected body-weight gain, had a wide confidence interval, with a relatively large BMDL / BMDU ratio.

B.6.6.2.2. Developmental toxicity in rabbits

An oral (gavage) developmental toxicity study has been conducted in rabbits at doses of 0, 5, 10 and 25 mg/kg bw/d (30-33 presumed-pregnant females / group). The doses were selected on the basis of three range-finding studies. In the first, doses of 50, 150 and 400 mg/kg bw/d were tested in three female non-pregnant New Zealand White rabbits per group; 2 / 3 does exposed to 50 mg/kg bw/d were sacrificed in a moribund condition, with signs of poor or reduced nutritional condition, no or reduced faeces (3 / 3 animals) and lateral position (1 / 3 animals). At 150 and 400 mg/kg bw/d, all animals either died or were sacrificed because of poor condition, with signs that were similar to those at the low-dose but that occurred earlier and were more severe. At all doses, food consumption was reduced to almost zero levels within 2-5 days of the commencement of the study. As a consequence, the animals constantly lost weight until their pre-terminal death or sacrifice. For these reasons, the dose level of 50 mg/kg bw/d was considered to be potentially lethal. In the second range-finding study, similar signs of toxicity (reduced food and water consumption, reduced faeces, body-weight loss) occurred in 1 / 3 females at 25 mg/kg bw/d, resulting in sacrifice of this animal on day 17. At necropsy, the small intestine, large intestine and rectum were empty of contents. All parameters in the remaining two animals were equivalent to the controls. Subsequently, groups of five pregnant New Zealand White rabbits were administered the test substance by oral gavage at doses of 0, 5, 10 and 20 mg/kg bw/d from gestation days 6 to 28; there were no consistent adverse effects at any dose. The chosen highest dose for the main study, 25 mg/kg bw/d, represented half the lethal dose in non-pregnant animals.

In the main study, animals were dosed from gestation days 6 to 28. At sacrifice on GD 29, 20 to 24 females per group had implantation sites. Only pregnant does were used for the calculations of mean maternal food consumption, body weight and body-weight change. Only pregnant does with scheduled sacrifice on GD 29 were taken for the calculation of mean gravid uterine weights, corrected body weight gain and summary of reproduction data. In accordance with the test guideline, each group contained at least 16 females with implantation sites at the time of necropsy.

One low-dose female and one mid-dose female were sacrificed after they had spontaneous abortions. One female from each of the control and high-dose groups was found dead following gavage errors. Otherwise, there were no deaths. There were no overt clinical signs of toxicity.

Food consumption of the high-dose group was transiently decreased (87 % of controls) on gestation days 6-7 and in the mid-dose group it was transiently increased (126 % of controls) on GDs 22-23.

Overall, however, the values were comparable in all the groups (treatment days 6-28: 100 %, 108 % and 96 % of control values at 5, 15 and 25 mg/kg bw/d). The mean body weights and body-weight change were also unaffected by exposure to BAS 750 F (body-weight change days 0-29: 91 %, 100 %, 87 % at 5, 15 and 25 mg/kg bw/d, not statistically significant; body-weight change during treatment, days 6-28: 89 %, 105 %, 89 % at 5, 15 and 25 mg/kg bw/d, not statistically significant). A slightly reduced body-weight change in the high-dose group compared with the controls during gestation days 0-6 (83 % of the control value, not statistically significant) was noted, but since this occurred before treatment commenced it cannot have been a treatment-related effect. This slight retardation of body-weight change pre-treatment might have had a knock-on effect on body-weight development during the treatment period.

Clinical pathology did not reveal any changes in haematology parameters. ALT and AST activities were decreased at 25 mg/kg bw/d; AST activity was also lower at 15 mg/kg bw/d. Since the decreases were slight (ALT by 24%; AST by 34% at 25 mg/kg bw/d) and were not accompanied by other changed liver parameters, the RMS concludes they were treatment-related but not adverse. The globulin level was lower in the high-dose rabbits but this, again, was likely to be adaptive rather than adverse, since no other changed clinical-chemistry parameters were noted.

Table B.6.6.2.10. Clinical-pathology – statistically significant changes

		BAS 750 F (mg/kg bw/d)			
Dose level [ppm]		**0**	**5**	**15**	**25**
ALT	[µkat/L]	0.68	0.55	0.55	**0.52****
AST	[µkat/L]	0.61	0.60	**0.43***	**0.40****
Globulin	[g/L]	9.57	9.49	9.18	**8.53****

Statistical evaluation: Kruskal-Wallis + Wilcoxon (2-sided); * $p \leq 0.05$; ** $p \leq 0.01$

At scheduled sacrifice, the mean carcass weights and corrected body weight (terminal body weight on gestation day 29 minus weight of the unopened uterus minus body weight on gestation day 6) were comparable between all the groups. The mean gravid uterus weights of the dosed rabbits were not influenced by the test substance. There were no treatment-related observations upon gross necropsy.

Table B.6.6.2.11. Uterus weight, carcass weight and corrected body-weight gain

	BAS 750 F Dose level (mg/kg bw/d)			
Parameter (g)	**0**	**5**	**15**	**25**
Gravid uterus	459 ± 142	464 ± 105	365 ± 163	450 ± 114
[% control]		101%	80%	97%
Carcass	3578 ± 308	3544 ± 243	3664 ± 413	3520 ± 342
[% control]		99%	102%	98%
Net weight change from GD 6	-64 ± 134	-123 ± 189	60 ± 247	-94 ± 230

* $p < 0.05$, ** $p < 0.01$ (Dunnett test, two-sided)
Carcass weight = terminal body weight minus uterine weight
Net weight change from GD 6 = carcass weight minus GD 6 body weight

The caesarean-section data showed that at least 20 pregnant females were available in each group. The number of females that were pregnant at terminal sacrifice was slightly reduced at 5 and 15 mg/kg bw/d compared with the control and high-dose groups, because of several animals that weren't pregnant and one in each of these (low- and mid-dose) groups that aborted; since there wasn't a dose-response relationship, the RMS concludes that this wasn't a response to BAS 750 F exposure. There were no treatment-related effects on the number of pre- and post-implantation losses or number of resorptions. At 15 mg/kg bw/d, the number of implantation sites (mean of 7.0 per dam) and subsequently the live-litter size (mean of 6.4 per dam) was statistically significantly lower than the controls and slightly below the historical control range. These lower values were the consequence of a mean number of corpora lutea that was below the historical control range and a higher pre-

implantation loss (within the historical control range) and hence were unrelated to BAS 750 F exposure. Furthermore, there was no dose-response relationship in the number of implantation sites and live-litter size, and no adverse findings in the reproductive organs of the affected animals. All other differences observed reflected the normal range of fluctuations for animals of this strain and age; this included a higher post-implantation loss in the control and 15 mg/kg bw/d groups, which were caused by single does in each group with spontaneously-resorbed litters. The mean number and weight of live foetuses, male and female foetuses, the sex ratio and placental weights were not affected by treatment.

Table B.6.6.2.12. Pregnancy status and caesarean section data

Dose level [mg/kg bw/d]		**0**	**5**	**15**	**25**
Pregnancy status					
Females					
- mated	[n]	27	30	33	30
- pregnant	[n]	25	21	23	23
conception rate	[%]	93	70	70	77
- aborted	[n]	0	1	1	0
- premature birth	[n]	0	0	0	0
- Does with viable fetuses	[n]	23	20	21	22
- Does with all resorptions	[n]	1	0	1	0
- Death		1	1	1	1
- Pregnant terminal sacrifice	[n]	24	**20***	**22***	22
Caesarean section data[a]					
- Corpora lutea	[n]	9.4 ± 2.67	8.9 ± 2.35	8.1 ± 2.41	9.1 ± 1.96
total number	[n]	225	179	178	201
- Implantation sites	[n]	8.9 ± 2.61	8.4 ± 2.54	**7.0*** ± 2.80	8.4 ± 2.52
total number	[n]	213	169	153	184
- Pre-implantation loss	[%]	4.8 ± 10.59	6.3 ± 9.99	14.5 ± 21.13	10.4 ± 16.18
- Post-implantation loss	[%]	12.6 ± 21.23	4.7 ± 7.46	12.4 ± 24.90	3.7 ± 6.25
- Resorptions	[n]	0.8 ± 0.78	0.4 ± 0.60	0.8 ± 1.97	0.4 ± 0.66
total number	[n]	19	8	18	8
- Early resorptions	[%]	10.8 ± 21.33	2.9 ± 6.65	11.4 ± 25.00	2.0 ± 4.45
number	[n]	0.6 ± 0.71	0.2 ± 0.41	0.7 ± 1.96	0.2 ± 0.39
total number	[n]	15	4	16	4
- Late resorptions	[%]	1.8 ± 5.62	1.8 ± 4.73	1.0 ± 3.22	1.6 ± 4.43
number	[n]	0.2 ± 0.48	0.2 ± 0.52	0.1 ± 0.29	0.2 ± 0.50
total number	[n]	4	4	2	4
- Dead fetuses	[n]	0	0	0	0
- Does with viable foetuses	[n]	23	20	21	22
- Live fetuses		8.4 ± 2.29	8.1 ± 2.42	**6.4*** ± 2.93	8.0 ± 2.31
total number	[n]	194	161	135	176
Mean	[%]	91.2 ± 10.42	95.3 ± 7.46	91.8 ± 15.77	96.3 ± 6.25
- Total live female foetuses	[n]	4.5 ± 1.83	4.4 ± 1.82	**3.0*** ± 1.75	3.8 ± 1.71
total number	[n]	104	89	64	84
Mean	[%]	49.5 ± 18.05	50.9 ± 18.24	42.7 ± 19.99	47.5 ± 20.13
- Total live male foetuses	[n]	3.9 ± 1.81	3.6 ± 1.31	3.4 ± 1.91	4.2 ± 1.87
total number	[n]	90	72	71	92
Mean	[%]	41.7 ± 14.49	44.4 ± 16.24	49.1 ± 21.89	48.8 ± 18.59
- Percent live females		53.6	55.3	47.4	47.7
- Percent live males		46.4	44.7	52.6	52.3
Placental weights	[g]	5.5 ± 0.81	5.5 ± 0.89	5.6 ± 0.93	5.3 ± 0.75
- male foetuses	[g]	5.5 ± 0.99	5.6 ± 0.94	5.6 ± 0.88	5.2 ± 0.56
- female foetuses	[g]	5.4 ± 0.81	5.4 ± 0.81	5.4 ± 0.91	5.2 ± 0.82
Mean foetal weight	[g]	39.4 ± 6.25	40.6 ± 5.00	42.6 ± 5.19	39.8 ± 6.55
- males	[g]	39.6 ± 7.32	39.9 ± 5.29	42.3 ± 5.03	39.9 ± 5.50

Table B.6.6.2.12. Pregnancy status and caesarean section data

Dose level [mg/kg bw/d]	0	5	15	25
- females [g]	38.7 ± 6.39	40.0 ± 4.84	41.9 ± 5.25	39.0 ± 6.97

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \leq 0.05$; ** $p < 0.01$ (Dunnett-test, two-sided)

Table B.6.6.2.13. Historical control data

Historical control data	Mean	±	SD	Range (per study) Minimum	Maximum
Corpora lutea	9.9	±	2.56	9.0	11.0
Implantation sites	8.8	±	2.98	7.1	10.3
Pre-implantation loss [mean %]	11.9	±	18.66	5.4	25.7
Post-implantation loss [mean %]	6.9	±	13.07	2.4	11.3
Resorptions [N]	0.6	±	0.99	0.3	1.1
Live litter size [N]	8.2	±	2.90	6.6	9.7

12 studies performed at test facility (Jan 2009– Sep 2013) with New Zealand White rabbits (Charles River); (303 pregnant dams; 285 litters with 2366 viable foetuses)

There were no treatment-related external malformations (foetal incidence: 2, 0, 0 and 0 at 0, 5, 15 and 25 mg/kg bw/d), external variations (1, 0, 0, 0 foetuses in the respective groups) or unclassified external findings (findings that could not be attributed to either the malformation or variation classifications).

There were no treatment-related visceral malformations (1 in each of the groups), visceral variations (foetal incidence: 4, 5, 2, 3 at 0, 5, 15 and 25 mg/kg bw/d) nor soft-tissue unclassified findings (2, 1, 1, 1 foetuses in the respective groups). The visceral malformations comprised absent subclavian at 0, 5, 25 mg/kg bw/d, together with small thymus in the high-dose group and diaphragmatic hernia at 15 mg/kg bw/d. The soft tissue variations included dilated cerebral ventricle (1 foetus at 25 mg/kg bw/d), cystic dilatation in the brain (1 foetus each at 5 and 15 mg/kg bw/d), malpositioned carotid branch (1 foetus in each of 0, 5, 25 mg/kg bw/d groups, 2 at 15 mg/kg bw/d), short innominate (1 foetus at 25 mg/kg bw/d) and absent lung lobe (3 control fetuses, 2 at 5 mg/kg bw/d).

Skeletal malformations were recorded in 7, 2, 1, and 3 foetuses at 0, 5, 15 and 25 mg/kg bw/d. The malformations observed in the high-dose group comprised one foetus with misshapen interparietal (compared with 2 controls) and two with a severely malformed sternum (3 in the controls); one of these high-dose foetuses also had absent subclavian and small thymus. Skeletal variations of different bone structures were observed, with or without effects on the corresponding cartilage, in all groups without a dose-response relationship; variations occurred in almost all animals, which is explained by the common finding of incomplete ossification: of the hyoid (foetal incidence = 43 % of controls, 40 % at 25 mg/kg bw/d), the cervical centrum (foetal incidence = 16 % of controls, 11 % at 25 mg/kg bw/d) and the sternebrae (45 % of control foetuses, 28 % at 25 mg/kg bw/d). One finding that was classified by the study authors as a skeletal variation (fused sternebra with unchanged cartilage) occurred in a higher incidence in the high-dose group than the controls (see table below), although without statistical significance; the incidences were slightly above the historical control ranges from the test facility. The RMS notes, however, that the litter incidence represented only one litter above the historical-control range (18 % = 4/22 litters in the high-dose group, compared with 14.3 % = 3/21 in the historical-control data). There were no treatment-related unclassified cartilage observations.

Table B.6.6.2.14. Skeletal malformation and variations (selected) in rabbit study

BAS 750 F (mg/kg bw/d)	0	5	15	25
Litters evaluated	23	20	21	22
Foetuses evaluated	194	161	135	176
Live	194	161	135	176
Dead	0	0	0	0

Fused sternebra; unchanged cartilage					
Foetal incidence	# (%)	1 (0.5)	3 (1.9)	1 (0.7)	7 (4.0)
Litter incidence	# (%)	1 (4.3)	2 (10)	1 (4.8)	4 (18)
Affected foetuses / litter	%	0.5 ± 2.32	3.3 ± 10.26	0.5 ± 2.42	5.5 ± 15.4
Severely malformed sternum					
Foetal incidence	# (%)	3 (1.5)	0	0	2 (1.1)
Litter incidence	# (%)	2 (8.7)			2 (9.1)
Affected foetuses / litter	%	1.6 ± 5.4			1.1 ± 3.5

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* : $p \leq 0.05$; ** : $p \leq 0.01$

Table B.6.6.2.15. Historical control data – foetal skeletal variations (rabbits)

	Foetuses (2894)			**Litters (349)**			**Affected foetuses / litter**	
	No.	%	Range	No.	%	Range	% Mean	Range
Fused sternebra; unchanged cartilage	25	0.9	0.0 – 1.9	22	6.3	0.0 – 14.3	0.8	0.0 – 2.1

12 studies performed at the test facility (2009–2013) with New Zealand White rabbits (Charles River) (349 litters with 2894 viable foetuses examined)

Three of the foetuses in the high-dose group that were diagnosed with fused sternebrae were in the same litter (number 80). Double-staining for bone and cartilage enabled an assessment of the magnitude of the morphologic change. The sternebral fusion of all these foetuses showed the same characteristics: two sternebrae (either the 3rd and 4th, or the 4th and 5th) linked by a weak bone bridge and, additionally, being misshapen. The litter size was 8 foetuses, so that 38 % of the litter had this variation. Dam number 80 showed no overt clinical signs of toxicity or findings at necropsy, but its carcass weight was 16 % below that of the mean of the high-dose group and 17 % below the mean control value; moreover, its body weight on day 0 (before the start of exposure) was 10 % lower than the group mean, such that it was one of the dams with the lowest body weight at the start of the study in any of the groups. Therefore, this variation might have resulted from a slight developmental delay, resulting from the lower maternal body weight of this dam. Across all the groups, many of the affected foetuses were amongst the smallest in their respective litters, which would support the finding being representative of a minor delay. The other four affected foetuses in the high-dose group (from three different litters) showed the same morphological characteristics, as did all the affected foetuses in the control, low- and mid-dose groups and controls from the historical control studies (see photographic comparison of the findings in Annex IV). The fusions were of minimal magnitude and confined to individual sternal embryonic areas rather than affecting the whole sternum; additionally, the pattern of sternal changes was identical between the affected control animals and those treated with BAS 750 F. In all cases, the underlying cartilage was normal, without any indication of a change. These characteristics indicate negligible consequences for post-natal development. In particular, the presence of normal underlying cartilage provides evidence of a simple delay or minor disturbance of ossification that is likely to disappear post-natally.

Skeletal examination of foetuses in developmental toxicity studies represents a single 'snapshot' in time; hence, an appreciation of the sequence and normal patterns of ossification aids in the differentiation of generalised delays and minor alterations from true skeletal dysplasia. In rodents and rabbits, the sternebrae are amongst the regions that ossify rapidly during late gestation: sternebrae 1 to 4 ossify first, followed by sternebra 6, with sternebra 5 being last. Variable ossification of these late-ossifying bones is normal in rodents and rabbits, with the incidence of foetuses with ossification in these sites being dependent upon the day of gestation at sacrifice and the criteria used by each laboratory for individual bones. In laboratories that perform Caesarean section on GD 29, as was the case in the present study, alterations of sternal elements (unossifications, misalignments, fusions, misshapes, attachments) are amongst the most commonly occurring developmental variations in New Zealand White rabbits. The slightly higher incidence in the high-dose group might have been a

secondary consequence of the marginally lower body-weight gain of the dams in this group during the pre-treatment period.

The RMS therefore considers that this finding represents a marginal variation that has no adverse consequences and would disappear over time. Taken together with the lack of a dose-response relationship and statistical significance for the findings, the RMS concludes that the slightly higher incidence of this variation in the high-dose group does not constitute evidence of a developmental effect. Furthermore, BAS 750 F clearly did not induce malformations of the sternum, since there were more cases of a malformed sternum in the controls than in the high-dose group.

Overall, exposure to BAS 750 F did not result in increased total foetal or litter incidences of malformations or variations in rabbits.

Table B.6.6.2.16. Total malformations and variations – rabbit study

BAS 750 F (mg/kg bw/d)		**0**	**5**	**15**	**25**
Litters evaluated		23	20	21	22
Foetuses evaluated		194	161	135	176
Total foetal malformations					
Foetal incidence	# (%)	8 (4.1)	3 (1.9)	2 (1.5)	3 (1.7)
Litter incidence	# (%)	6 (26)	3 (15)	2 (9.5)	3 (14)
Affected foetuses / litter	%	3.8	2.1	1.4	1.7
Total foetal variations					
Foetal incidence	# (%)	191 (98)	160 (99)	134 (99)	173 (98)
Litter incidence	# (%)	23 (100)	20 (100)	21 (100)	22 (100)
Affected foetuses / litter	%	98.7	99.4	99.5	98.4

Statistics, litter incidence: Fisher's Exact Test (1-sided); affected fetuses/litter: Wilcoxon-Test (1-sided)
* $p < 0.05$, ** $p < 0.01$

Discussion and conclusion

The developmental toxicity of BAS 750 F in rabbits has been investigated in a study in which the test substance was administered orally at doses up to 25 mg/kg bw/d from the time of implantation to one day prior to the expected day of parturition (GD 0-28).

The highest dose in this study was chosen on the basis of two range-finding studies in non-pregnant female rabbits, in which 2 / 3 animals dosed with 50 mg/kg bw/d and 1 / 3 animals dosed with 25 mg/kg bw/d BAS 750 F were sacrificed because of poor condition, no or reduced faeces and almost zero food consumption, such that they lost weight throughout the study (21 days' duration). No treatment-related adverse effects occurred in a second range-finding study when 20 mg/kg bw/d was administered to pregnant rabbits. Hence, the study authors determined 50 mg/kg bw/d to be a lethal dose, with 25 mg/kg bw/d representing half this dose. This dose in the main study did not induce signs of maternal toxicity: there were no overt clinical signs or treatment-related necropsy findings, and food consumption, body weight, body-weight gain and carcass weights were unaffected by exposure to BAS 750 F. It is unclear why there was a difference in toxicity in the range-finding study in non-pregnant animals at 25 mg/kg bw/d and in the main study at the same dose. However, the difficulty of selecting appropriate doses for developmental toxicity studies is acknowledged, particularly in rabbits, which are susceptible to abortion and death when food intake is drastically reduced (Matsuoka *et al*., 2006[16]).

[16] Matsuoka, T., Mizoguchi, Y., Serizawa, K., Ishikura, T., Mizuguchi, H., Asano, Y. (2006). Effects of stage and degree of restricted feeding on pregnancy outcome in rabbits. *Journal of Toxicological Sciences*, **31**, 169-175.

In the main study, doses up to 25 mg/kg bw/d BAS 750 F did not affect reproduction parameters or foetal weights, and thus did not exhibit any embryo- or foetal-toxicity. There were no statistically significant or dose-related increases in any type of malformation, variation, or unclassified observation. The total numbers of malformations and variations were lower than or the same as the control values. This study is appropriate for risk assessment, since a clear reference point was identified. Administration of BAS 750 F at doses up to 400 mg/kg bw/d in rats did not indicate that the substance had an adverse effect on development (section B.6.6.2.1.). Overall, the RMS considers that there is sufficient information from both studies to conclude on developmental toxicity.

In conclusion, BAS 750 F was not a reproductive toxicant under the conditions of the study. The RMS proposes a NOAEL of 25 mg/kg bw/d for both maternal and developmental toxicity. BMDL values have not been derived, since no adverse effects were observed.

The co-RMSs (AT and FR) consider that the maternal NOAEL is 15 mg/kg bw/d, based upon early body-weight effects in the does and that the developmental NOAEL is also 15 mg/kg bw/d, based upon skeletal variations at 25 mg/kg bw/d.

B.6.6.3. Summary and conclusion on reproductive toxicity

The reproductive toxicity of BAS 750 F has been assessed in a two-generation study in rats and developmental toxicity studies in rats and rabbits. The two-generation study and the developmental toxicity study in rats were acceptable for hazard identification and risk assessment purposes. The developmental toxicity study in rabbits was acceptable for the identification of a point of departure for risk assessment, although it is noted that maternal toxicity was not induced at the highest dose administered (25 mg/kg bw/d). The doses chosen were based on range-finding studies in pregnant and non-pregnant rabbits, in which severe toxicity was observed in one of three non-pregnant rabbits at 25 mg/kg bw/d and all three animals at 50 mg/kg bw/d. The RMS thus considers the doses administered in the main study to be adequate.

In the two-generation study, BAS 750 F administered orally at doses up to 200 mg/kg bw/d did not affect mating or fertility, nor was it embryo- or foeto-toxic. It did not have a specific effect on post-natal pup survival; a small number of early post-natal litter losses appeared to result from inadequate nursing, arising from reduced maternal food intake. Also consequent to maternal toxicity were lower foetal body weights in the high-dose group and some delays in development and markers of sexual development.

In the rat developmental toxicity study, BAS 750 F was administered orally at doses up to 400 mg/kg bw/d, at which maternal toxicity was evident (reduced food consumption, body weight and body-weight gain). There were no effects on embryo / foetal survival or foetal weights, and there were no clearly treatment-related increases in any malformation or variation.

In the rabbit developmental toxicity study, oral administration of BAS 750 F at doses up to 25 mg/kg bw/d did not result in any developmental toxicity or indicate a potential to induce malformations.

It is proposed that BAS 750 F should not be classified for reproductive toxicity or effects on or via lactation (please see CLH report).

Table B6.6.3.1. Summary of reference points for risk assessment from reproductive toxicity studies

Study	Species	NOAEL mg/kg bw/d	Response
Two-generation	Rat	Parental systemic = 25	50 % ↑ alkaline phosphatase
		Fertility = 200	No adverse effects
		Development = 75	↓ pup body weight / body-weight gain
Developmental	Rat	Maternal = 150	10 % ↓ corrected body-weight gain
		Developmental = 400	No adverse effects
Developmental	Rabbit	Maternal = 25	No adverse effects
		Developmental = 25	No adverse effects

B.6.7. NEUROTOXICITY

B.6.7.1. Neurotoxicity studies in rodents

The acute neurotoxicity of BAS 750 F has been investigated in an oral study in rats (summarised below). Information on neurotoxicity following repeated exposure of BAS 750 F was provided by the 90-day oral study in rats (section B.6.3), in which there were no adverse findings in the functional observation battery, motor-activity measurements and histopathology of neuronal tissues when the test substance was administered at doses up to 314 mg/kg bw/d.

Table B.6.7.1.1 Summary of acute neurotoxicity study with BAS 750 F

Study Batch / purity Intakes (mg/kg bw/d)	Doses	NOAEL / BMDL (mg/kg bw)	Main adverse effects
Acute neurotoxicity **Wistar rat** 10 / sex / group Oral gavage; vehicle: 1% CMC OECD 424 GLP Purity 98.6% Report CA 5.7.1/1: [redacted] *al.*, 2015 (2014/1170759)	0, 200, 600, 2000	200 (NOAEL) 342 ($BMDL_{20}$)	<u>2000 mg/kg bw:</u> ↓ body-weight gain (males & females) during days 0-7 ↑ unsteady gait of 5/10 males and 3/10 females on day 0 ↓ motor activity (males & females) on day 0 ↓ male forelimb grip strength on day 0 ↑ male landing foot splay on day 0 <u>600 mg/kg bw/d</u>: ↓ motor activity (females) on day 0

The acute neurotoxicity of BAS 750 F was investigated in groups of 10 male and 10 female Wistar rats (Crl:WI(Han) after a single administration by gavage at dose levels of 0, 200, 600, and 2000 mg/kg bw. The animals were observed for 14 days after administration of the test substance.

There were no deaths or clinical signs of toxicity during the clinical examinations in any group. At 2000 mg/kg bw, mean body-weight gains were lower in males (-29%, statistically significant) and females (-20%, not statistically significant) between study days 0 to 7. Mean body weights of treatment groups were not significantly different from controls. The animals recovered between study days 8 to 14, such that by the end of the study period neither body-weight gain nor body weight was affected. There were no effects at 200 and 600 mg/kg bw.

Functional observation battery (FOB) measurements were taken prior to administration and on study days 0, 7 and 14. Home-cage observations were negative at all time-points for all groups. On study-day 0, slight impairment of coordination, i.e. unsteady gait, was observed at 2000 mg/kg bw in five male and three female animals, as compared to none in male and female control animals. These slight changes were related to the bolus dosing of the relatively high, limit-dose level. It did not occur on study days 7 and 14. No changes were observed on study-days 7 and 14, nor at dose levels of 200 or 600 mg/kg bw on any study day. There were no treatment-related effects in the sensorimotor tests and reflexes.

On the day of test-substance administration, treatment-related effects in the quantitative test parameters were observed in the high-dose male animals. In this group, grip strength of the forelimbs was lower than the controls (-22 %). In addition, the landing foot-splay test revealed a statistically significantly increased distance between the hind-limbs (12.5 cm compared with 9.3 cm in the controls). Both these findings indicated a lower body tension. Since there were no (histo-)pathological findings and the effect was not evident on days 7 and 14 after administration, the RMS concludes that the change was related to general toxicity and impaired well-being on the day of treatment, not to structural neuronal damage. In mid-dose males, landing foot-splay distance was increased between the hind-limbs, but the mean value of 11.6 cm was within the historical control range (10 acute neurotoxicity studies conducted between 2010-2013, range of study means in male Wistar rats (Crl:WI(Han)) = 7.5 - 12.0 cm). The change was thus assessed to be incidental. No findings were observed for female animals at any dose.

Motor activity was measured on the same days as the FOB was performed. The animals were placed in cages with 18 beams per cage. The number of beam interrupts was counted over 12 intervals for five minutes per interval. The measurement period began when the first beam was interrupted and finished exactly one hour later. The only treatment-related changes occurred on the day of BAS 750 F administration. The mean values for overall motor activity were statistically significantly reduced in male and female animals at 2000 mg/kg bw. Comparing the single intervals of this test group with the control group, significantly decreased values were measured for male animals at intervals 1, 2, 3, and 4 as well as for female animals at intervals 1, 2 and 3. Overall motor activity were also reduced in females at 600 mg/kg bw. However, the only interval with a statistically significantly decreased value was interval 6, an interval that was not changed at 2000 mg/kg bw. Because of its isolated occurrence and the lack of other associated findings, the applicant considers that this is not a treatment-related adverse effect. A dose-related decrease is noted for both sexes, however, leading the RMS to conclude that the statistically significant decrease in females of the mid-dose group was treatment related.

Table B.6.7.1.2. Overall mean motor activity in rats administered BAS 750 F once and observed for 14 days [mean number of beam interruptions]

	Males				**Females**			
Dose level [mg/kg bw]	**0**	**200**	**600**	**2000**	**0**	**200**	**600**	**2000**
Body weight [g]								
- Day -7	2858 ±524	3167 ±546	2924 ±753	3418 ±735	4039 ±1001	3350 ±947	3899 ±889	3708 ±1148
- Day 0	3019 ±807	2990 ±505	2454 ±533	**1436**** **±446**	4510 ±784	4240 ±1360	**3480**** **±523**	**2233**** **±639**
- Day 7	2679 ±587	2829 ±380	3225 ±618	3162 ±809	3812 ±938	4551 ±1251	5573 ±1804	4837 ±2016
- Day 14	2933 ±704	2930 ±546	3140 ±597	2891 ±933	4037 ±1005	3959 ±1399	5889 ±3692	4552 ±1923

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Kruskal-Wallis + Wilcoxon test (two-sided)

Neuropathology evaluation was performed on five animals per sex per group. Terminal body weight and absolute and relative brain weights were unaffected at all doses. There were no treatment-related gross or histopathology findings.

In conclusion, in an acute oral neurotoxicity study, administration of BAS 750 F at a dose of 2000 mg/kg bw resulted in some neuro-behavioural effects on the day of dosing. These effects comprised unsteady gait in the open-field examinations, reduced motor activity (also in females at 600 mg/kg bw), reduced grip—strength of the fore-limbs (males) and increased distance between the hind-limbs in the landing foot-splay test (males). All these effects were transient and unrelated to structural or functional neuronal damage. The RMS therefore concludes that they were related to systemic toxicity and impaired well-being subsequent to the application of a high-dose bolus application of test substance and that no specific neurotoxicity was observed.

The RMS has identified a systemic NOAEL of 200 mg/kg bw/d, based upon reduced motor activity in females at 600 mg/kg bw/d (day 0 only). *The applicant proposed a NOAEL of 600 mg/kg bw, based upon transient systemic toxicity and neuro-behavioural effects at 2000 mg/kg bw*. The NOAEL for neurotoxicity was ≥ 2000 mg/kg bw/d.

To refine the assessment, the RMS has calculated BMDL values based on the observed neuro-behavioural effects on day 0. Response levels of 20 % were set for motor activity and hind-limb foot-splay to reflect the variable nature of these measurements.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Unsteady gait	10%	Males	739	1980	2.7
		Females			
Motor activity	20 %	Males	341.8	967	2.9
		Females			
Reduced fore-limb grip strength	5 %	Males	0	Inf	-
		Females			
Foot-splay	20 %	Males	447.1	15631000	349609
		Females			

The value of 341.8 mg/kg bw for a 20 % reduction in motor activity (as an effect secondary to systemic toxicity, not specific neurotoxicity) is consistent with the NOAEL and reflects the dose spacing in the study.

B.6.7.2. Delayed polyneuropathy studies

Regulation 283/2013 states that these studies shall be conducted for active substance of similar or related structures to those capable of inducing delayed polyneuropathy, such as organophosphorous compounds. BAS 750 F does not belong to those chemical classes that are suspected of causing delayed neurotoxicity. Therefore, no acute delayed neurotoxicity study was performed. The RMS agrees that a study is not required.

B.6.8. OTHER TOXICOLOGICAL STUDIES

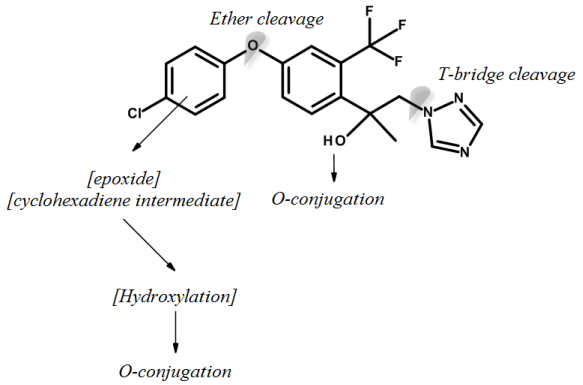
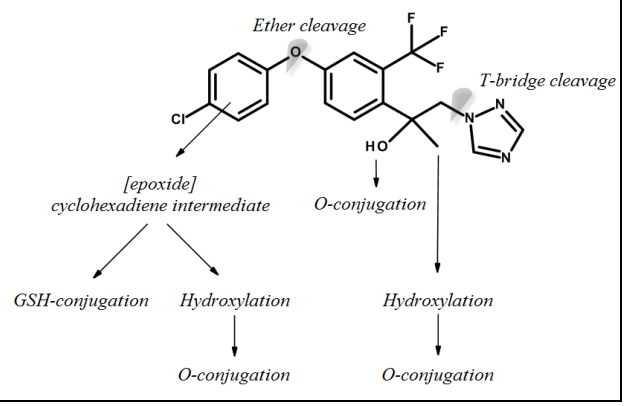
B.6.8.1. Toxicity studies on metabolites and relevant impurities

B.6.8.1.1. Animal and plant metabolites

BAS 750 F is extensively metabolised in plants and animals (see figure below and volume 3 B7). The metabolism of BAS 750 F comprises hydroxylation mainly at the chlorophenyl ring with or without subsequent conjugate formation, and cleavage of the three-ring molecule either at the ether bridge or

by release of the triazole-ring. Some of the resulting smaller molecules are themselves hydroxylated/oxidised and/or conjugated.

Figure B.6.8.1.1. BAS 750 F transformation reactions

Plants	Animals (goat, hen, rat)
	

Metabolites that are found in feed and edible food items were grouped according to their chemical similarity and common metabolic pathways and the extent of their occurrence in BAS 750 F studies of rat metabolism was determined.

For the toxicological relevance assessment of BAS 750 F metabolites that were identified in food commodities, a grouping approach was applied, and, if applicable and considered necessary, key structures were selected for further in-depth evaluation. The applied grouping of metabolites took into account chemical similarity and common metabolism pathways; and coverage by mammalian toxicity studies conducted with the parent BAS 750 F.

With regard to evaluation of chemical similarity, the general proposals given by, for example, the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment [EFSA Journal 2012;10(07):2799] were followed, taking into consideration:

- metabolic steps that were identified to probably not cause additional 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 than the parent molecule
  - conjugation of metabolite with amino acid
- consideration of conjugated metabolites being bioavailable as their unconjugated products (cleavage of glutathione, sulphate, O-glucuronides or sugar conjugates in the human gastrointestinal tract).

Comparison was made to the parent as well as to the grouped metabolites in order to select key metabolites for testing. In addition, consideration of increased hydrophilicity and thus faster excretion of the grouped metabolites as compared to the tested key metabolites and/or parent was taken into account.

The species comparison showed an overlap in the metabolism of BAS 750 F in animals and plants. Therefore, general conclusions drawn from the animal metabolism of BAS 750 F as well as overlaps of metabolite structures were taken into consideration when grouping the metabolites with regard to human exposure from the source livestock, plant or rotational crop. It was considered whether either the metabolites under consideration or similar structures were formed in the metabolism studies conducted in mammals. Moreover, it was considered that the uptake of food commodity metabolites of BAS 750 F could subsequently be transformed by known metabolic pathways into structures that have been identified in the mammalian metabolism studies.

Phase II conjugation of hydroxylates (O-bound sugar conjugation, glucuronidation or sulfation were considered to generally increase the excretability of the metabolite of concern. Hydroxylated, glucuronidated and sulfate-conjugated metabolites of BAS 750 F are commonly found in excreta of rats, and also glutathione conjugation was demonstrated to occur in rat metabolism (section B.6.1.). Thus, these conjugates are covered by the rat metabolism of the parent molecule. With regard to O-bound sugar conjugates, which are specific to plant metabolism, these O-glycosylated conjugates are known to be readily cleaved in the mammalian gastrointestinal tract, back-transforming the conjugated metabolite to the respective hydroxylates, which are assessed accordingly.

All metabolites but one (M750F022) were formed in the rat at > 10 % of the administered dose and were therefore sufficiently addressed in the toxicological studies performed with BAS 750 F (see annex III for the levels of plant and animal metabolites in the rat metabolism studies). M750F022 (*syn.* Reg.No. 6011210) was identified as a residue in a hen metabolism study. The RMS thus concluded that the proposed residue for risk assessment in animals (except poultry) be BAS 750 F. In poultry the proposed residue definition is the sum of parent BAS 750 F, metabolite M750F022 and fatty acid conjugates of M750F022, expressed as parent equivalents (see volume 3 CA B7). Compared with BAS 750 F, M750F022 has a hydroxyl group instead of the triazole ring as a result of cleavage of the T-bridge. In the rat metabolism studies with BAS 750 F, M750F022 was not found in significant amounts (<< 10 %). Therefore, toxicological studies were performed with this metabolite; these comprised an acute oral toxicity study, *in vitro* genotoxicity studies and a 28-day oral study.

B.6.8.1.1.1. **Livestock metabolite M750F022: acute oral toxicity**

The acute oral toxicity of M750F022 in rats has been investigated by the acute-toxic-class method.

Table 6.8.1.1.1 Summary of acute toxicity studies with M750F022

Study **Batch / purity** **Dose levels (ppm)** **Intakes (mg/kg bw/d)**	**Species**	**Doses**	**LD_{50}**	**Main results**
Acute oral toxicity (acute toxic class) OECD 423 (2001) GLP L85-116 / 99.0% CA 5.8.1/1: [redacted] 2015a (2015/1175551)	Female Wistar rat 3 / group Two groups	2000 mg/kg bw in corn oil Observation period: 14 days	> 2000 mg/kg bw	No deaths. Clinical signs from 2 hours to 3 days after exposure: impaired general state, piloerection; in 3 rats: apathy, cowering (day of exposure), dyspnoea (day after exposure)

In an acute oral toxicity study, two groups of 3 young adult female Wistar rats were sequentially administered a single oral gavage dose M750F022 suspended in corn oil at a dose level of 2000 mg/kg bw. Animals were observed for 14 days.

All animals survived until the termination of the study. Accordingly, the oral LD_{50} was greater than 2000 mg/kg bw. Clinical signs of general toxicity (piloerection, impaired general state, apathy, dyspnoea) were observed from two hours to three days after administration of the test substance. There were no effects on body weight development throughout the 14-day post-exposure period, nor macroscopic pathology findings.

The acute oral LD_{50} was therefore > 2000 mg/kg bw in rats.

B.6.8.1.1.2. Livestock metabolite M750F022: genotoxicity

The *in vitro* genotoxicity of M750F022 has been investigated in mutagenicity studies in bacteria and mammalian cells and a micronucleus test in human cells.

Table B.6.8.1.1.2. Summary of *in vitro* genotoxicity studies with M750F022

Study type	Test system	Concentration range	Result
Mutagenicity in bacteria: Ames test L85-106 / 98.3% OECD 471 (1997) GLP CA 5.8.1/4: Woitkowiak, 2015b (2015/1174564) CA 5.8.1/5: Becker & Kamp, 2015a (2015/1186975)	TA 100, TA 1535, TA 1537, TA 98, WP2urA With/without S9-mix	Standard-plate test = 1 to 5000 µg/plate in DMSO Pre-incubation tests = 1 to 1000 µg/plate in DMSO Triplicate plates	Negative
Mutagenicity in mammalian cells: mouse lymphoma assay OECD 476 GLP L85-116 / 99.0% CA 5.8.1/6: Schulz & Landsiedel, 2015a (2015/1174532)	L5178Y mouse lymphoma cells (TK+/- locus) With/without S9-mix	1.56 to 50 µg/mL in DMSO Treatment intervals: 4 hours (+/- S9) or 24 hours (experiment II -S9) Duplicate plates	Negative
Clastogenicity in mammalian cells: micronucleus test *in vitro* OECD 487 GLP L85-106 / 98.3% CA 5.8.1/7: Sokolowski, 2015b (2015/1038964)	Human lymphocytes (one donor for each experiment)	Concentrations in range 6.1 to 32.7 µg/ml evaluated Treatment intervals: 4 (± S9 mixture) or 20 hours (-S9 mixture) 1000 binucleated cells / culture evaluated for micronuclei Two independent experiments	Negative

Mutagenicity in bacteria: Ames test

S. typhimurium and *E. coli* strains were exposed to M750F022 in dimethyl sulfoxide (DMSO) in the presence and absence of metabolic activation (hepatic S9-mix of phenobarbital/ß-naphthoflavone induced rats) in standard-plate (SPT) and pre-incubation tests (PIT). Triplicate plates were used per concentration and per test condition. Vehicle and positive controls were included in each experiment. Tested concentrations ranged from 1.0 to 5000 µg/plate and from 1.0 to 1000 µg/plate for SPT and PIT, respectively.

A bacteriotoxic effect, evident by reduced his^- or trp^- background growth and/or a decrease in the number of his^+ or trp^+ revertants, was observed from about 333 and 100 µg/plate onwards in SPT and PIT, respectively. No test-substance precipitation occurred either with or without S9 mix.

No biologically-relevant increase in the number of revertant colonies was observed in any strain under any test condition. The expected results were obtained for the positive and negative controls.

Table B.6.8.1.1.3. Ames test (SPT) with M750F022

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	21	21	110	74	10	10	7	6	18	16
Test substance										
33 µg/plate	12	20	107	77	8	10	8	8	19	20
100 µg/plate	8	18	123	89	8	6	7	6	15	15
333 µg/plate	5 B	0 B	94 B	83 B	6 B	7 B	0 B	0 B	18 B	17 B
1000 µg/plate	0 B	0 B	0 B	0 B	0 B	4 B	0 B	0 B	17 B	17 B
2500 µg/plate	0 B	0 B	0 B	0 B	0 B	0 B	0 B	0 B	11 B	3 B
5000 µg/plate	0 B	0 B	0 B	0 B	0 B	0 B	0 B	0 B	7 B	2 B
Pos. control	109	343	1830	4454	173	5221	109	905	69	908
Experiment 2: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537			
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9		
Neg. control (DMSO)	30	16	102	101	9	8	8	6		
Test substance										
1.0 µg/plate	21	19	98	89	12	7	8	8		
3.3 µg/plate	21	17	94	100	9	13	6	4		
10 µg/plate	23	19	95	95	8	7	7	6		
33 µg/plate	20	17	103	93	10	9	8	5		
100 µg/plate	16	17	96	83	8	10	6	5		
333 µg/plate	14 B	14 B	87 B	84 B	12 B	6 B	5 B	0 B		
Pos. control	1701	443	1996	4274	293	4996	151	1238		

B = reduced background growth

Table B.6.8.1.1.4. Ames test (PIT) with M750F022

Experiment 3: Pre-incubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537			
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9		
Neg. control (DMSO)	24	18	111	96	10	11	9	6		
Test substance										
1.0 µg/plate	24	20	102	103	10	11	8	7		
3.3 µg/plate	31	15	104	97	8	12	10	7		
10 µg/plate	34	16	103	95	8	11	11	8		
33 µg/plate	30	15	96	95	8	10	6	8		
100 µg/plate	30	15 B	92	59 B	8	9 B	7 B	2 B		
333 µg/plate	21 B	0 B	66 B	0 B	4 B	0 B	0 B	0 B		
Pos. control	1702	375	1616	2728	194	2145	139	803		
Experiment 3: Pre-incubation assay										
Strain									E. coli	
Metabol. activation									+S9	-S9
Neg. control (DMSO)									21	16
Test substance										
3.3 µg/plate									26	20
10 µg/plate									19	18
33 µg/plate									17	19
100 µg/plate									23	21
333 µg/plate									21	23 B
1000 µg/plate									17 B	17 B
Pos. control									87	1145

B = reduced background growth

In conclusion, M750F022 was not mutagenic in bacteria under the conditions of this study.

Mutagenicity in mammalian cells: mouse lymphoma assay

M750F022 was tested *in vitro* in L5178Y $TK^{+/-}$ mouse lymphoma cells (MLTK) for its ability to induce forward mutations of the heterozygous autosomal thymidine kinase (TK) locus and structural chromosome aberrations at chromosome 11 carrying the functional TK gene. Two independent experiments, with duplicate cultures for each test condition, were conducted in the presence or absence of metabolic activation (S9 from rat livers induced with phenobarbital and β-naphthoflavone). Based on the results of a preliminary cytotoxicity assay, in which precipitation occurred at ≥ 218.8 µg/mL and relative suspension growth (RSG) was ≤ 20 % at ≥ 54.7 µg/mL, concentrations from 1.56 to 50 µg/mL were used in the main experiments. The treatment intervals for both experiments in the presence and absence of metabolic activation were generally 4 hours, except in experiment II (in the absence of metabolic activation) where a treatment interval of 24 h was applied. Methylmethanesulfonate (MMS) or 7,12-dimethylbenz[a]anthracene (DMBA) and cyclophosphamide (CPA) served as positive controls in the experiments without or with metabolic activation, respectively. DMSO was used as the vehicle control. After the incubation period, treatment media were replaced by culture medium in both experiments and the cells were incubated for 48 h for expression of mutant cells. This was followed by incubation of cells in selection medium containing 5-trifluorothymidine (TFT) for about 10 days.

The criteria for a positive response were: exceedance of a threshold of 126 colonies per 10^6 cells (global evaluation factor) above the corresponding solvent control in the induced mutation frequency; evidence of reproducibility of any increase in mutant frequencies; statistically significant dose-related increase in mutant frequencies when analysed with an appropriate statistical trend test.

No biologically relevant increase in mutant colony numbers was observed either with or without metabolic activation in either main experiment. Relevant cytotoxic effects, indicated by a relative total growth of less than 50% and/or reduced cloning efficiency of less than 80% as compared with the respective vehicle control, were observed in the first experiment at 50 µg/mL in the presence and absence of metabolic activation. In the second experiment cytotoxic effects were noted at 25 µg/mL after 24 hours of exposure without metabolic activation. Appropriate responses were recorded with the positive controls and the solvent controls.

Table B.6.8.1.1.5. Results of mouse lymphoma assay - main experiments

Test group	conc. [µg/mL]	S9 mix	Cytotoxicity[#] RCE$_1$ [%]	RTG [%]	Genotoxicity MF$_{corr.}$ [colonies/ 10^6 cells]	Threshold[##]
Experiment I / 4 h treatment						
DMSO	-	-	100	100	51.8	178
M750F022	3.13	-	111.9	85.2	46.7	
	6.25	-	122.9	96.9	53.7	
	12.50	-	98.5	83.6	67.3	
	25.00	-	95.0	72.9	36.0	
	50.00	-	91.0	40.3	78.1	
	100.00	-	2.2	n.c.	n.c.	
	200.00	-	n.c.	n.c.	n.c.	
MMS	15.0	-	80.0	21.2	1218.9	
Experiment I / 4 h treatment						
DMSO	-	+	100.0	100	50.3	176
M750F022	3.13	+	108.3	94.0	40.2	
	6.25	+	99.2	95.9	47.5	
	12.50	+	98.5	89.0	45.0	
	25.00	+	100.8	70.8	50.7	
	50.00	+	68.3	29.3	61.0	
	100.00	+	n.c.	n.c.	n.c.	
	200.00	+	n.c.	n.c.	n.c.	
CPA	2.5	+	100.0	52.8	362.5	
DMBA	2.5	+	89.9	52.8	354.2	
	4.0	+	85.4	55.6	315.2	
Experiment II / 24 h treatment						
DMSO	-	-	100	100	40.8	167
M750F022	1.56	-	79.3	82.7	32.3	
	3.13	-	116.9	78.5	56.3	
	6.25	-	121.7	81.1	51.7	
	12.50	-	92.0	56.4	48.0	
	25.00	-	79.3	43.7	53.1	
	50.00	-	12.2	n.c.	n.c.	
	100.00	-	n.c.	n.c.	n.c.	
MMS	5.0	-	90.5	31.7	588.6	
Experiment II / 4 h treatment						
DMSO	-	+	100	100	50.0	176
M750F022	2.34	+	97.0	114.6	36.3	
	4.69	+	114.4	110.2	33.8	
	9.38	+	110.7	105.5	37.3	
	18.75	+	92.1	91.2	39.2	
	37.50	+	92.1	76.8	37.6	
	75.00	+	2.4	n.c.	n.c.	
	150.00	+	n.c.	n.c.	n.c.	

Table B.6.8.1.1.5. Results of mouse lymphoma assay - main experiments

Test group	conc. [µg/mL]	S9 mix	Cytotoxicity[#] RCE$_1$ [%]	RTG [%]	Genotoxicity $MF_{corr.}$ [colonies/ 10^6 cells]	Threshold[##]
CPA	2.5	+	88.1	62.2	399.9	
DMBA	2.5	+	93.4	61.0	331.9	
	4.0	+	100.0	71.5	421.0	

[#] = cytotoxicity related to the respective vehicle control
[##] = number of mutant colonies per 10^6 cells of current vehicle control plus 126 (rounded value)
RCE = relative cloning efficiency
RTG = relative total growth
n.c. = Culture not continued owing to strong cytotoxicity

Thus, under the experimental conditions described, M750F022 did not induce forward mutations or structural chromosome aberrations *in vitro* in the mouse lymphoma assay with L5178Y $TK^{+/-}$ cells either in the absence or the presence of metabolic activation.

Clastogenicity in mammalian cells: micronucleus test

M750F022 was tested for its potential to induce micronuclei in human lymphocytes *in vitro* in the absence and presence of hepatic S9-mix from phenobarbital/β-naphthoflavone-induced rats. Two independent experiments were performed where the cells were incubated for 4 (±S9-mix) or 20 hours (-S9-mix). The concentrations applied in the main experiments were based on a pre-test, in which 2035 µg/ml was the highest concentration employed. Because of excessive cytotoxicity in this test, 100 µg/ml was chosen as the highest concentration in the main experiments. The vehicle DMSO served as negative control, mitomycin C (MMC; 4 hours) and demecolcin (20 hours) as positive controls in the absence of metabolic activation and cyclophosphamide (CPA) as the positive control in the presence of metabolic activation. Exposure was started after a 48-hour stimulation period with phytohaemagglutinine. Thereafter, cytochalasin B was added and the cultures were fixed and stained finally after another 20 hours. Cytokinesis-block proliferation index (CBPI) and cytostasis were determined in 500 binucleated cells/culture as cytotoxicity parameters and the number of micronucleated cells were determined in 1000 binucleated cells/culture for the evaluation of clastogenicity.

In line with the test guideline, the highest concentrations evaluated were those that resulted in approximately 55 ± 5 % cytotoxicity. Clear cytotoxicity was observed in both experiments at the highest evaluated concentrations of 18.7 µg/ml (without metabolic activation) and 32.7 µg/mL (with metabolic activation); cytotoxicity was excessive at concentrations higher than these, and hence the cultures were not evaluated for genotoxicity. Osmolarity and pH values were not influenced by test substance treatment. No precipitation of the test item in the culture medium at the end of treatment was observed.

Incubation with M750F022 did not lead to an increase in the number of micronucleated cells under any experimental condition. The positive control substances resulted in distinct increases in cells with micronuclei, and thus demonstrated the sensitivity of the test system. The number of micronucleated cells induced by the vehicle control, DMSO, was within the range of the historical control data.

Table B.6.8.1.1.6. Results of the *in vitro* micronucleus test in human lymphocytes

Test group	conc. [µg/mL]	S9 mix	Cytotoxicity[#]		Genotoxicity
			Proliferation index (CBPI)	Cytostasis [%][a]	Micronucleated cells [%][b]
Experiment I / 4 h exposure (preparation at 40 h)					
DMSO [0.5% (v/v)]	-	-	1.77		0.45
M750F022	6.1	-	1.12	4.7	0.65
	10.7	-	1.74	28.8	0.55
	18.7	-	1.55	55.8	0.45
	32.7	-	1.12	84.8	n.e.
	57.1	-	1.03	95.9	n.e.
	100.0	-	n.e.	n.e.	n.e.
MMC	2.0	-	1.12	84.5	**13.30***
DMSO	-	+	1.58		1.30
M750F022	10.7	+	1.62	n. c.	0.75
	18.7	+	1.54	7.4	1.10
	32.7	+	1.34	41.7	0.65
	57.1	+	1.22	62.3	n. e.
	100.0	+	n.e.	n.e.	n.e.
CPA	17.5	+	1.28	51.6	**4.50***
Experiment II / 20-h exposure (preparation at 40 h)					
DMSO	-	-	1.93		0.70
M750F022	6.1	-	1.68	26.7	0.45
	10.7	-	1.65	30.0	0.25
	18.7	-	1.36	61.7	0.50
	32.7	-	1.34	63.4	n.e.
	57.1	-	1.10	89.0	n.e.
	100.0	-	n.e.	n.e.	n.e.
Demecolcin	125.0	-	1.49	47.9	**3.90***
Experiment II / 4-h exposure (preparation at 40 h)					
DMSO	-	+	1.98		0.85
M750F022	10.7	+	1.79	19.7	0.35
	18.7	+	1.61	37.8	0.20
	32.7	+	1.30	69.6	0.25
	44.9	+	1.08	91.7	n.e.
	57.1	+	1.09	91.3	n.e.
	100.0	+	n.e.	n.e.	n.e.
CPA	15.0	+	1.51	48.3	**6.15***

[a]: the values are related to the solvent controls
[b]: The number of micronucleated cells was determined in a sample of 2000 binucleated cells
*: statistically significantly higher than corresponding control values ($p \leq 0.05$)
n. c.: Not calculated as the CBPI is equal or higher than the solvent control value
n. e.: Not evaluated owing to a reduction of the cell number and most of the cells did not show a clear visible cytoplasm area, and therefore did not met the acceptance criteria for evaluation

In conclusion, M750F022 was not clastogenic in this *in vitro* micronucleus test when tested up to cytotoxic concentrations on human lymphocytes.

B.6.8.1.1.3. Livestock metabolite M750F022: 28-day oral repeated-dose toxicity

The repeated-dose toxicity of M750F022 has been investigated in a 28-day dietary study in mice.

Table B.6.8.1.1.7. Summary of repeated-dose toxicity study with M750F022

Study **Batch / purity** **Dose levels (ppm)** **Intakes (mg/kg bw/d)**	**Doses**	**NOAEL/ BMD (mg/kg bw/d)**	**Main results**
28-day oral (diet) C57BL/6 Rj mouse 5 / sex / group OECD 407 GLP L85-116 / 99.0% Report CA 5.8.1/8 [redacted] 2015a (2016/1000646)	0, 87, 872, 2500 ppm Equivalent to males: 0, 20, 180, 587 mg/kg bw/d Females: 0, 32, 249, 718 mg/kg bw/d	Male: 20 Females: 249	<u>≥ 872 ppm:</u> ↓ Triglycerides (males) ↑ slight liver weight (+9%) with hypertrophy ↑ multifocal necrosis in 2 / 5 males <u>2500 ppm:</u>
		$BMDL_{15}$ 171.8 (relative liver weight)	↓ bw (females), bw gain (males & females) and food intake (females) ↑ ALP (males & females), ALT (males) ↓ Cholesterol, total protein and albumin (males) ↑ liver weight (rel. +52% males, +64% females) with heptatocellular hypertrophy /fine granular eosinophilic cytoplasm; multifocal necrosis in 4 males and 1 female

M750F022 was administered to mice at dietary concentrations of 0, 87, 872 and 2500 ppm for at least 28 days. The low- and the mid-dose levels corresponded to equimolar BAS 750 F dietary concentrations of 100 and 1000 ppm, which were tested in the same mouse strain for 28 days; this allowed for a comparison of the toxicity of the parent and the metabolite.

Two control animals (one male and one female) died during blood collection on study day 14. There were no treatment-related <u>deaths</u> or <u>clinical signs of toxicity</u>. Analysis of plasma collected on study day 14 demonstrated that M750F022 was bioavailable after oral administration, with mean plasma levels of 149, 326, 261 ng/ml in males and 180, 304, 641 in females at 87, 872 and 2500 ppm.

Administration of 2500 ppm resulted in marked reductions in <u>body weight</u> (maximum reduction of 23 % on study day 21) and <u>food intake</u> (up to -88 %) in females, and reduced body-weight gain in both sexes; in females, a continuous body-weight loss occurred between days 0 and 21 (maximum decrease of -161 % on day 21). There were no toxicologically-relevant, statistically-significant changes in body weight, body-weight gain and food consumption in either sex at the low- and mid-dose levels, nor in food consumption in the high-dose males.

Table B.6.8.1.1.8. Body weight and body weight change

	Males				Females			
Dose level [ppm]	**0**	**87**	**872**	**2500**	**0**	**87**	**872**	**2500**
Body weight [g]								
- Day 0	21.8	21.9	22.3	22.0	18.3	18.4	18.5	18.1
- Day 28	25.7	25.2	25.7	24.3	21.8	21.1	21.3	**17.1****
% (compared to control)		-1.9	±0.0	-5.2		-2.9	-2.0	**-21.4**
Body weight change (g)								
- Day 0-7	0.9	0.5	1.2	**-0.3***	1.0	0.4	0.7	**-0.2****
Δ% (compared to control)		-43.2	+36.4	**-130**		-56.0	-34.0	**-120**
- Day 0-14	1.9	1.7	2.2	0.6	2.1	1.3	1.6	**-0.8****
Δ% (compared to control)		-13.5	+13.5	-68.7		-40.6	-23.6	**-136**
- Day 0-21	3.3	2.5	2.5	**1.0***	2.7	1.8	1.9	**-1.6****
Δ% (compared to control)		-24.8	-24.2	**-68.5**		-34.2	-27.5	**-161**
- Day 0-28	4.0	3.2	3.4	2.3	3.0	2.7	2.8	**-1.0****
Δ% (compared to control)		-19.0	-15.5	-41.5		-8.7	-6.7	**-133**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; Dunnett test (two-sided)

There were no treatment-related changes in <u>haematology</u> parameters.

Treatment-related, adverse clinical chemistry changes at 2500 ppm were indicative of impaired liver function in both male and female mice, comprising increased serum ALP in both sexes and, additionally in males, increased ALT, decreased triglycerides (also at 872 ppm), cholesterol, total protein and albumin levels.

Table B.6.8.1.1.9. Clinical chemistry parameters – 28-day mouse study

		Males				Females			
Dose level [ppm]		**0**	**87**	**872**	**2500**	**0**	**87**	**872**	**2500**
ALT		0.78	0.69	0.81	**1.34**	1.03	0.71	0.96	1.55
ALP	[µkat/l]	2.12	1.77*	2.10	**3.95***	2.38	2.26	2.31	**4.76***
Cholesterol	[mmol/l]	2.61	2.22	2.53	**2.00***	1.76	1.74	2.22*	2.23
						Historical control range: 1.46 - 2.37			
Triglycerides	[mmol/l]	0.78	0.61	**0.54***	**0.29***	0.75	0.59	0.31	0.37
Total protein	[g/l]	50.73	49.19	49.46	**45.47***	46.74	48.19	46.28	46.73
Albumin	[g/l]	31.02	30.85	31.10	**28.54***	30.08	31.22	29.97	29.88

Statistical significance - * = $p \leq 0.05$; ** = $p \leq 0.01$ (Kruskal-Wallis / Kruskal-Wallis+ Wilcoxon, 2-sided)

Treatment-related, specific effects on <u>organ weights</u> (i.e., not secondary to a decreased terminal body weight) were confined to the liver. At 872 ppm, the increase in relative liver weight was statistically significant in both sexes and above the historical control range in males; in females, the value was at the upper limit of the historical control range (13 studies conducted 2010 to 2015, same test facility and mouse strain). Because of the marked liver-weight increase in females at 2500 ppm, the applicant performed an additional assessment to obtain a more accurate terminal body weight, i.e., one in which the differences in liver weights from the controls was taken into account. When the terminal body weight excluding the liver of the high-dose females was compared with that of the control females, the decrease ranged between -11.8 % and -27.7 % (mean of 13.92 g, range = 12.33 to 15.03 g; mean value for controls = 17.04 g, range = 16.25 to 17.47 g).

Absolute and relative epididymides, ovary, testes and uterus weights were unaffected in all dose groups.

Table B.6.8.1.1.10. Organ weights (selected)

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ%	Absolute weight	Δ%	Relative weight [% of bw]	Δ%
Terminal weight [g]	0	21.35				17.875			
	87	20.88	(-2)			17.14	(-4)		
	872	20.74	(-3)			17.78	(-1)		
	2500	19.44	(-9)			**15.08**	**(-16)**		
Liver (mg)	0	973.0		4.559		838.0		4.682	
	87	916.2	(-6)	4.386	(-4)	770.4	(-8)	4.497	(-4)
	872	1033.6	(+6)	**4.989***	**(+9)**	**966.2***	**(+15)**	**5.44***	**(+16)**
	2500	**1347.8***	**(+39)**	**6.939***	**(+52)**	**1155.8***	**(+38)**	**7.666***	**(+64)**
Historical ctrl data (liver wt)		*854 - 1040*		*3.716 – 4.664*		685.0 – 886.6		4.172 – 5.447	

* $p \leq 0.05$ (Kruskal-Wallis and Wilcoxon-test, two sided)

The only treatment-related gross pathology finding was a dark-brown discolouration of the liver. At 872 ppm, this was reported in 2/5 females; and at 2500 ppm, in 5/5 males and 4/5 females.

Upon histopathology, treatment-related findings were recorded in the liver of both sexes and in the kidney of males. The kidney finding comprised an increased incidence of basophilic tubules of at 2500 ppm, with only a very few tubules affected (minimal severity). The study authors stated that basophilic tubules of minimal grade can be a common spontaneous occurrence, which is supported by 3/5 females of the control group presenting with this finding. Notwithstanding, since the incidence in males was dose-related, this is considered by the RMS to potentially be a consequence of treatment. However, the RMS concludes that the very few tubules affected would be unlikely to have a functional effect on kidney function and therefore clear adversity is not indicated.

Hepatocellular hypertrophy was observed in the liver of all males and females of the mid- and high-dose groups. The distribution pattern changed at the two dose levels, with the hypertrophy being diffuse at 2500 ppm and centrilobular at 872 ppm. Additionally, at 2500 ppm the hepatocytes showed a fine granular eosinophilic cytoplasm that might have correlated with the macroscopically observed dark-brown discoloration. The hypertrophy in the mid- and high-dose groups was associated with a decrease in fat storage, manifested as a decreased incidence of fatty change in these groups. (Multi)focal necroses were seen in two males at 872 ppm and in four males and one female at 2500 ppm. One of the affected males of the high-dose group showed some grade 3 (large) areas of necrosis, whereas the other affected animals had grade 1 (very few, very small) areas of necrosis.

Table B.6.8.1.1.11. Histopathology findings

	Males				Females			
Dose level [ppm]	**0**	**87**	**872**	**2500**	**0**	**87**	**872**	**2500**
No. of animals	5	5	5	5	5	5	5	5
KIDNEYS								
examined	5	5	5	5	5	5	5	5
Tubules, basophilic Grade 1	1	1	2	5	3		1	2
	Males				**Females**			
Dose level [ppm]	**0**	**87**	**872**	**2500**	**0**	**87**	**872**	**2500**
No. of animals	5	5	5	5	5	5	5	5
LIVER								
examined	5	5	5	5	5	5	5	5
Hypertrophy, centrilobular Gr. 2			5				5	
Hypertrophy, diffuse Gr. 2				5				5
Necrosis, (multi)focal			2	4				1
Grade 1			2	3				1
Grade 3				1				
Fatty change, diffuse	4	5			4	5		
Grade 1	2	3			2	4		
Grade 2	2	2			2	1		
Fatty change, (multi)focal			2					

In the evaluated ovarian sections, no corpora lutea were present in 3/5 females of the high-dose group. The uterus in these three females was diffusely atrophic and of decreased size, with the size of the smooth muscle cells of the myometrium in particular being reduced. The atrophy in two of the females was slight, whilst that in the third affected female was moderate; additionally, this female showed a slight hypertrophy with mucification in the cervix and vagina.

The terminal body weights of this group were greatly reduced compared with the controls. When the terminal body-weights excluding the liver were assessed (see above), it was demonstrated that the two females without findings in the genital tract had values that were 11.8 % and 13.68 % below the controls. Meanwhile, the value was 19 % lower for the two females with slight findings and 27.66 % lower than the control mean in the animal with the most severe findings. The RMS thus concludes that the findings in the genital tract of some high-dose females were secondary to the dramatically reduced terminal body weight in these animals and did not indicate a specific toxic effect on the female reproductive tract.

Discussion and conclusion

In a 28-day study in mice, the metabolite M750F022 was administered in dietary concentrations of 0, 87, 872 and 2500 ppm. The low- and the mid-dose levels corresponded to equimolar BAS 750 F dietary concentrations of 100 and 1000 ppm.

Administration of 2500 ppm M750F022 resulted in reduced food intake and body weight of females; the effect on terminal body-weight was particularly marked when adjustment for the greatly increased liver weights was made. Body-weight gain was reduced in both sexes at this dose, with continuous body-weight loss in females.

Liver changes were recorded at 872 and 2500 ppm. These comprised statistically significant increases in weight at 872 ppm (relative increases of 9 % in males and 16 % in females), which became marked at the higher dose (relative increases of 52 % in males and 64 % in females). The weight increases correlated with hepatocellular hypertrophy. Other histopathology findings included a fine granular eosinophilic cytoplasm at 2500 ppm, which might have explained the dark-brown discolouration of the

liver that was observed macroscopically, and hepatocellular necrosis in males at 872 and 2500 ppm and one female at 2500 ppm. Clinical chemistry changes in both sexes at 2500 ppm, with one finding also at 872 ppm (decreased triglyceride in males), were indicative of impaired liver function.

In males, a dose-related increase in basophilic tubules of the kidney was recorded, with 5/5 animals at 2500 ppm being affected. The severity was graded as minimal, meaning that very few tubules were basophilic; hence, this is regarded by the RMS as a treatment-related but non-adverse finding.

The identified NOAEL values were 87 ppm in males (20 mg/kg bw/d), based upon hepatocellular hypertrophy and necrosis at 872 ppm, and 872 ppm in females (249 mg/kg bw/d).

The RMS has conducted BMD analysis on the following parameters: terminal body weight (including the liver, since weights excluding the liver were only available for the control and high-dose groups), overall body-weight change, relative liver weight and incidence of hepatocellular necrosis.

Parameter	Response level	Covariate	Lowest BMDL (mg/kg/d)	Highest BMDU (mg/kg/d)	BMDU / BMDL ratio
Terminal body weight	10 %	Males Females	553.0	700.4	1.3
Overall body-weight gain	10 %	Males Females	433.4	489.5	1.1
Relative liver weight	15 %	Males Females	171.8	257.8	1.5
Hepatocellular necrosis	10 %	Males Females	5.67	247	43.6

The lowest BMDL, for a 10 % extra risk of hepatocellular necrosis, was associated with a considerable amount of uncertainty, reflecting the small sample size. Therefore, the BMDL of 171.8 mg/kg bw/d for a 15 % increase in relative liver weight will be taken as the relevant value from this study and takes account of the dose spacing; a comparison of the same parameter obtained from the parent demonstrates that M750F022 is less potent than the parent.

B.6.8.1.1.4. Conclusion on animal and plant metabolites

The only plant or animal metabolite that was not covered by the toxicological database on BAS 750 F was M750F022, which was a major residue in hens. Therefore, the toxicological profile of this metabolite was investigated in an acute oral toxicity study in rats, three *in vitro* genotoxicity studies and a 28-day repeated-dose study in mice. The aromatase inhibiting-activity of the metabolite was also investigated in an *in vitro* assay (see section B.6.8.3.). A comparison of the toxicology of M750F022 and BAS 750 F is presented in the table below.

Table B.6.8.1.1.12. Comparison of the toxicological profile of M750F022 and BAS 750 F

Study	M750F022	BAS 750 F
Acute oral toxicity in rats	LD_{50} > 2000 mg/kg bw	LD_{50} > 2000 mg/kg bw
Mutagenicity in bacteria	Negative	Negative
Mutagenicity in mammalian cells	Negative	Negative
Clastogenicity in mammalian cells	Negative	Negative
28-day oral (diet) in mice	$BMDL_{15}$ = 172 mg/kg bw/d (15 % increase in relative liver weight)	$BMDL_{15}$ = 15 mg/kg bw/d (15 % increase in relative liver weight)
Aromatase inhibition *in vitro*	At 316 µM remaining aromatase	IC_{50} = 0.92 µM

Table B.6.8.1.1.12. Comparison of the toxicological profile of M750F022 and BAS 750 F

Study	M750F022	BAS 750 F
study	activity was 46 % of maximum	

The information from the 28-day repeated-dose study in mice and the aromatase assay indicates that M750F022 had a lower potency than BAS 750 F. Furthermore, the nature of the toxicity in the 28-day study with the metabolite was consistent with that of the parent compound, with effects on body weight and liver toxicity constituting the observed adverse effects. The acute toxicity of both parent and metabolite was low, and there was no indication of a genotoxic potential for either.

The applicant used Derek Nexus for the *in silico* analysis of M750F022 toxicity (document ID 2015/1112684). No alerts were triggered for chromosome damage, genotoxicity or mutagenicity, nor for carcinogenicity. The metabolite triggered an equivocal "rapid-prototype alert" for nephrotoxicity; this alert describes the nephrotoxicity of 1,2-ethyleneglycol and its derivatives. This alert type of Derek Nexus represents only an indication of potential toxicity and does not carry the same weight as a full alert. There were no indications of adverse kidney effects in the available 28-day study. No other human relevant endpoints were triggered by these structures.

In view of the toxicological profiles of the parent and this metabolite, it is proposed to apply the reference values of BAS 750 F to the risk assessment of M750F022.

B.6.8.1.2. Groundwater metabolites

There are no relevant groundwater metabolites (document ID 2016/1000838).

Although two metabolites (M750F002 (1,2,4-triazole) and M750F003) were detected in laboratory studies investigate the metabolism of BAS 750 F in soil, there was no substantial formation of either metabolite during field studies. Notwithstanding, the potential for groundwater contamination was further assessed for M750F001 by prediction of the environmental concentrations in groundwater. Since the modelled PEC_{gw} was << 0.1 µg/L in all FOCUS groundwater scenarios, the applicant concluded that no further data was necessary.

B.6.8.2. Supplementary studies on the active substance

Supplementary studies on the active substance might be required where they are necessary to further clarify observed effects. The applicant has provided mechanistic studies to investigate the mode of action of the liver toxicity, and has discussed the immunotoxicity potential of BAS 750 F.

Mechanistic investigations of liver enzyme induction and cell proliferation

The applicant conducted mechanistic studies to explore the mode of action of the liver toxicity in rodents [redacted], 2015a (CA 5.8.2/1; 2014/1170760); [redacted] 2016a (CA 5.8.2/2; 2014/1170771); [redacted] 2015c (CA 5.8.2/3; 2015/1037704); [redacted], 2016b (CA 5.8.2/4; 2015/1040901); [redacted] 2015d (CA5.8.2/5; 2014/1170772); [redacted] 2015b (CA 5.8.2/6; 2014/1170773); Elcombe, 2016a (CA 5.8.2/7; 2015/1037705); Elcombe, 2016a (CA 5.8.2/8; 2015/103773)). These studies have not been presented in full in this report, but the applicant's summary is provided below.

> Studies of liver-enzyme and cell-proliferation induction were performed in Wistar rats and in C57BL/6J mice (wild-type and PXR knockout (KO) / CAR KO strains) after dietary exposure to the same dose levels as those used in the carcinogenicity studies. In addition, *in vitro* investigations in primary hepatocyte cultures from human donors and from wild-type / knock-out mice were performed.

In the rat, mefentrifluconazole administered for treatment periods of 3-28 days at a dose of 3600 ppm caused only weak responses: there was no induction of liver-cell proliferation and very little impact on CYP enzyme activities. In wild-type mice, a marked induction of mainly CYP2B10 was found, and a dose-dependently increased induction of liver-cell proliferation (in male mice at all dose levels tested (20 – 200 ppm) and in females at 50 and 250 ppm). The findings were associated with increased serum ALT, liver-weight increases and hypertrophy, but there was no evidence for degenerative changes. None of these findings, except for slight liver-weight increases, were observable in CAR / PXR double-knockout mice. *In vitro* studies with primary hepatocyte cultures from human donors and from wild-type and CAR / PXR double-knockout mice provided further evidence that the main nuclear hormone receptor in BAS 750 F-mediated liver activation is CAR, whilst PXR did not seem to be involved. In human male and female hepatocytes, neither BAS 750 F nor the reference compound phenobarbitone had an impact on replicative DNA synthesis. The assessment of CYP3A4 and CYP2B6 revealed a small increase in BROD activity in male human hepatocytes exposed to BAS 750 F, which, however, was not confirmed by increased CYP2B6 mRNA levels. Phenobarbitone induced CYP2B6 and CYP3A4 at the levels of both enzyme activity and mRNA in both sexes.

The applicant concluded that this data indicated that liver effects in C57BL/6J mice, comprising increased serum ALT levels, increased liver weight, hypertrophy and liver cell proliferation, are CAR-mediated and therefore of limited relevance for human risk assessment. The RMS notes that necrosis was observed in some repeated-dose mouse studies with BAS 750 F, albeit of minimal or slight severity. Notwithstanding, it is generally accepted that the CAR mode of action does not result in cytotoxicity. Furthermore, increased liver weight, hypertrophy and changes in clinical chemistry parameters associated with impairment of liver function were observed in the dog studies. Therefore, the RMS concludes that the liver effects observed in the regulatory studies with BAS 750 F in several species cannot be attributed to CAR-mediated processes and should thus be considered to be potentially of relevance to humans.

Immunotoxicity

The standard sub-chronic and chronic toxicity studies on BAS 750 F have incorporated measurements of a number of potential immune-related end-points.

There were no treatment-related changes in white blood cell (WBC) count, select differential blood cell counts (lymphocytes, neutrophils, basophils, monocytes) or histology of the spleen, thymus, lymph node or bone marrow in any study. There was also no evidence of a specific immunotoxic effect on any immune-related parameter. Organ-weight changes, for example of the spleen and thymus in some of the high-dose groups, were secondary to decreased body weights and occurred together with general systemic toxicity. The only finding related to immune parameters that occurred without indications of systemic toxicity was a decrease in globulin levels in the high-dose rabbit developmental toxicity study; this observation was not considered to be adverse by the study authors and was most likely to be related to changes in liver function (site of synthesis) in these animals.

There was therefore no evidence of a specific effect on immune-related parameters in the available studies. The RMS concludes that specific investigations into the immunotoxicity potential of BAS 750 F are not required.

B.6.8.3. Studies on endocrine disruption

At the time of evaluation, the interim ED criteria were in application. The interim criteria are laid down in Point 3.6.5 of Annex II to Regulation 1107/2009 as follows.

- Substances that are or have to be classified, in accordance with the provisions of Regulation 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine-disrupting properties.
- In addition, substances such as those that are or have to be classified, in accordance with the provisions of Regulation 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have endocrine-disrupting properties.

BAS 750 F did not meet the criteria for classification for carcinogenicity or for reproductive toxicity, nor did it demonstrate toxic effects on the endocrine organs in a complete data-set of repeated-dose, carcinogenicity and reproductive toxicity studies. Therefore, it does not meet the interim criteria for the identification of a substance with endocrine-disrupting properties under Regulation EC 1107/2009. Furthermore, BAS 750 F does not meet the World Health Organisation / International Programme on Chemical Safety definition of an endocrine disruptor (WHO / IPCS, 2002)[17], since there were no alterations in the function(s) of the endocrine system that resulted in adverse effects in intact organisms.

In accordance with Regulation 283/2013, additional information or specific studies shall be required if there is evidence that the active substance may have endocrine-disrupting properties. Although the interim criteria were not met, the applicant has provided information on an *in vitro* aromatase inhibition assay because of the chemical class of the active substance: BAS 750 F belongs to the triazole class of fungicide compounds that act by blockage of sterol biosynthesis; the inhibition of mammalian aromatase (CYP19) is a known side effect of this chemical class. This assay is summarised below. The assay also provides information on the aromatase inhibition potential of the R- and S-enantiomers of BAS 750 F and on the livestock metabolite Reg. No. 6011210 (hereafter referred to as M750F022).

Table B.6.8.3.1. Summary of *in vitro* aromatase inhibition assays

Method	Concentrations	Test materials	Results
In vitro human recombinant aromatase inhibition assay Human CYP19 supersomes Guideline: EPA 890.1200 adapted to non-radioactive assay Not GLP CA 5.8.3/1: Mentzel, 2016c (2015/1261377) CA 5.8.3/2: Mentzel, 2016d (2016/1001905) (validation study)	10^{-10} to 3.16 x 10^{-4} M	BAS 750 F: 99.4 % purity, batch L85-12 S-enantiomer: 99.5 % pure, batch L84-256 R-enantiomer: 98.9 % pure, batch L84-254 M750F022: 99 % pure, batch L85-116 Positive control: 4-hydroxy-androstenedione (OH-ASDN) Negative control: solvent (DMSO)	IC_{50} concentrations: S-enantiomer = 0.58 µM BAS 750 F = 0.92 µM R-enantiomer = 2.97 µM M750F022 = at 316 µM remaining aromatase activity 46 % of maximum
In vitro rat recombinant aromatase inhibition assay Rat CYP10 supersomes	10^{-10} to 3.16 x 10^{-4} M	BAS 750 F: 99.4 % purity, batch L85-12 S-enantiomer: 99.5 % pure, batch L84-256	IC_{50} concentrations: S-enantiomer = 0.294 µM BAS 750 F = 0.402 µM

[17] WHO / IPCS, 2002: Global assessment of the state-of-the-science of endocrine disruptors. WHO/IPCS/EDC/02.2.

Guideline: EPA 890.1200 adapted to non-radioactive assay Not GLP Mentzel, 2017a (2016/1035281) Mentzel, 2017b (2017/1035249) (validation study)		R-enantiomer: 98.9 % pure, batch L84-254 Epoxiconazole: 99 % pure, batch SZBD099XV Positive control: 4-hydroxy-androstenedione (OH-ASDN) Negative control: solvent (DMSO)	R-enantiomer = 0.719 µM Epoxiconazole = 0.0082 µM

B.6.8.3.1. In vitro human recombinant aromatase inhibition assay

BAS 750 F, the S-enantiomer, the R-enantiomer and the metabolite M750F022 were tested *in vitro* for their effect on human aromatase activity (CYP 19). The analysis of CYP19-enzyme activity can be used to identify chemicals that can inhibit the catalytic activity of aromatase through an interaction with the substrate binding-site on the enzyme. The present study used recombinant aromatase and the fluorometric substrate O-benzyl fluorescein benzyl ester (DBF) (Stresser *et al*., Analyt. Biochem., 284, 427-430, 2000). A validation study with several reference chemicals, including proficiency chemicals recommended by the test guideline, confirmed the suitability of the method for the detection of aromatase-inhibiting activity (Mentzel, 2016d).

In the main study (Mentzel, 2016c), human CYP19 supersomes (aromatase + reductase) were exposed to the test substances or the positive reference substance 4-OH-ASDN at concentrations that ranged from 10^{-10} to 3.16 x 10^{-4} M, or to the solvent DMSO. The assay comprised four individual test runs with four replicates for each concentration and substance per run. Activity values were fitted with a four-parameter regression model, which allowed for the calculation of half-maximum inhibition concentration (IC_{50}) values.

BAS 750 F and both enantiomers had a measurable effect on CYP19 activity. The high reproducibility of the individual measurements, and thus the accuracy of the comparisons, was demonstrated by the small confidence intervals and high correlation coefficients obtained. Under the study conditions, the S-enantiomer had the lowest IC_{50} concentration of 0.58 µM, followed by the racemate BAS 750 F with an IC_{50} of 0.92 µM and then the R-enantiomer with an IC_{50} of 2.97 µM. This graduated response of aromatase inhibition for BAS 750 F and its enantiomers was reproduced in all individual test runs. Overall, the IC_{50} values obtained for the racemate and the two enantiomers were all within the same order of magnitude (difference less than a factor of 10).

The metabolite M750F022 had a very weak effect on aromatase activity. Complete inhibition of aromatase activity could not be achieved: even at the solubility limit concentration of 316 µM, the remaining enzyme activity was still 46 % of the maximum value. At a concentration of 3.2 µM, no aromatase inhibition occurred. The calculated IC_{50} value of 715 µM estimated on the basis of the modelled dose-response curve was not considered to be a sufficiently precise estimate since, statistically, it was no different from zero. The test guideline recommends a classification as a specific inhibitor only for compounds that achieve > 50 % enzyme inhibition; therefore, given the errors in the measurements, the metabolite was not securely identified as an inhibitor of CYP19 enzyme activity.

Table B.6.8.3.2. Effect of test compounds on human aromatase enzyme activity

	Human aromatase IC_{50} [µM]		
	Mean	**SE**	**95% confidence interval**
Test substance			
BAS 750 F	0.92	0.05	0.82 – 1.03
S-enantiomer	0.58	0.03	0.51 – 0.65
R-enantiomer	2.97	0.02	2.52 – 3.14
M750F022 (metabolite)	715	1432	–
4-OH ASDN (positive control)	0.0196	0.00169	0.0162 – 0.0230

The reference compound 4-hydroxy-androstenedione (4-OH ASDN) gave the expected result.

The validation study (Mentzel, 2016d) gave IC_{50} values of 0.0525 µM for epoxiconazole, 0.0033 µM for letrozole and 0.0039 µM for econazole.

In conclusion, under the conditions of this human aromatase inhibition study, the S-enantiomer of BAS 750 F had the highest activity (lowest IC_{50} concentration), followed by BAS 750 F and then the R-enantiomer. Nevertheless, the activity of BAS 750 F and both enantiomers was at least 30-fold lower than that of the reference chemical. Furthermore, aromatase inhibition in this *in vitro* system did not translate to endocrine-mediated adverse effects in intact organisms. The metabolite M750F022 had a very weak inhibitory effect on aromatase activity.

B.6.8.3.2. In vitro rat recombinant aromatase inhibition assay

The *in vitro* rat recombinant aromatase inhibition assay followed the same non-radioactive method as the *in vitro* human recombinant assay. A validation assay (Mentzel, 2017b) demonstrated the reproducibility and reliability of the method. In the main study (Mentzel, 2016c), rat CYP19 supersomes (aromatase + reductase) were exposed to the test substances or the positive reference substance 4-OH-ASDN at concentrations that ranged from 10^{-10} to 3.16 x 10^{-4} M, or to the solvent DMSO.

BAS 750 F and each of the enantiomers had a measurable effect on rat aromatase activity. The small confidence intervals and high correlation coefficients that were obtained underlined the high reproducibility of the individual measurements and allowed for an accurate comparison of the different chemicals. The S-enantiomer had the lowest IC_{50} value, followed by the racemate and then the R-enantiomer (see the table below). The IC_{50} values of epoxiconazole and the reference substance were several orders of magnitude below those of BAS 750 F and the enantiomers.

Table B.6.8.3.3. Effect of test compounds on rat aromatase enzyme activity

	Rat aromatase IC_{50} [µM]		
	Mean	**SE**	**95% confidence interval**
Test substance			
BAS 750 F	0.402	0.015	0.371 – 0.432
S-enantiomer	0.294	0.0131	0.268 – 0.32
R-enantiomer	0.719	0.0399	0.639 – 0.800
Epoxiconazole	0.0082	0.000576	0.00704 – 0.00936
4-OH ASDN (positive control)	0.0417	0.00126	0.0392 – 0.0442

In the validation study, letrozole gave an IC_{50} value of 0.0013 µM.

In conclusion, under the conditions of this rat aromatase inhibition study, the S-enantiomer of BAS 750 F had the highest activity (lowest IC_{50} concentration), followed by BAS 750 F and then the R-enantiomer. This study indicated that BAS 750 F and its enantiomers had a slightly stronger inhibitory effect on rat aromatase activity than on the human enzyme. The activity of BAS 750 F and both enantiomers was many-fold lower than that of the reference chemical and of epoxiconazole. Furthermore, aromatase inhibition in this *in vitro* system did not translate to endocrine-mediated adverse effects in intact organisms.

B.6.9. MEDICAL DATA AND INFORMATION

BAS 750 F has not yet been sold commercially and aside from pilot-scale preparations, it has been handled by only a small number of employees or contract scientists involved in regulatory and field biological testing. Therefore, there is only very limited human data at present.

The applicant's literature search did not return any relevant results.

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for the monitoring of BAS 750 F effects. Thus, the medical monitoring programme is designed as a general health check, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.

The surveillance programme includes a general physical examination including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to BAS 750 F exposure have not been observed.

B.6.9.2. Data collected on humans

No reports of adverse effects have been identified during routine monitoring of production plant workers and amongst personnel involved in the experimental biological testing or field trials with BAS 750 F or BAS 750 F-containing products. There is no evidence or data available to support any findings in relation to poisoning with BAS 750 F.

B.6.9.3. Direct observation

No human cases of intoxication or poisoning deriving from BAS 750 F exposure are known to the applicant.

B.6.9.4. Epidemiological studies

There are no data on the exposure of the general public to BAS 750 F, nor are epidemiological studies available.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

An analytical method for the determination of BAS 750 F in blood plasma of rats and mice is available. Clinical tests are not available. No specific symptoms of poisoning are expected or have been identified in animal studies (see below).

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

See the safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known.

B.6.10. REFERENCES RELIED ON

A literature search on BAS 750 F was performed by the BASF Group Information Centre. The first step of the search result processing based on summary records was done by the Information Centre and involved the separation into "hits" and "ballast" (obviously irrelevant records). The "ballast" was not further processed.

The "hits" were further evaluated by the scientific experts and categorised into "not relevant", "not reliable", and "used for dossier". The hits in toxicology did not contribute to the risk assessment and were therefore not further discussed in the dossier.

A literature search on TDMs was performed for the TDMG. The hits in toxicology did not contribute to the risk assessment and were therefore not further discussed in the dossier.

The RMS considers the literature-search approach to be acceptable.

Data Point	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	Previous evaluation
KCA 5.1.1/ 1	[redacted]	2015 a	14C-BAS 750 F (14C-Chlorophenyl and Trifluoromethylring-U-14C labels): Study on kinetics and excretion in Wistar rats after single and repeated oral administration 2015/1208128 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.1.1/ 2	[redacted]	2016 a	14C-BAS 750 F (triazole-3(5)-C14) - Study on the biokinetics in rats 2015/1078847 [redacted]	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
			[redacted] yes Unpublished					
KCA 5.1.1/ 3	[redacted]	2016 a	Excretion and metabolism of 14C-BAS 750 F (Reg.No. 5834378) after oral administration in rats 2015/1107610 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.1.1/ 4	[redacted]	2014 a	14C-BAS 750 F - Study on plasma kinetics in C57BL/6 J Rj mice 2014/1018105 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.1.2/ 1	Funk D. et al.	2016 a	Comparative in-vitro-metabolism with 14C-BAS 750 F 2015/1020123 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.	No	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
			yes Unpublished					
KCA 5.2.1/ 1	[redacted]	2013 c	BAS 750 F - Acute oral toxicity study in rats 2013/1149656 [redacted]. yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.2.1/ 2	Becker M. Kamp H.	2013 a	BAS 750 F - Homogeneity and concentration control analyses in corn oil 2013/1395622 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.2.2/ 1	[redacted]	2013 b	BAS 750 F - Acute dermal toxicity study in rats 2013/1149657 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
KCA 5.2.2/ 2	Becker M. Kamp K.	2013 a	Analytical report - BAS 750 F - Homogeneity and concentration control analyses in corn oil 2013/1395620 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.2.3/ 1	[redacted]	2014 a	BAS 750 F - Acute inhalation toxicity study in Wistar rats - 4-hour dust exposure (head-nose only) 2014/1127433 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.2.4/ 1	Remmele M.	2012 a	Reg.No. 5834378 - EpiDerm skin corrosion / irritation test 2012/1367952 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
KCA 5.2.4/2	[redacted]	2013 a	BAS 750 F - Acute dermal irritation / corrosion in rabbits 2013/1150122 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.2.5/1	Remmele M.	2012 b	Reg.No. 5834378 - EpiOcular eye irritation test 2012/1367953 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	NA
KCA 5.2.5/2	Remmele M.	2012 c	Reg.No. 5834378 - Bovine corneal opacity and permeability test (BCOP test) 2012/1367954 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	NA
KCA 5.2.5/3	[redacted]	2013 a	BAS 750 F - Acute eye irritation in rabbits	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
			2013/1150121 [redacted]. yes Unpublished					
KCA 5.2.6/ 1	[redacted]	2013 a	BAS 750 F - Test for skin sensitization using the guinea pig maximization test (GPMT) 2013/1150123 [redacted]. yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.2.6/ 2	Grauert E. Kamp H.	2014 a	Analytical report - BAS 750 F - Homogeneity and concentration control analyses in paraffinum subliquidum 2014/1116448 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.2.6/	Stinchcombe S.	2016 a	Assessment of the skin sensitization potential of BAS 750 F -	No	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
3			Consideration of the appropriate sub-category for GHS classification as skin sensitizer 2016/1028946 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished					
KCA 5.2.7/1	Cetto V. Landsiedel R.	2015 a	BAS 750 F - In vitro 3T3 NRU phototoxicity test 2015/1117503 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.3.1/1	[redacted]	2015 a	BAS 750 F - Repeated dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1170747 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA	[redacted]	2015	Amendment No. 1 to	Yes	Yes	Data for first	BASF	NA

Data Point	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	Previous evaluation
5.3.1/ 2	[redacted]	a	the report - BAS 750 F - Repeated dose 28-day toxicity study in Wistar rats - Administration via the diet 2015/1249664 [redacted] yes Unpublished			Approval		
KCA 5.3.1/ 3	[redacted]	2014 a	BAS 750 F - Repeated-dose 28-day toxicity study in C57BL/6 Rj mice - Administration via the diet 2013/1110704 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.3.1/ 4	[redacted]	2015 a	BAS 750 F - Repeated-dose 28-day oral toxicity study in beagle dogs - Oral administration (capsule) 2014/1170748 [redacted] yes	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
			Unpublished					
KCA 5.3.2/ 1	[redacted]	2015 b	Final amended report - BAS 750 F - Repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet 2015/1198721 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.3.2/ 2	[redacted]	2015 a	90-day oral dietary toxicity study with BAS 750 F in C57BL/6JRj mice 2014/1046542 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.3.2/ 3	Becker M. Kamp H.	2014 a	BAS 750 F - Plasma analysis for external studies 2014/1177165 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
KCA 5.3.2/ 4	Becker M.	2015 a	Amendment No. 1 to the report: BAS 750 F - Plasma analysis for external studies 2015/1240217 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.3.2/ 5	[redacted]	2015 a	BAS 750 F - Repeated-dose 90-day oral toxicity study in beagle dogs - Oral administration (capsule) 2015/1000530 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.3.2/ 6	[redacted]	2016 b	BAS 750 F - Repeated-dose 12-month toxicity study in Beagle dogs - Oral administration (capsule) [redacted] 2016/1273716 yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
KCA 5.3.3/ 1	[redacted]	2015 b	BAS 750 F - Repeated dose 28-day dermal toxicity study in Wistar rats 2014/1170751 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.4.1/ 1	Woitkowiak C.	2014 a	BAS 750 F - Salmonella typhimurium / Escherichia coli reverse mutation assay 2014/1128030 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.4.1/ 2	Becker M.,Kamp H.	2013 b	BAS 750 F - Stability analysis in Dimethyl sulfoxide 2015/1040886 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.4.1/	Woitkowiak C.	2015 a	BAS 750 F - Salmonella	No	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
3			typhimurium / Escherichia coli - Reverse mutation assay 2015/1116956 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished					
KCA 5.4.1/ 4	Wollny H.-E.	2015 a	BAS 750 F: In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2015/1112683 Harlan Cytotest Cell Research GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.4.1/ 5	Wollny H.-E.	2015 b	BAS 750 F: In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2015/1101908 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes	No	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
			Unpublished					
KCA 5.4.1/6	Schulz M.,Landsiedel R.	2014a	BAS 750 F - In vitro micronucleus assay in V79 cells (Cytokinesis Block Method) 2013/1375108 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.4.1/7	Sokolowski A.	2015a	BAS 750 F: Micronucleus test in human lymphocytes in vitro 2015/1101907 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.4.2/1	[redacted]	2014a	BAS 750 F - Micronucleus test in bone marrow cells of the mouse 2014/1043159 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
KCA 5.5/1	[redacted]	2016 b	BAS 750 F - Combined chronic toxicity/carcinogenicity study in Wistar rats - Administration via the diet up to 24 months 2015/1000531 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.5/2	[redacted]	2015 a	Historical control data - Compilation from 24-month studies in Wistar rats (CrI:WI (Han)) - Performed at BASF SE Experimental Toxicology and Ecology, Ludwigshafen, Germany 2015/1261375 [redacted] no Unpublished	No	No	Not applicable	BASF	NA
KCA 5.5/3	[redacted]	2015 b	18-month carcinogenicity study with BAS 750 F in male and female C57BL/6JRJ mice 2015/1000532 [redacted]	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
			[redacted] yes Unpublished					
KCA 5.5/4	[redacted]	2015 a	Historical control data mouse study - Compilation from 18-month studies in male and female C57BL/6JRj mice - Performed at BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany 2015/1261376 [redacted] no Unpublished	No	No	Not applicable	BASF	NA
KCA 5.5/5	Becker M. Kamp H.	2015 a	BAS 750 F - Plasma analysis for external studies 2015/1186254 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.6.1/	[redacted]	2015 c	BAS 750 F - Two-generation reproduction toxicity	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
1			study in Wistar rats - Administration via the diet 2014/1170754 [redacted] yes Unpublished					
KCA 5.6.2/ 1	[redacted]	2015 a	BAS 750 F - Prenatal developmental toxicity study in Wistar rats - Oral administration (gavage) 2014/1170755 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.6.2/ 2	[redacted]	2015 b	BAS 750 F - Prenatal developmental toxicity study in New Zealand white rabbits - Oral administration (gavage) 2014/1170757 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA	[redacted]	2016	Report Amendment:	Yes	Yes	Data for first	BASF	NA

Data Point	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	Previous evaluation
5.6.2/ 3	[redacted]		BAS 750 F - Prenatal developmental toxicity study in New Zealand white rabbits - Oral administration (gavage) 2016/1321110 [redacted] yes Unpublished			Approval		
KCA 5.7.1/ 1	[redacted]	2015 c	BAS 750 F - Acute oral neurotoxicity study in Wistar rats - Administration by gavage 2014/1170759 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
KCA 5.8.1/ 1	[redacted]	2015 a	Reg.No. 6011210 - Acute oral toxicity study in rats 2015/1175551 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.8.1/ 2	Schmitt D.	2015 a	Concentration control analysis and homogeneity control analysis of Reg.No. 6011210 in vehicle corn oil 2015/1186900 Institut Kuhlmann GmbH Analytik-Zentrum Ludwigshafen, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.8.1/ 3	Lawson S.	2015 a	Toxicological analysis of BAS 750 F and a metabolite using Derek Nexus 2015/1112684 ForthTox Ltd., Linlithgow West Lothian EH49 7YU, United Kingdom	No	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
			no Unpublished					
KCA 5.8.1/ 4	Woitkowiak C.	2015 b	Reg.No. 6011210 - Salmonella typhimurium / Escherichia coli - Reverse mutation assay 2015/1174564 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.8.1/ 5	Becker M. Kamp M.	2015 a	Reg.No. 6011210 - Stability analysis in dimethyl sulfoxide 2015/1186975 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.8.1/ 6	Schulz M. Landsiedel R.	2015 a	Reg.No. 6011210 - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK+/- Locus assay, microwell version) 2015/1174532 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.	No	Yes	Data for first Approval	BASF	NA

Data Point	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	Previous evaluation
			yes Unpublished					
KCA 5.8.1/7	Sokolowski A.	2015b	Reg.No. 6011210: Micronucleus test in human lymphocytes in vitro 2015/1038964 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	NA
KCA 5.8.1/8	[redacted]	2016a	Reg.No. 6011210: Repeated-dose 28-day toxicity study in C57BL/6 J Rj mice - Administration via the diet 2016/1000646 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.8.2/1	[redacted]	2015a	A 28 day dietary study with BAS 750 F in male and female C57BL mice: Elucidation of hepatic mode of action and time course 2014/1170760 [redacted]	Yes	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
			[redacted] no Unpublished					

Data Point	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	Previous evaluation
KCA 5.8.2/ 2	[redacted]	2016 a	BAS 750 F - S-phase response study in livers (histopathological examination) of mice for the study: A 28 day dietary study with BAS 750 F in male and female C57BL mice: Elucidation of hepatic mode of action and time course 2014/1170771 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.8.2/ 3	[redacted]	2015 c	A 7 day dietary study with BAS 750 F in male and female C57BL/6 wild-type and Pxr KO/Car KO mice 2015/1037704 [redacted] no Unpublished	Yes	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
KCA 5.8.2/ 4	[redacted]	2016 b	BAS 750 F - S-phase response study in livers (histopathological examination) of mice for the study: A 7 day dietary study with BAS 750 F in male and female C57BL/6 wild-type and Pxr KO/Car KO mice 2015/1040901 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.8.2/ 5	[redacted]	2015 d	BAS 750 F- S-Phase response study in Wistar rats - Administration via the diet for 3, 7, 14 and 28 days 2014/1170772 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	NA
KCA 5.8.2/ 6	[redacted]	2015 b	Ex vivo analysis of liver samples taken at termination of a 3, 7, 14 and 28 day dietary study administering BAS 750 F in the diet to male and female Wistar rats	Yes	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
			2014/1170773 [redacted] no Unpublished					
KCA 5.8.2/7	Elcombe B. et al.	2016a	BAS 750 F - Enzyme and DNA-Synthesis induction in cultured male and female wild-type and Pxr KO/Car KO mouse hepatocytes 2015/1037705 CXR Biosciences, Dundee DD1 5JJ, United Kingdom no Unpublished	No	No	Not applicable	BASF	NA
KCA 5.8.2/8	Elcombe B.	2016a	BAS 750 F - Enzyme and DNA-Synthesis induction in cultured male and female human hepatocytes 2015/1037703 CXR Biosciences, Dundee DD1 5JJ, United Kingdom no Unpublished	No	No	Not applicable	BASF	NA
KCA 5.8.3/1	Mentzel T.	2016c	Reg.No. 5834378 (BAS 750 F, R,S-racemate), Reg.No. 5934588 (S-	No	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
			enantiomer), Reg.No. 5934591 (R-enantiomer), Reg.No. 6011210 (metabolite) - Human recombinant aromatase assay 2015/1261377 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished					

Data Point	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	Previous evaluation
KCA 5.8.3/ 2	Mentzel T.	2016 d	Letrozole – Econazole nitrate – 4-OH ASDN – Fenarimol – Epoxiconazole – Nitrofen – Atrazine – Bis(2-ehtylhexyl)phthalate – Human recombinant aromatase assay 2016/1001905 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
	Mentzel T.	2017a	Reg.No. 5834378 (BAS 750 F, R,S-racemate), Reg.No. 5934588 (S-enantiomer), Reg.No. 5934591 (R-enantiomer), Epoxiconazole Rat recombinant aromatase assay 2017/1035281	No	No	Not applicable	BASF	NA

Data Point	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	Previous evaluation
	Mentzel T.	2017 b	Letrozole, Econazole nitrate, 4-OH-ASDN, Fenarimol, Nitrofen, Atrazine, Bis(2-ethylhexyl)phthalate Rat recombinant aromatase assay 2017/1035249	No	No	Not applicable	BASF	NA

B.6.11. ANNEX I: PROPOSED METABOLIC PATHWAY OF BASF 750 F

Figure B6.11.1. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 1)

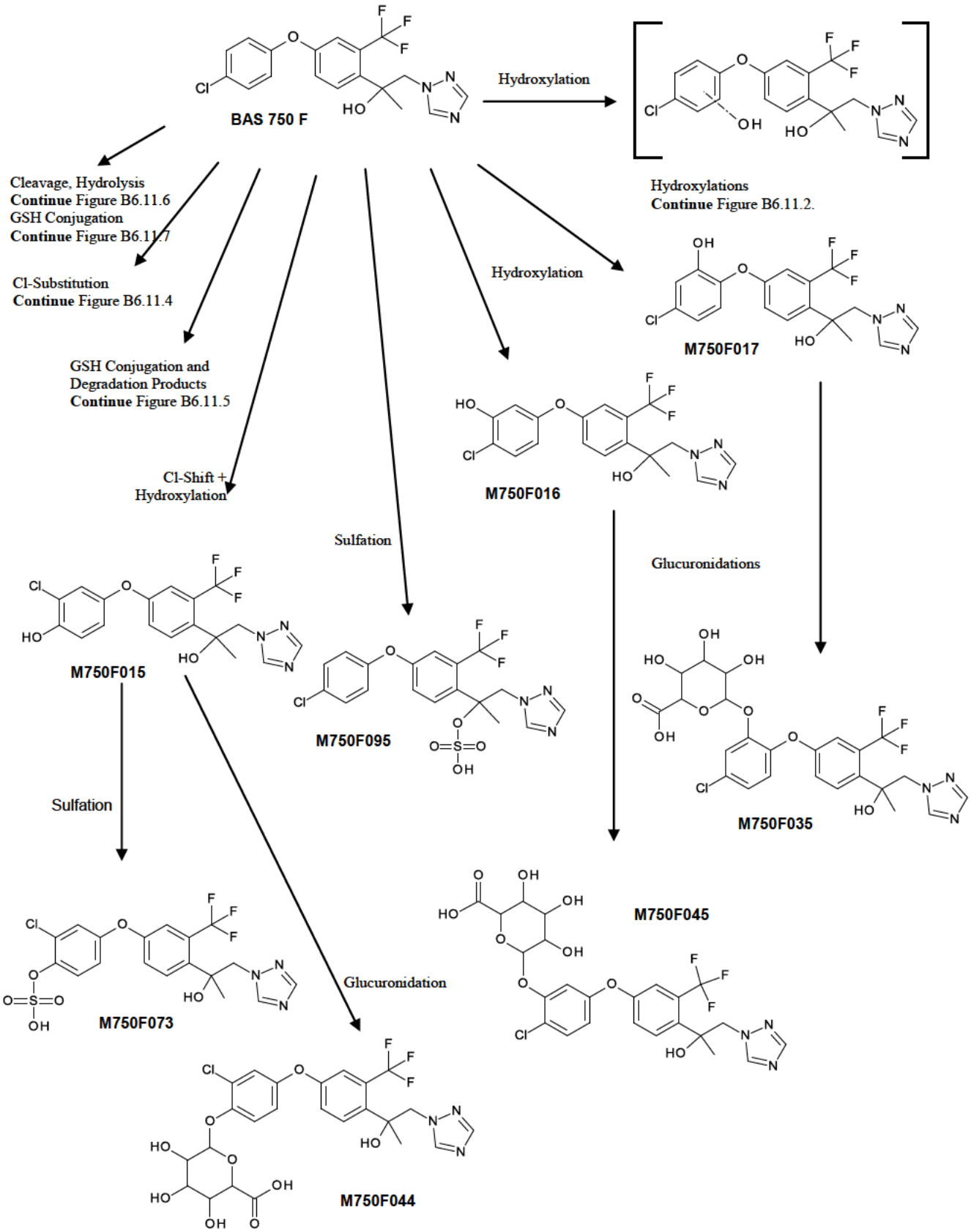


Figure B6.11.2. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 2, First and Second Hydroxylation, Multiple Phase I and Phase II)

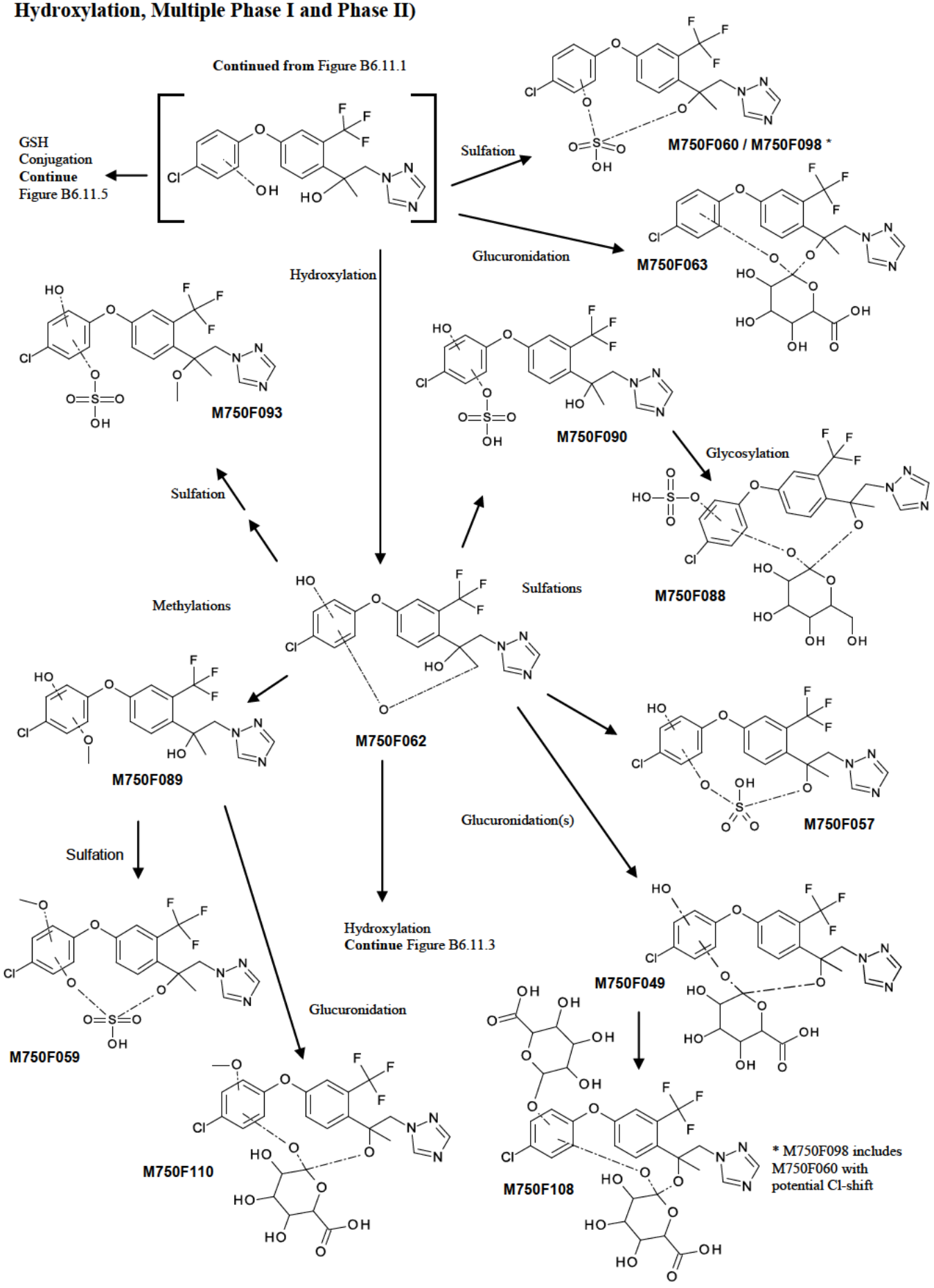


Figure B6.11.3. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 3, Third Hydroxylation, Multiple Phase I and Phase II)

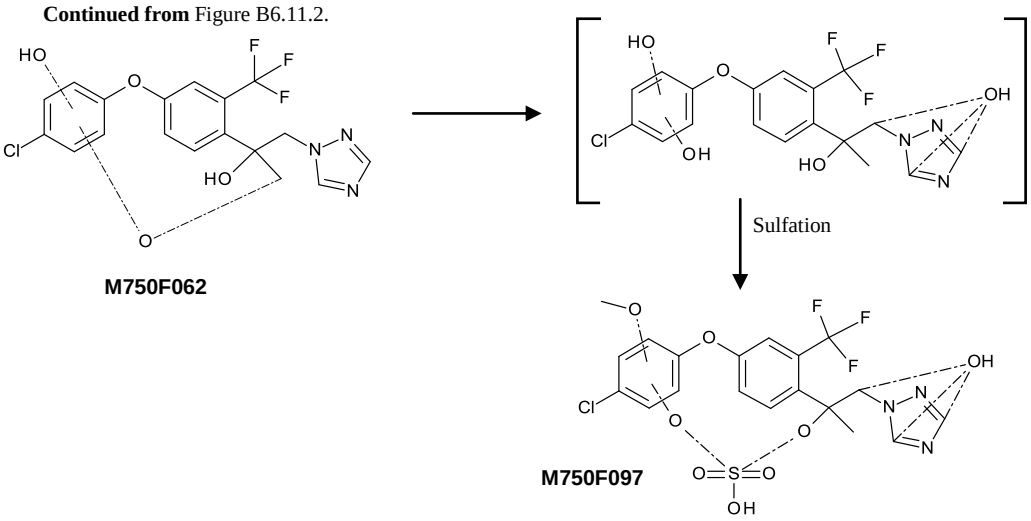


Figure B6.11.4. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 4, Cl-Substitution, Multiple Phase I and Phase II)

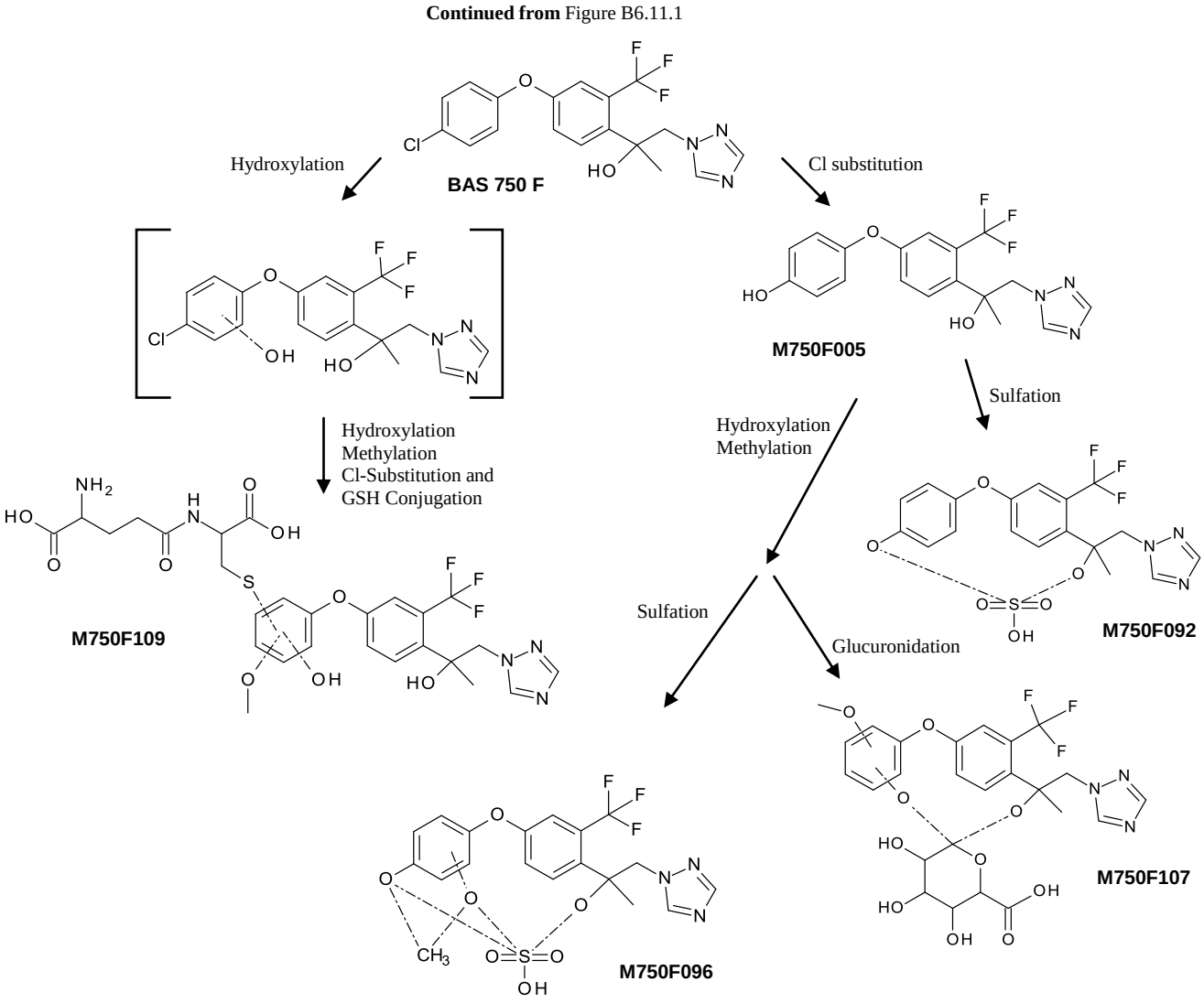


Figure B6.11.5. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 5, GSH Conjugation)

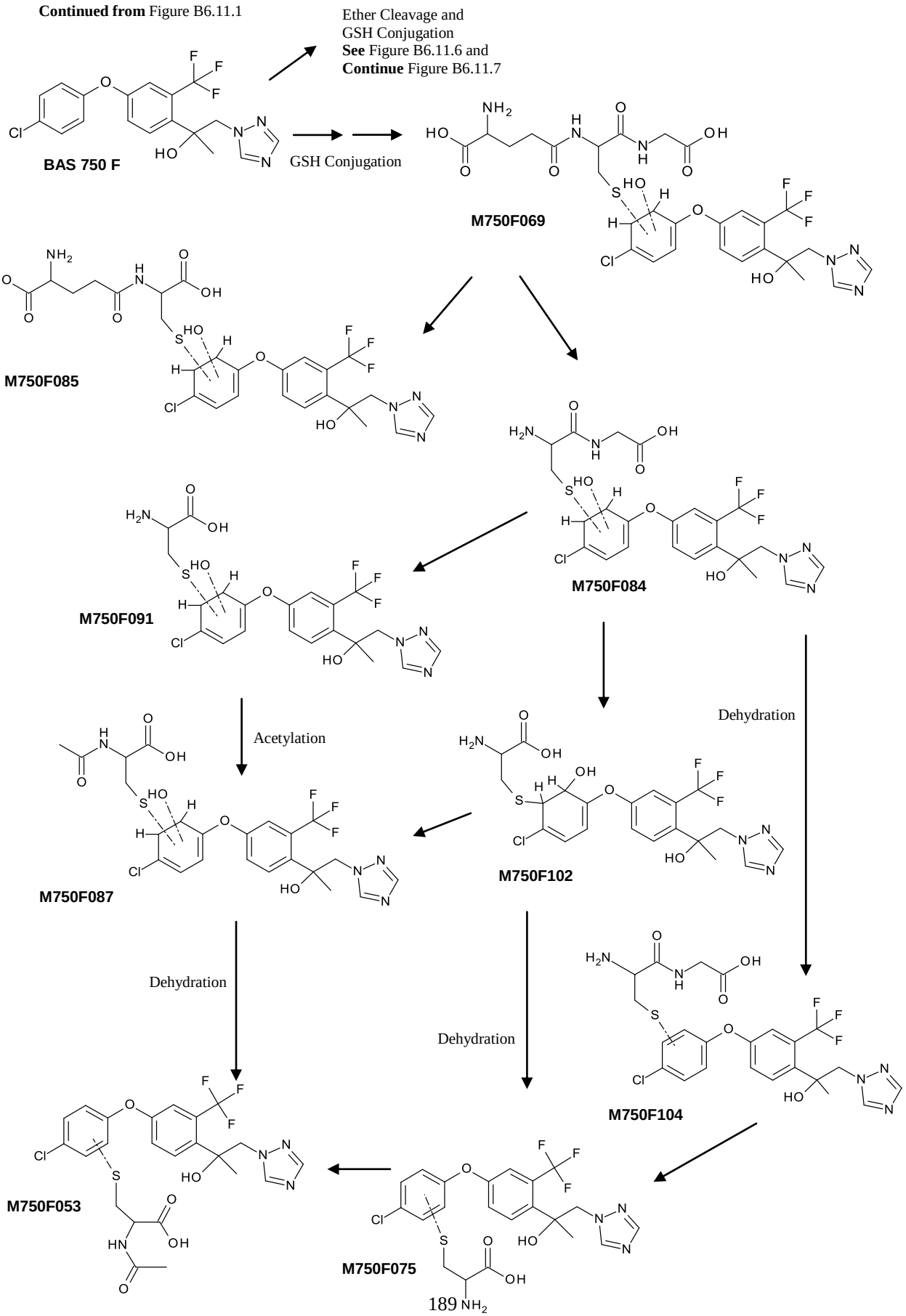


Figure B6.11.6. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 6, Ether Cleavage and Hydrolysis)

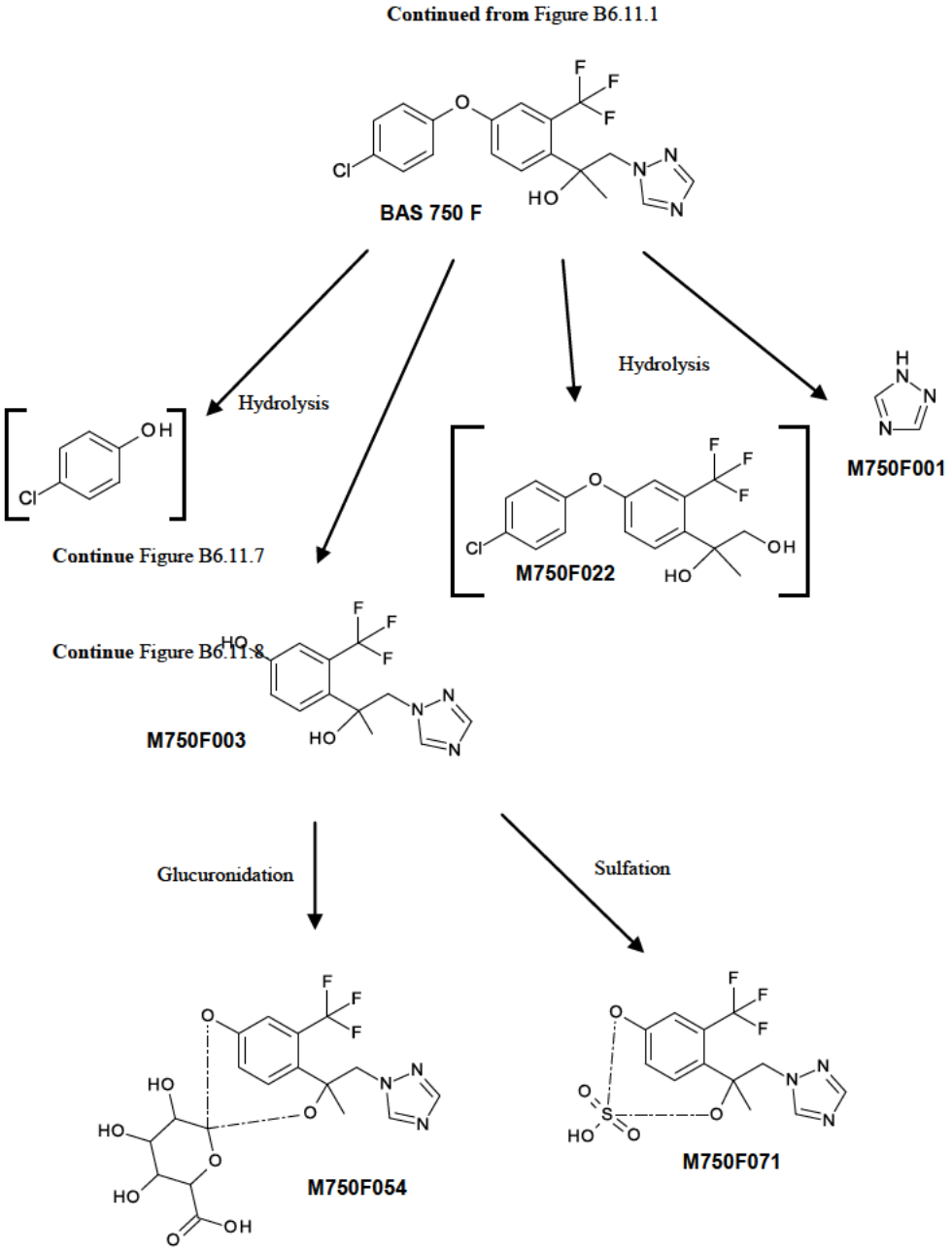


Figure B6.11.7. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 7, Ether Cleavage, GSH Conjugation)

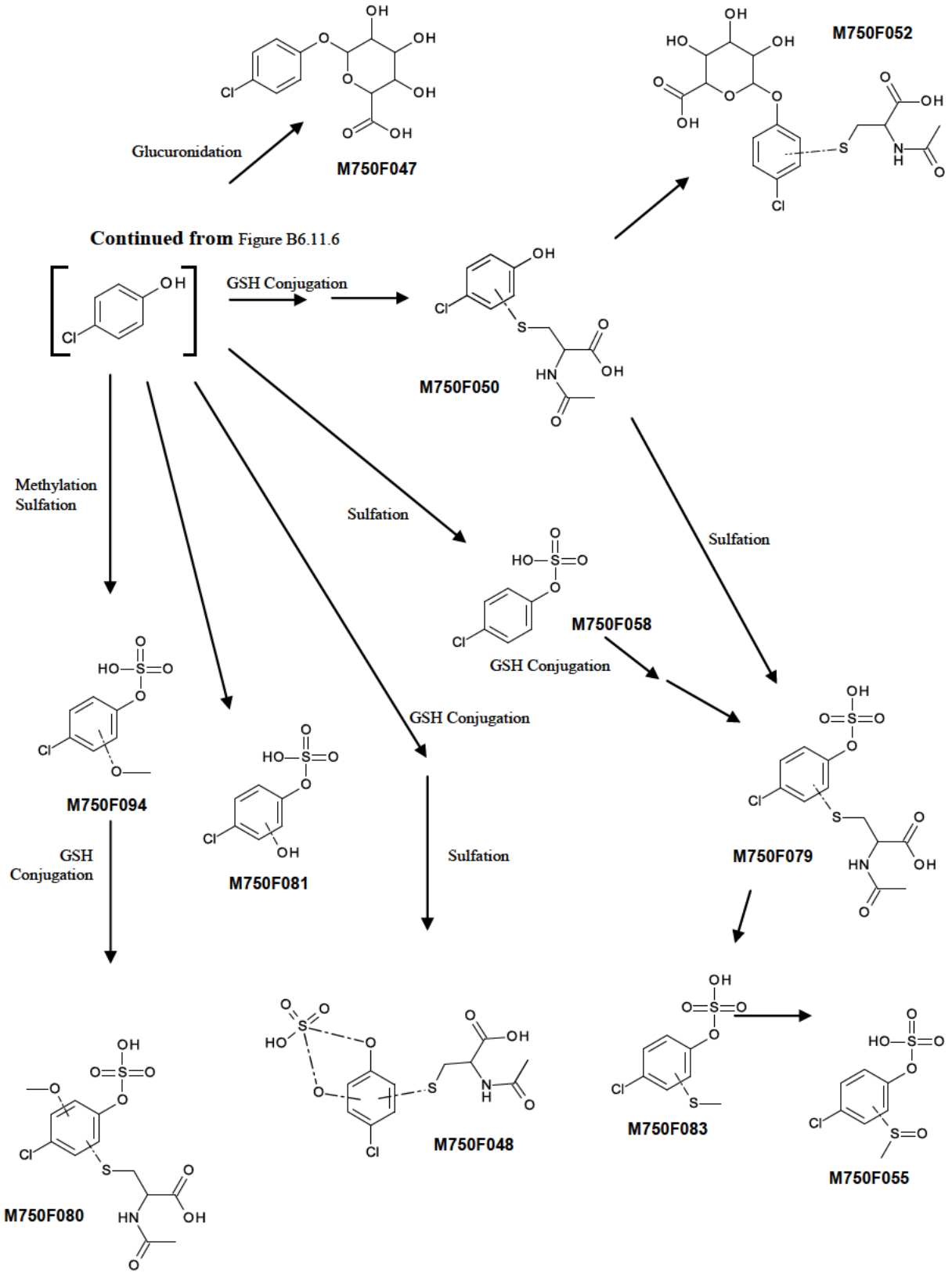


Figure B6.11.8. Proposed Metabolic Pathway of BAS 750 F in Rats (Part 8, Multiple Phase I and Phase II after Hydrolysis)

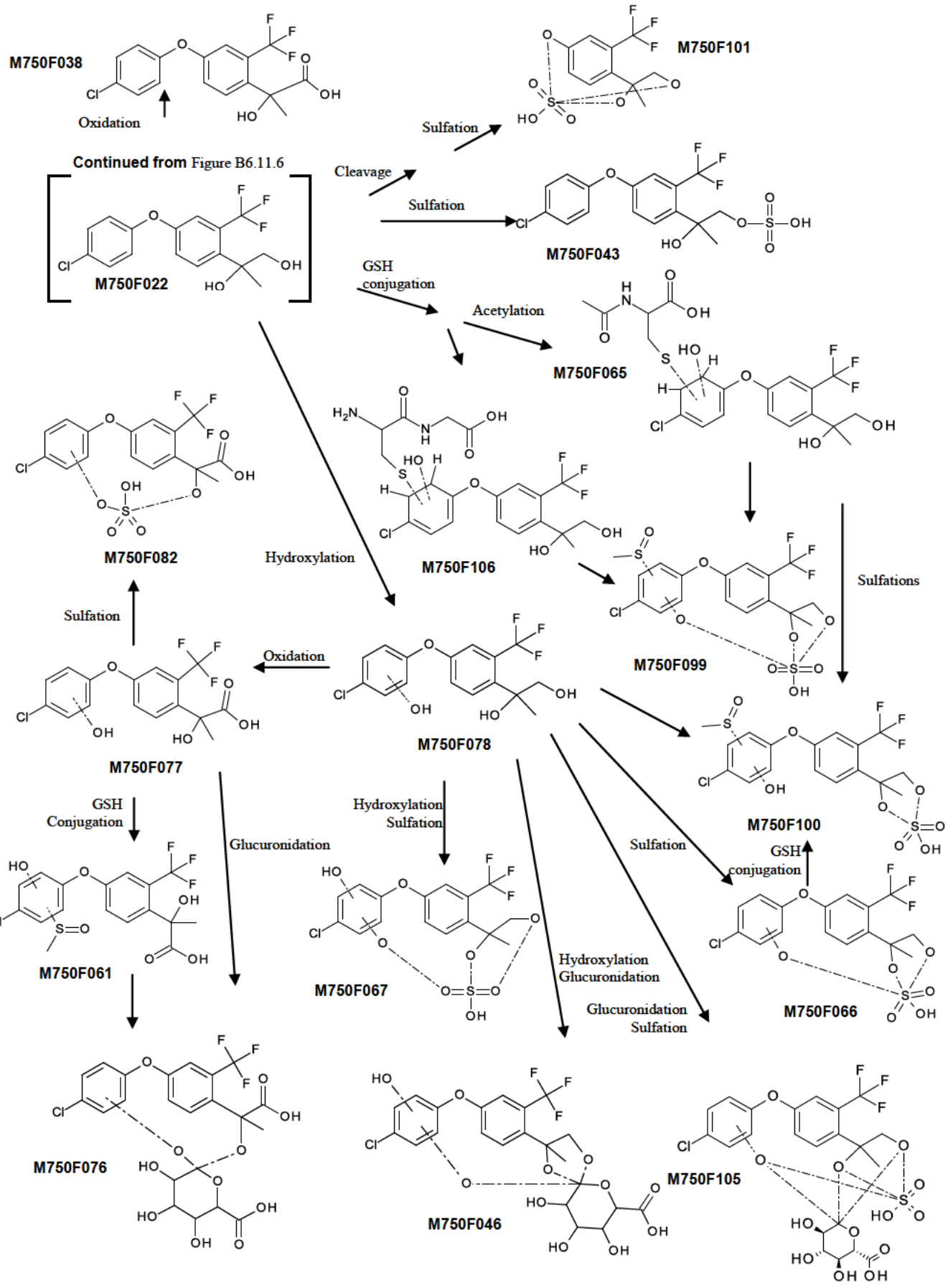
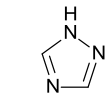


Table B6.11.1 Summary of identified and quantified metabolites



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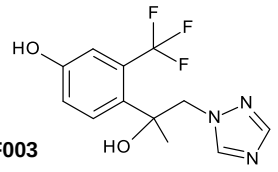

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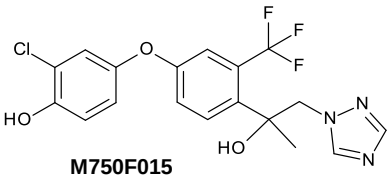

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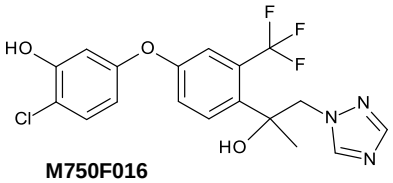

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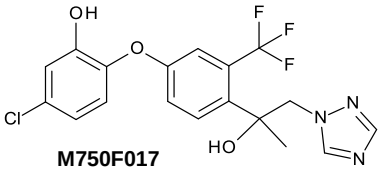

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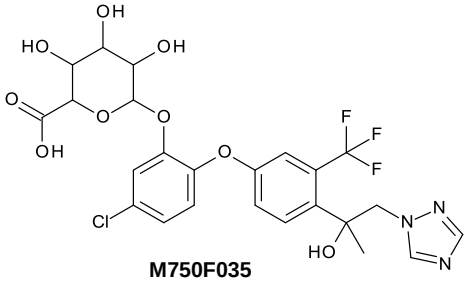

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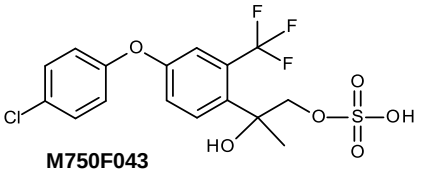

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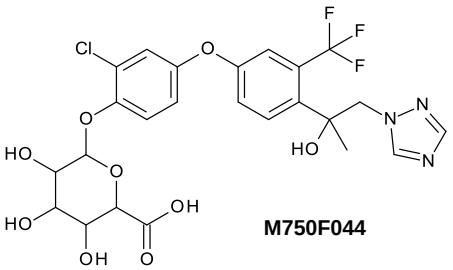

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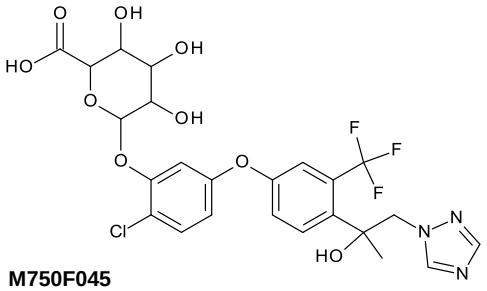

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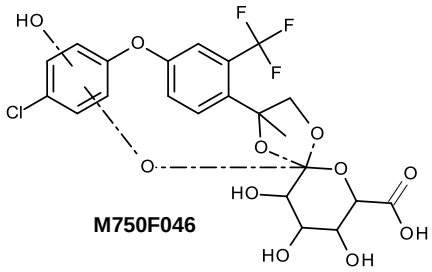

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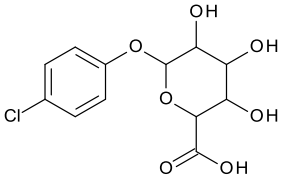

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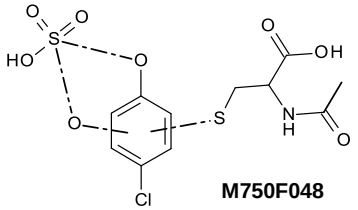

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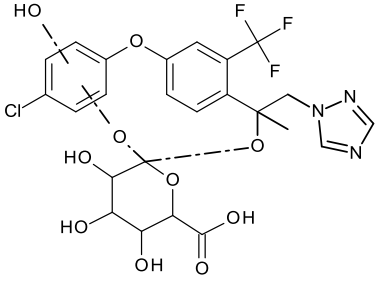

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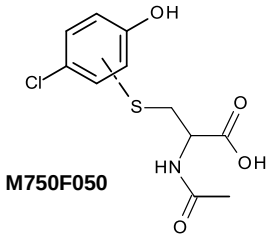

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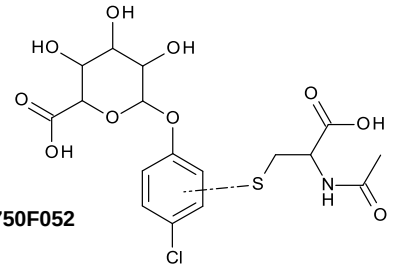

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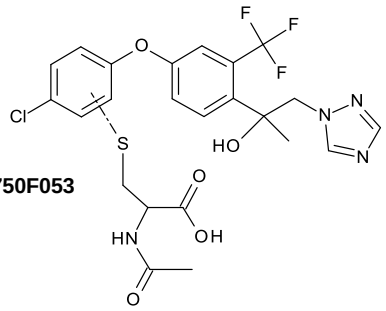

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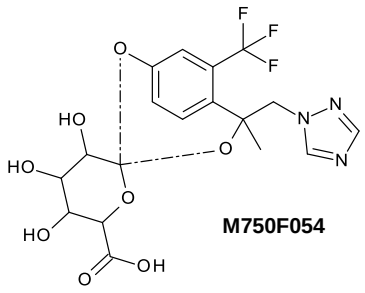

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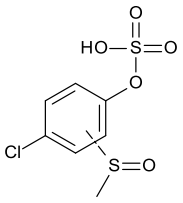

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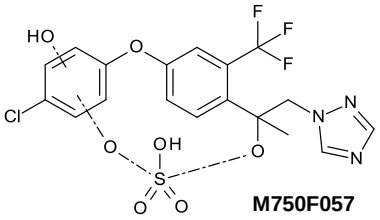

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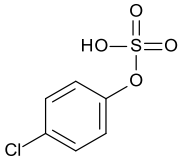

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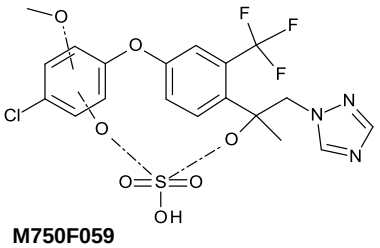

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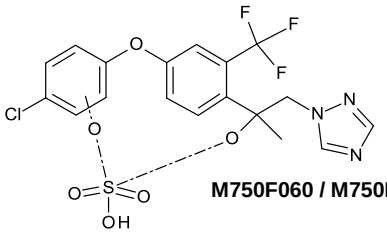
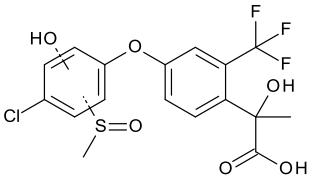


M750F060 / M750F098 *



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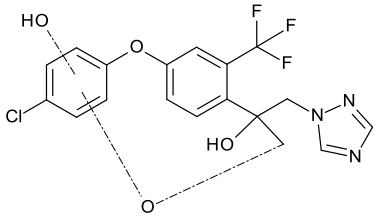
* M750F098 includes M750F060 with potential Cl-shift



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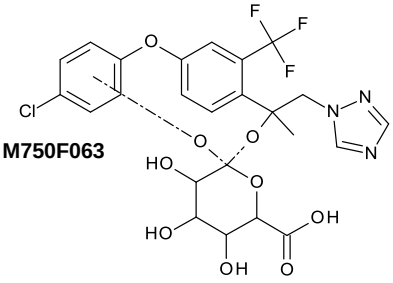

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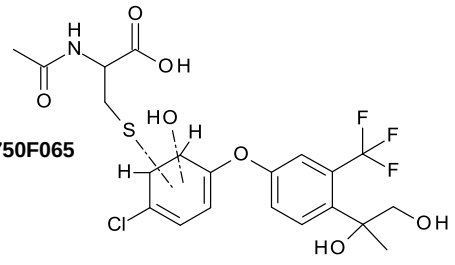

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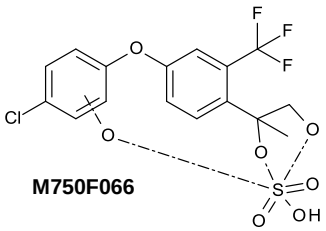

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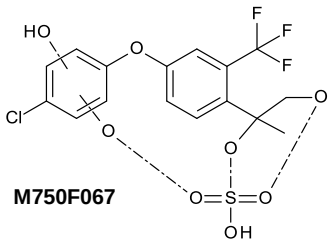

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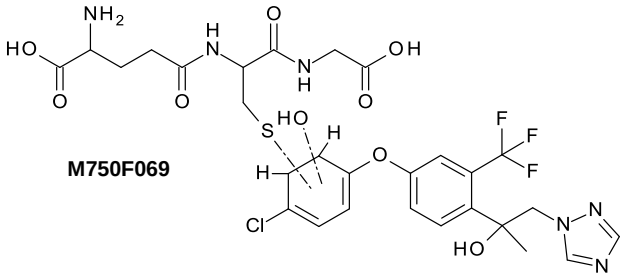

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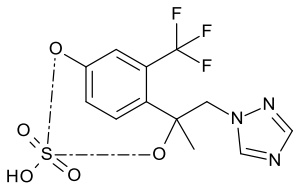

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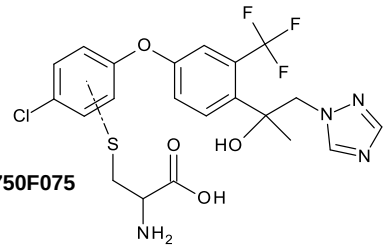

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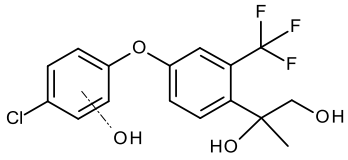

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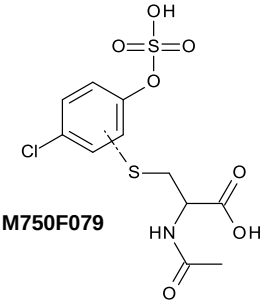

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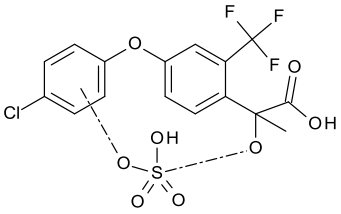

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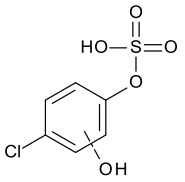

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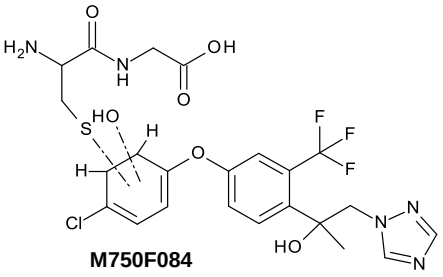

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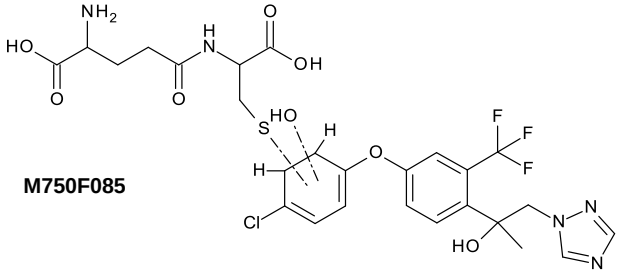

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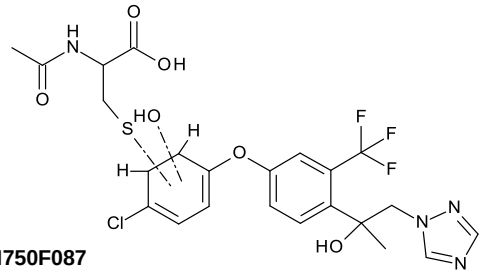

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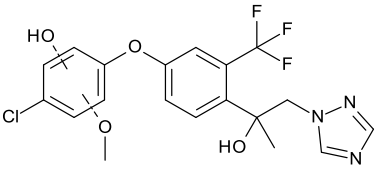

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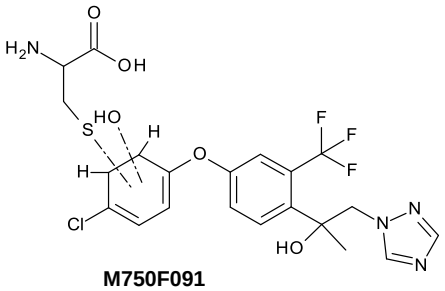

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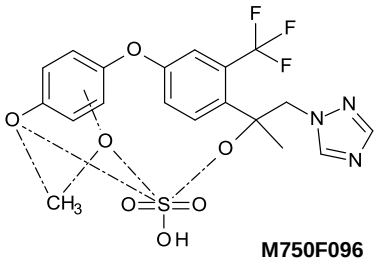

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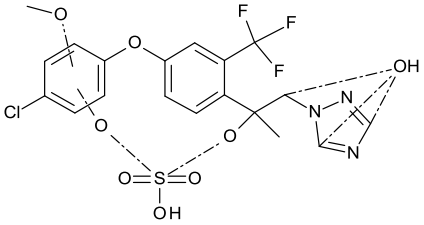
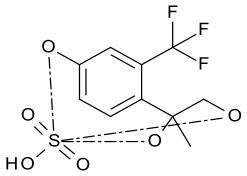


M750F097

M750F098: see M750F060



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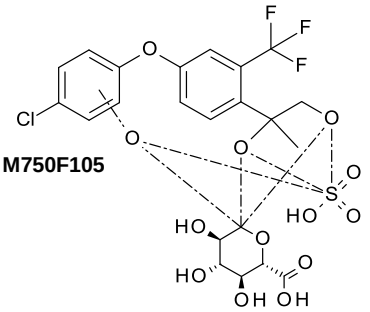

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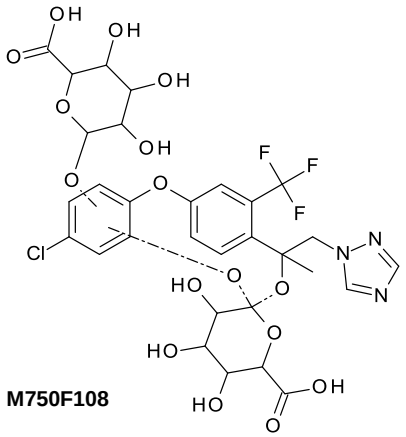


M750F108

Table B6.11.2. Proportions of metabolites identified in rat urine (biokinetics study, C-label)

Dose	**Percent of administered dose** **Group B** **Single low dose** **(5 mg/kg bw)**		**Group D** **Single high dose** **(180 mg/kg bw)**		**Group C** **Repeated high dose** **(14 + 1 x 180 mg/kg bw)**	
Compound M750-	Males (0-48 h)	Females (0-48 h)	Males (0-48 h)	Females (0-48 h)	Males (0-48 h)	Females (0-48 h)
F052	n.d.	n.d.	0.139	n.d.	0.476	n.d.
F049 [a]	2.603	2.156	0.951	n.d.	0.450	n.d.
F052 / F049 (19.0)	n.d.	n.d.	n.d.	n.d.	n.d.	0.906
F050	n.d.	n.d.	0.725	0.468	0.675	0.661
F079 (30.0)	n.d.	n.d.	0.099	0.338	0.231	n.d.
F063 (22.3) / F063 (23.1)	n.d.	2.363	n.d.	n.d.	n.d.	n.d.
F063 (22.3) / F079 (30.0) / F087 (21.4)	n.d.	n.d.	n.d.	n.d.	n.d.	2.221
F015 / F057 (26.9) - isomer 1	n.d.	0.161	n.d.	0.619	n.d.	0.243
F015 / F058 / F067 (31.2)	n.d.	n.d.	n.d.	n.d.	0.369	n.d.
F058 / F081 (34.4)	n.d.	n.d.	n.d.	n.d.	n.d.	0.025
F016	n.d.	n.d.	n.d.	n.d.	0.715	n.d.
F017	n.d.	n.d.	0.596	n.d.	n.d.	n.d.
F016 / F017 / F057 (26.9) - isomer 2	n.d.	0.769	n.d.	n.d.	n.d.	n.d.
F016 / F017 / F059 (28.9)	n.d.	n.d.	n.d.	1.054	n.d.	n.d.
F016 / F017 / F096 (32.6)	n.d.	n.d.	n.d.	n.d.	n.d.	0.656
F098 (31.1)	n.d.	0.568	n.d.	n.d.	n.d.	n.d.
F067 (31.2)	n.d.	n.d.	0.292	n.d.	n.d.	n.d.
F058	n.d.	n.d.	n.d.	0.634	n.d.	n.d.
F058 / F081 (34.4)	0.902	n.d.	0.500	n.d.	n.d.	n.d.
F082 (39.6)	n.d.	n.d.	0.128	n.d.	n.d.	n.d.
F082 (43.5)	n.d.	n.d.	0.256	n.d.	n.d.	n.d.
F083	n.d.	0.303	0.858	0.988	n.d.	n.d.
F082 (39.6) / F083	n.d.	n.d.	n.d.	n.d.	1.104	n.d.
F098 (34.5)	n.d.	0.744	n.d.	n.d.	n.d.	n.d.
F038 / F066 (36.9) / F083 / F098 (31.1)	n.d.	n.d.	n.d.	n.d.	n.d.	0.763
F066 (41.4)	n.d.	0.079	n.d.	n.d.	n.d.	n.d.
F059 (35.5) / F066 (36.9)/ F066 (39.9) / F098 (34.5) / F099 (44.0) - isomer 1	n.d.	n.d.	n.d.	0.092	n.d.	n.d.
F059 (35.5) / F059 (41.4) / F066 (39.9) / F066 (41.4) / F082 (39.6) / F098 (34.5) / F099 (44.0) - isomer 1 / F099 (44.0) - isomer 2	n.d.	n.d.	n.d.	n.d.	n.d.	2.585
F099 (44.0) - isomer 2	n.d.	n.d.	n.d.	0.239	n.d.	n.d.
F059 (41.4)	n.d.	1.521	n.d.	n.d.	n.d.	n.d.
F043	n.d.	n.d.	n.d.	n.d.	n.d.	0.298
Total identified	**3.505**	**8.662**	**4.544**	**4.433**	**4.020**	**0.298**

n.d. = not detected / identified

() numbers in brackets denote retention times of isomers

[a] includes isomer of metabolite M750F049 at 19.0 min

Table B6.11.3. Proportions of metabolites identified in rat urine (metabolism study, C-label)

Dose	**Percent of administered dose** **Group DX** **single high dose (180 mg/kg bw), C-label**	
Compound M750-	Males (0-168 h)	Females (0-168 h)
F047	0.297	n.d.
F047 / F048 (18.0)	n.d.	0.206
F046 / F048 (18.4)	0.269	0.147
F048 (17.3) / F052	0.169	0.089
F049 (19.0)	0.640	0.530
F048 (19.7)	0.102	0.093
F050	n.d.	0.228
F050 / F076	0.501	n.d.
F044 / F045 / F087 (21.4) / F087 (21.9)	n.d.	1.008
F053 (25.1) / F062 (24.8)	n.d.	0.044
F077 / F078 (25.9)	0.065	n.d.
F078	n.d.	n.d.
F015 / F055 (26.1) / F078 (26.8)	0.203	0.330
F016 / F017 / F078 (27.2)	0.312	n.d.
F016 / F017 / F061 (27.2) / F078 (27.2) / F089 (27.2)	n.d.	0.920
F067 (31.2) / F079 (31.5) / F090 / F095 / F097 (32.1)	n.d.	0.176
F058	0.760	n.d.
F058 / F092 (33.0) / F093 / F094 / F096 (33.6)	n.d.	0.383
F081 (34.4)	0.067	n.d.
F057 (36.9) / F066 (36.9) / F073	n.d.	0.849
F055 (40.5) / F082 (39.6) / F083	0.244	n.d.
F060 (39.7) / F066 (39.9) / F083 / F097 (39.7)	n.d.	0.220
F055 (40.5) / F057 (40.5) / F060 (40.5)	n.d.	0.365
F057 (41.4) / F059 (41.4) / F066 (41.4) / F100	n.d.	0.529
F082 (44.2) / F099	n.d.	0.064
Total identified	**3.628**	**6.180**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.4. Proportions of metabolites identified in rat urine (biokinetics study, TFMP-label)

Dose	**Percent of administered dose** **Group D** **Single high dose** **(180 mg/kg bw)**		**Group S** **Single high dose** **(180 mg/kg bw)**	
Compound M750-	Males (0-168 h)	Females (0-168 h)	Males (0-72 h)	Females (0-72 h)
F054	1.650	2.074	0.410	0.479
F084	n.d.	n.d.	n.d.	0.133
F091	n.d.	n.d.	0.841	0.779
F101 (17.3) / F101 (18.1)	0.567	n.d.	n.d.	n.d.
F003 / F049 (19.0)	2.836	2.074	n.d.	n.d.
F003 / F049 (19.0) / F071 (20.4)	n.d.	n.d.	0.465	n.d.
F003 / F049 (19.0) / F108 (19.1)	n.d.	n.d.	n.d.	0.609
F063 (22.3)/ F063 (23.1)/ F087 (21.4)	n.d.	0.632	n.d.	n.d.
F063 (22.3) / F087 (21.4)	n.d.	n.d.	2.572	n.d.
F063 (23.1)	n.d.	n.d.	2.656	n.d.
F035 / F044 / F045 / F065 / F087 (21.4) / F087 (21.9)	n.d.	n.d.	n.d.	4.253
F071 (19.4) / F071 (20.4)	3.052	n.d.	n.d.	n.d.
F053 (25.1) / F062 (24.8)	n.d.	n.d.	n.d.	0.328

Dose	**Percent of administered dose** **Group D** **Single high dose** **(180 mg/kg bw)**		**Group S** **Single high dose** **(180 mg/kg bw)**	
F053 (25.1) / F067 (26.2)	n.d.	n.d.	n.d.	0.054
F015	0.680	0.680	1.163	n.d.
F015 / F089 (26.3)	n.d.	n.d.	n.d.	0.240
F015 / F067 (26.2)	n.d.	n.d.	n.d.	0.974
F016 / F017	0.400	1.446	1.911	n.d.
F016 / F017 / F089 (27.2)	n.d.	n.d.	n.d.	1.892
F098 (31.1)	n.d.	0.845	n.d.	n.d.
F059	n.d.	1.949	n.d.	n.d.
Total identified	**9.185**	**9.700**	**10.018**	**9.739**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers
[a] includes isomer of metabolite M750F049 at 19.0 min

Table B6.11.5. Proportions of metabolites identified in rat urine (biokinetics study, T-label)

Dose	**Percent of administered dose** **Group B** **Single low dose** **(5 mg/kg bw)**		**Group D** **Single high dose** **(180 mg/kg bw)**		**Group C** **Repeated high dose** **(14 + 1 x 180 mg/kg bw)**	
Compound M750-	Males (0-48 h)	Females (0-48 h)	Males (0-48 h)	Females (0-48 h)	Males (0-48 h)	Females (0-48 h)
F001	19.964	3.193	9.583	3.097	13.834	7.700
F054	4.342	2.705	1.134	1.455	0.748	1.575
F049 [a]	n.d.	3.744	n.d.	n.d.	n.d.	n.d.
F003	n.d.	n.d.	1.526	n.d.	1.863	n.d.
F003 / F049 (19.0)	3.118	n.d.	n.d.	1.096	n.d.	1.374
F063 (22.3) / F063 (23.1)	n.d.	1.756	n.d.	n.d.	n.d.	n.d.
F063 (22.3) / F087 (21.9)	n.d.	n.d.	n.d.	0.733	n.d.	n.d.
F071	6.698	n.d.	2.061	n.d.	1.585	n.d.
F015	n.d.	0.412	n.d.	0.402	n.d.	0.380
F016 / F017	n.d.	1.315	n.d.	n.d.	n.d.	1.042
F016 / F017 / F059 (28.9)	n.d.	n.d.	n.d.	1.060	n.d.	n.d.
F098 (31.1)	n.d.	0.089	n.d.	n.d.	n.d.	0.178
F098 (31.1) / F098 (33.8)	n.d.	n.d.	n.d.	0.505	n.d.	n.d.
F098 (33.8) / F098 (34.5)	n.d.	0.270	n.d.	n.d.	n.d.	n.d.
F098 (34.5)	n.d.	n.d.	n.d.	n.d.	n.d.	0.171
F057 (26.9)	n.d.	n.d.	n.d.	0.010	n.d.	n.d.
F059 (41.4)	n.d.	0.596	n.d.	0.601	n.d.	n.d.
Total identified	**34.123**	**14.080**	**14.304**	**8.958**	**18.030**	**12.420**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers
[a] includes isomer of metabolite M750F049 at 19.0 min

Table B6.11.6. Proportions of metabolites identified in rat urine (metabolism study, T-label)

Dose	**Percent of administered dose** **Group DX** **single high dose, (180 mg/kg bw), T-label**	
Compound M750-	Males (0-170 h)	Females (0-168 h)
F001	10.491	3.320
F054	2.282	1.837
F049 [a]	0.962	0.777
F044 / F087 (21.4) / F045 / F087 (21.9)	n.d.	1.217

Dose	**Percent of administered dose Group DX single high dose, (180 mg/kg bw), T-label**	
F071	2.021	n.d.
F015	n.d.	0.309
F016 / F017	n.d.	0.649
F073	n.d.	1.013
F057 (41.4) / F059 (41.4)	n.d.	0.229
F057 (42.1)	n.d.	0.341
Total identified	**15.756**	**9.692**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers
[a] includes isomer of metabolite M750F049 at 19.0 min

Table B6.11.7. Proportions of metabolites identified in rat faeces (biokinetics study, C-label)

Dose	**Percent of administered dose Group B Single low dose (5 mg/kg bw)**		**Group D Single high dose (180 mg/kg bw)**		**Group C Repeated high dose (14 + 1 x 180 mg/kg bw)**	
Compound M750-	Males (0-72 h)	Females (0-72 h)	Males (0-72 h)	Females (0-72 h)	Males (0-48 h)	Females (0-48 h)
F062 (24.8)	3.972	3.663	n.d.	n.d.	n.d.	n.d.
F015	25.252	26.787	12.776	23.391	21.019	30.239
F016 / F017	32.121	23.621	23.754	17.896	31.686	29.139
F000	4.546	5.435	27.865	21.102	19.580	15.544
Total identified	**65.891**	**59.506**	**64.395**	**62.390**	**72.285**	**74.922**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.8. Proportions of metabolites identified in rat faeces (biokinetics study, TFMP-label)

Dose	**Percent of administered dose Group D Single high dose (180 mg/kg bw)**	
Compound M750-	Males (0-48 h)	Females (0-48 h)
F003	2.451	2.576
F015	14.722	30.181
F016 / F017	28.955	21.781
F000	17.220	21.142
Total identified	**63.348**	**75.680**

n.d. = not detected / identified

Table B6.11.9. Proportions of metabolites identified in rat faeces (biokinetics study, T-label)

Dose	**Percent of administered dose Group B Single low dose (5 mg/kg bw)**		**Group D Single high dose (180 mg/kg bw)**		**Group C Repeated high dose (14 + 1 x 180 mg/kg bw)**	
Compound M750-	Males (0-72 h)	Females (0-72 h)	Males (0-72 h)	Females (0-72 h)	Males (0-48 h)	Females (0-48 h)
F003	4.319	4.314	1.796	3.454	1.290	1.087
F062 (24.8)	6.016	4.578	n.d.	n.d.	n.d.	n.d.
F062 (25.1)	0.833	1.615	n.d.	n.d.	n.d.	n.d.
F015	10.198	41.037	13.931	25.488	10.947	19.898
F016 / F017	21.702	26.499	23.763	18.148	20.390	19.957
F000	3.079	1.381	25.842	29.889	28.914	23.898

Dose	**Percent of administered dose** **Group B** **Single low dose** **(5 mg/kg bw)**		**Group D** **Single high dose** **(180 mg/kg bw)**		**Group C** **Repeated high dose** **(14 + 1 x 180 mg/kg bw)**	
Total identified	**46.147**	**79.424**	**65.332**	**76.979**	**61.541**	**64.840**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.10. Proportions of metabolites identified in rat faeces (metabolism study, C- and T-label)

Dose	**Percent of administered dose** **Group DX** **single high dose** **(180 mg/kg bw)** **C-label**		**Group DX** **single high dose** **(180 mg/kg bw)** **T-label**	
Compound M750-	Males (0-72 h)	Females (0-72 h)	Males (0-72 h)	Females (0-72 h)
F003	n.d.	n.d.	1.371	1.164
F062 (24.8)	3.032	3.772	n.d.	n.d.
F015	16.663	26.510	11.349	20.372
F016 / F017	28.491	20.652	19.980	15.500
F000	12.726	7.560	35.233	30.069
Total identified	**60.912**	**58.494**	**67.933**	**67.105**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers
[a] includes isomer of metabolite M750F049 at 19.0 min

Table B6.11.11. Proportions of metabolites identified in rat bile (biokinetics study, C-label)

Dose	**Percent of administered dose** **Group R** **Single low dose** **(5 mg/kg bw)**		**Group S** **Single high dose** **(180 mg/kg bw)**		
Compound M750-	Males (0-24 h)	Females (0-24 h)	Males (0-24 h)	Females, 1 (0-24 h)	Females, 2 (0-24 h)
F084	2.477	4.012	2.281	1.164	0.929
F091	1.904	n.d.	n.d.	n.d.	n.d.
F069 (18.1)	n.d.	n.d.	n.d.	0.392	5.475
F069 (18.1) / F091	n.d.	n.d.	4.928	n.d.	n.d.
F049 (19.0) / F104	2.389	3.067	n.d.	n.d.	0.532
F104	n.d.	n.d.	n.d.	n.d.	n.d.
F075 (19.7) / F075 (20.1)	2.110	n.d.	n.d.	n.d.	0.563
F049 (19.0) / F104 / F075 (19.7) / F075 (20.1)	n.d	n.d.	3.147	n.d.	n.d.
F044 / F049 (21.5) / F087 (21.4) / F035 / F045 / F049 (21.9) / F049 (22.2)	53.172	49.069	22.040	37.494	45.401
F105	0.445	n.d.	0.673	n.d.	n.d.
F015	n.d.	n.d.	n.d.	0.139	n.d.
F060 (31.4)	0.578	n.d.	0.652	n.d.	n.d.
Total identified	**62.984**	**56.148**	**33.720**	**39.189**	**52.900**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.12. Proportions of metabolites identified in rat bile (biokinetics study, TFMP-label)

Dose	**Percent of administered dose Group S Single high dose (180 mg/kg bw)**	
Compound M750-	Males (0-24 h)	Females (0-24 h)
F054	0.551	n.d.
F084	2.069	n.d.
F069 (18.1) / F085	6.933	n.d.
F069 (18.3)	0.606	n.d.
F069 (18.1) / F085 / F069 (18.3)	n.d.	3.616
F049 (19.0)	1.811	3.263
F075 (19.7)	n.d.	0.484
F075 (19.7) / F075 (20.1)	1.302	n.d.
F044 / F087 (21.4)	21.523	n.d.
F035 / F045 / F049 (21.9)	18.815	n.d.
F044 / F049 (21.5) / F087 (21.4) / F035 / F045 / F049 (21.9) / F049 (22.2)	n.d.	47.556
F015	1.451	n.d.
F110 (22.5)	n.d.	1.638
Total identified	**55.061**	**56.557**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.13. Proportions of metabolites identified in rat bile (biokinetics study, T-label)

Dose	**Percent of administered dose Group R Single low dose (5 mg/kg bw)**		**Group S Single high dose (180 mg/kg bw)**	
Compound M750-	Males (0-15 h)	Females (0-21 h)	Males (0-24 h)	Females (0-24 h)
F001	0.203	0.040	0.232	0.024
F054	1.489	0.709	0.609	n.d.
F084	n.d.	1.572	n.d.	n.d.
F091	3.315	3.314	3.959	0.146
F069 (17.2)	n.d.	0.305	n.d.	n.d.
F003 / F049 (19.0)	7.035	5.497	1.819	0.537
F075 (19.7) / F075 (20.1)	10.503	5.192	5.452	5.437
F044 / F049 (21.5)	10.395	n.d.	n.d.	n.d.
F044 / F049 (21.5)/ F087 (21.4)	n.d.	23.980	13.010	n.d.
F044 / F049 (21.5)/ F087 (21.4) / F035 / F045 / F049 (21.9) / F049 (22.2) / F087 (21.9)	n.d.	n.d.	n.d.	30.691
F035 / F045 / F049 (21.9)	12.230	n.d.	n.d.	n.d.
F035 / F045 / F049 (21.9) / F049 (22.2)	n.d.	17.464	12.134	n.d.
F015	9.485	9.966	3.020	3.812
F016 / F017	12.323	6.507	3.131	2.306
Total identified	**66.977**	**74.547**	**43.367**	**42.954**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.14. Proportions of metabolites identified in rat liver analyzed at tmax of plasma level (1 hour, metabolism study, C-label)

Dose	**Percent of administered dose** **Group V** **Single low dose** **(5 mg/kg bw)**		**Group W** **Single high dose** **(180 mg/kg bw)**	
Compound M750-	**VM**	**VF**	**WM**	**WF**
F049 (19.0)	0.463	n.d.	0.281	0.131
F015 / F055 (26.1) / F067 (26.2) / F078 (26.8)	3.597	2.359	1.727	1.825
F016 / F017 / F061 (27.2) / F078 (27.2) / F089 (27.2)	1.610	2.068	0.608	0.884
BAS 750 F	1.664	1.011	1.881	2.025
Total Identified	**7.334**	**5.438**	**4.496**	**4.866**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.15. Proportions of metabolites identified in rat liver analyzed at tmax of plasma level (1 hour, metabolism study, T-label)

Dose	**Percent of administered dose** **Group V** **Single low dose** **(5 mg/kg bw)**		**Group W** **Single high dose** **(180 mg/kg bw)**	
Compound M750-	**VM**	**VF**	**WM**	**WF**
F049 (19.0)	0.513	n.d.	n.d.	n.d.
F015	3.252	2.664	0.948	0.907
F016 / F017	1.663	2.202	0.438	0.509
BAS 750 F	1.412	1.243	1.075	1.231
Total Identified	**6.840**	**6.109**	**2.460**	**2.647**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.16. Proportions of metabolites identified in rat kidney analyzed at tmax of plasma level (1 hour, metabolism study, C-label)

Dose	**Percent of administered dose** **Group V** **Single low dose** **(5 mg/kg bw)**		**Group W** **Single high dose** **(180 mg/kg bw)**	
Compound M750-	**VM**	**VF**	**WM**	**WF**
F015 / F055 (26.1) / F067 (26.2) / F078 (26.8)	0.055	0.063	0.046	0.042
F016 / F017 / F061 (27.2) / F078 (27.2) / F089 (27.2)	n.d.	0.030	n.d.	0.015
BAS 750 F	0.112	0.106	0.188	0.237
Total Identified	**0.167**	**0.199**	**0.234**	**0.293**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.17. Proportions of metabolites identified in rat kidney analyzed at tmax of plasma level (1 hour, metabolism study, T-label)

Dose	**Percent of administered dose** **Group V** **Single low dose** **(5 mg/kg bw)**		**Group W** **Single high dose** **(180 mg/kg bw)**	
Compound M750-	**VM**	**VF**	**WM**	**WF**
F001	0.049	n.d.	0.007	n.d.
F054	n.d.	0.008	n.d.	n.d.

Dose	**Percent of administered dose** **Group V** **Single low dose** **(5 mg/kg bw)**		**Group W** **Single high dose** **(180 mg/kg bw)**	
F049 (19.0)	0.016	n.d.	0.005	n.d.
F015	0.037	0.040	0.017	0.024
F016 / F017	0.012	0.032	n.d.	n.d.
BAS 750 F	0.082	0.092	0.130	0.144
Total Identified	**0.196**	**0.171**	**0.159**	**0.168**

n.d. = not detected / identified
() numbers in brackets denote retention times of isomers

Table B6.11.18. Proportions of metabolites identified in rat fat analyzed at tmax of plasma level (1 hour, metabolism study, C-label)

Dose	**Percent of administered dose** **Group W** **Single high dose** **(180 mg/kg bw)**	
Compound	**WM**	**WF**
BAS 750 F	0.135	0.224
Total Identified	**0.135**	**0.224**

Table B6.11.19. Proportions of metabolites identified in rat plasma (1 hour, metabolism study, C-label)

Dose	**Percent of administered dose** **Group V** **Single low dose** **(5 mg/kg bw)**		**Group W** **Single high dose** **(180 mg/kg bw)**	
Compound	**VM**	**VF**	**WM**	**WF**
BAS 750 F	0.030	0.007	0.052	0.067
Total Identified	**0.030**	**0.007**	**0.052**	**0.067**

Table B6.11.20. Proportions of metabolites identified in rat plasma (1 hour, metabolism study, T-label)

Dose	**Percent of administered dose** **Group V** **Single low dose** **(5 mg/kg bw)**		**Group W** **Single high dose** **(180 mg/kg bw)**	
Compound M750-	**VM**	**VF**	**WM**	**WF**
F001	0.037	0.002	0.014	0.006
F015	0.009	0.003	0.003	0.006
F000	0.016	0.003	0.025	0.026
Total Identified	**0.061**	**0.009**	**0.042**	**0.038**

B.6.12. ANNEX II: BENCHMARK DOSE ANALYSIS

RAT 28-DAY STUDY

Dose	Sex	Grp_size	Liverwt_rel	Liver_sd	Final_bw	bw_sd
0	1	5	2.693	0.164	300.2	9
47	1	5	2.736	0.114	293	17.3
135	1	5	2.818	0.222	289.3	15.7
388	1	5	2.844	0.15	265.5	14.1
0	2	5	2.644	0.152	185.6	12.3
47	2	5	2.84	0.287	184.5	7
138	2	5	2.849	0.269	188.8	5
334	2	5	3.241	0.16	169.4	8.3

This report was generated by on `2017-03-06` using PROAST version `63.5` and `R version 3.3.2 (2016-10-31)`.

Dose

The dose variable was `'Dose'`.

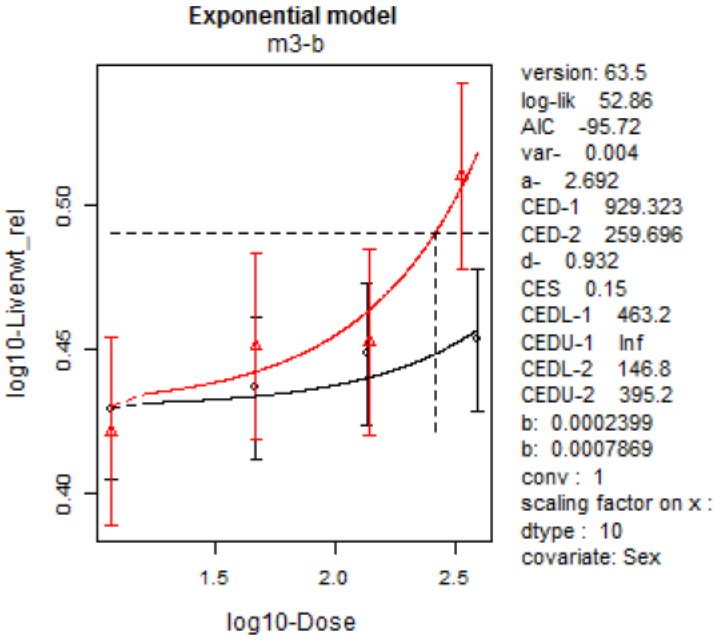
Response

The response variable was `'Liverwt_rel'`.

Covariate

The covariate was 'Sex' with levels '1', '2'.

Fitted Models



Exponential model

model	converged	loglik	npar	AIC
full	1	54.09	9	-90.18
full-v	1	54.82	10	-89.64
m1-	1	41.74	2	-79.48
m1-a	1	42.81	3	-79.62

m2-ab	1	53.00	5	-96.00
m3-	1	48.00	4	-88.00
m3-a	1	49.66	5	-89.32
m3-b	1	52.86	5	-95.72
m3-ab	1	53.06	6	-94.12
m5-a	1	49.68	6	-87.36
m5-b	0	52.81	6	-93.62
m5-ab	0	53.01	7	-92.02

The chosen exponential model was m3-b.

Hill model

model	converged	loglik	npar	AIC
m3-	1	47.99	4	-87.98
m3-a	1	49.66	5	-89.32
m3-b	1	52.92	5	-95.84
m3-ab	1	53.11	6	-94.22
m5-a	1	49.67	6	-87.34
m5-b	1	52.81	6	-93.62
m5-ab	1	53.01	7	-92.02

The chosen Hill model was m3-b.

Benchmark dose

	Covariate	Lowest BMDL	Highest BMDU
1	1	461.95	Inf
2	2	146.77	395.25

MOUSE 28-DAY STUDY

Dose	Sex	Grpsize	Rel_livwt	Rel_livwt_sd	necrosis	Focus	bw	bw_sd
0	1	5	4.067	0.212	0	0	24.2	1.7
4.8	1	5	4.552	0.183	0	0	25.5	1.2
15.5	1	5	4.818	0.206	0	0	25.2	0.7
47.9	1	5	4.958	0.523	0	1	26	0.4
128	1	5	6.959	0.316	4	2	21	1
0	2	5	4.469	0.08	0	0	20.3	0.5
5.8	2	5	4.68	0.038	0	0	20.1	0.5
18.5	2	5	5.185	0.432	0	0	21.4	0.7
61	2	5	5.939	0.223	0	0	20.3	0.9
145	2	5	7.696	0.207	3	2	19	0.4

Analysis name: 28d mouse liver

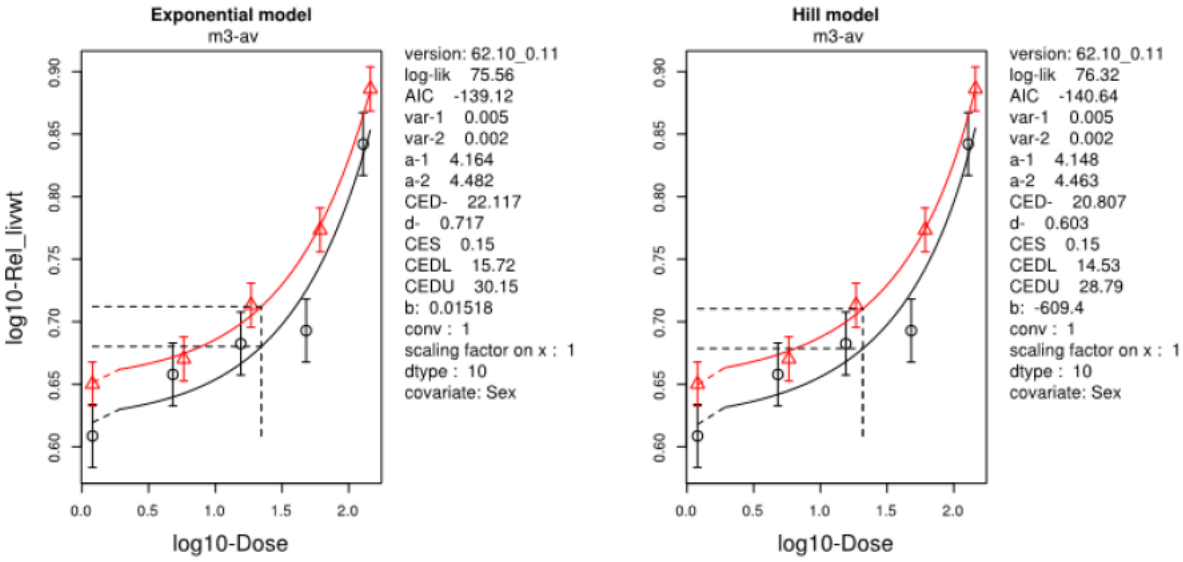
This report was generated by Anonymous on 12/20/2016 12:36:53 PM (CET). PROAST version 62.10_0.11

Input values

Type of response data

Continuous, summary data
Dose column
Dose
Response column
Rel livwt
Litter effect
No
Dispersion measure column
Rel livwt sd
Relating to
Standard deviation
Group size column
Grpsize
Covariate column
Sex
Benchmark response
0.15
AIC criterion
2

Fitted Models



Exponential Model

model	converged	loglik	npar	AIC
full	1	80.95	11	-139.90
full-v	1	82.52	12	-141.04
m1-v	1	9.57	3	-13.14
m1-av	1	11.06	4	-14.12
m2-ab	1	69.26	5	-128.52
m3-av	1	75.56	6	-139.12
m3-abv	1	75.57	7	-137.14
m5-av	0	74.54	7	-135.08
m5-abv	0	74.61	8	-133.22

The chosen Exponential model was m3-av

Hill Model

model	converged	loglik	npar	AIC
m3-av	1	76.32	6	-140.64
m3-abv	1	76.32	7	-138.64
m5-av	1	74.54	7	-135.08
m5-abv	1	74.61	8	-133.22

The chosen Hill model was m3-av

BMD confidence interval

Sex	Lowest BMDL	Highest BMDU
1	14.528	30.153
2	14.528	30.153

RAT 90-DAY STUDY

DOSE	GRPSIZE	SEX	BODYWT	BODYWT_SD	BWG	BWG_SD	RELLIVWT	RELIVWT_SD
0	10	1	395.2	331.3	227.8	26.8	2.248	0.087
27	10	1	409.4	27.6	242	24.2	2.31	0.112
76	10	1	395.1	31.5	227.9	28.8	2.376	0.153
256	10	1	369.6	25.5	202.2	24.8	2.502	0.106
0	10	2	233.9	17.6	108	14.1	2.486	0.187
30	10	2	228.9	11.4	98.7	11.6	2.359	0.281
91	10	2	225.6	8.8	99.7	11.3	2.541	0.189
314	10	2	213	11.8	86	9.4	2.779	0.098

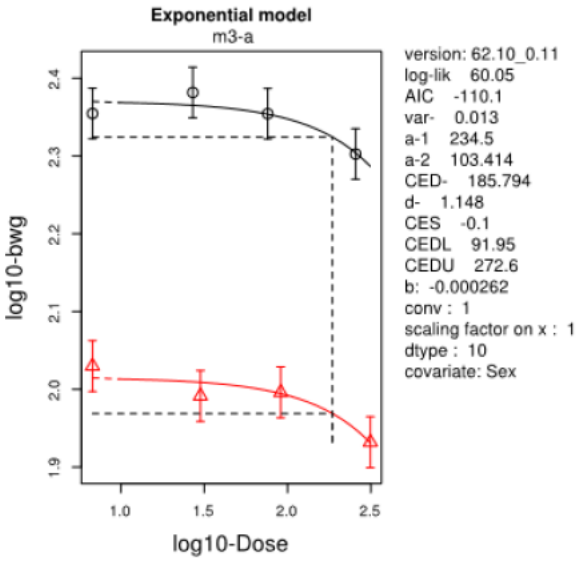
Analysis name: 90d rat bwg
This report was generated by **Anonymous** on **12/20/2016 12:58:32 PM (CET)**. PROAST version 62.10_0.11

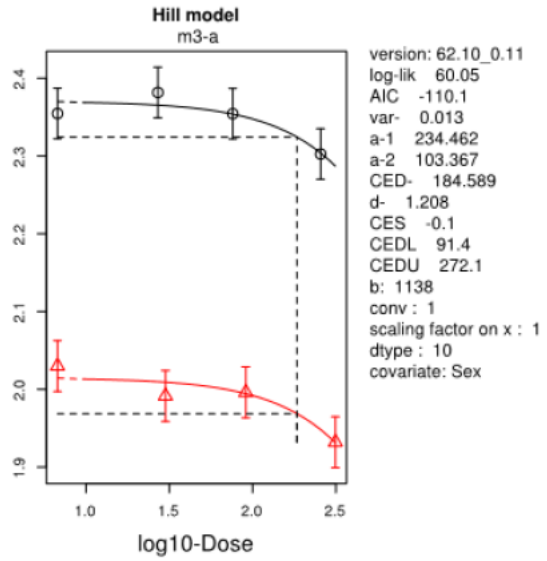
Input values

Type of response data
Continuous, summary data
Dose column
Dose
Response column
bwg
Litter effect
No
Dispersion measure column
bwg sd
Relating to
Standard deviation
Group size column
Grpsize
Covariate column
Sex

Benchmark response
0.1
AIC criterion
2

Fitted Models





Exponential Model

model	converged	loglik	npar	AIC
full	1	62.27	9	-106.54
full-v	1	62.27	10	-104.54
m1-	1	-47.21	2	98.42
m1-a	1	47.61	3	-89.22
m2-ab	1	60.02	5	-110.04
m3-a	1	60.05	5	-110.10
m3-b	0	-6.45	5	22.90
m3-ab	1	60.05	6	-108.10
m5-a	1	60.05	6	-108.10
m5-b	1	-6.95	6	25.90
m5-ab	1	60.06	7	-106.12

The chosen Exponential model was **m3-a**

Hill Model

model	converged	loglik	npar	AIC
m3-a	1	60.05	5	-110.10
m3-b	0	-6.50	5	23.00
m3-ab	1	60.05	6	-108.10
m5-a	1	60.05	6	-108.10
m5-b	0	-6.43	6	24.86
m5-ab	1	60.05	7	-106.10

The chosen Hill model was **m3-a**

BMD confidence interval

Sex	Lowest BMDL	Highest BMDU
1	91.4	272.59
2	91.4	272.59

MOUSE 90-DAY STUDY

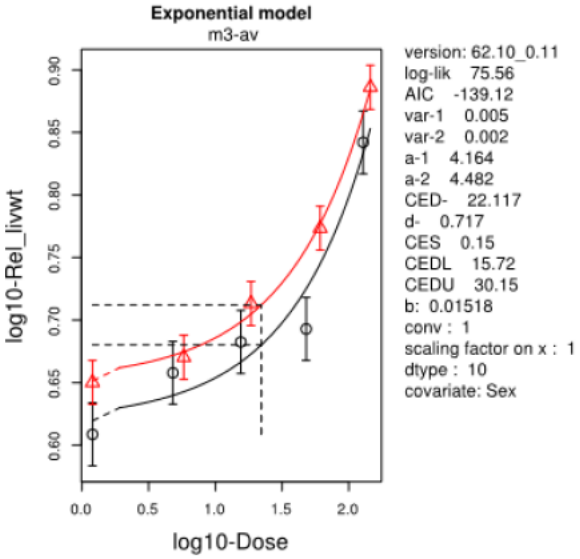
DOSE	GRP SIZE	SEX	NECROSIS SC	NECROSIS MF	BW	BW_SD	RELLIVWT	RELLIVWT SD	HAEM GRP	HB	HB_SD	HT	HT_SD
0	10	1	0		28.1	1.7	4.45	0.32	6	9.1	0.2	0.455	0.019
2	10	1	0		27.5	1.4	4.69	0.34	7	9.3	0.1	0.468	0.013
11	10	1	0		28.5	1.9	5.09	0.32	8	9.5	0.2	0.48	0.005
58	10	1	2		27.4	1.2	6.12	0.18	9	9.8	0.2	0.484	0.02
174	10	1	8		26.7	1.2	8.34	0.39	2	9.2	0.4	0.471	0.028
0	10	2		0	23	0.8	4.87	0.32	9	9.3	0.4	0.457	0.019
3	10	2		0	22.5	0.7	4.81	0.21	10	9.4	0.3	0.475	0.014
15	10	2		0	23.3	1.6	5.03	0.47	10	9.3	0.2	0.468	0.007
67	10	2		0	22.8	1.2	6.12	0.29	10	9.7	0.3	0.484	0.014
211	10	2		6	22.1	1.4	8.12	0.55	6	9	0.6	0.458	0.019

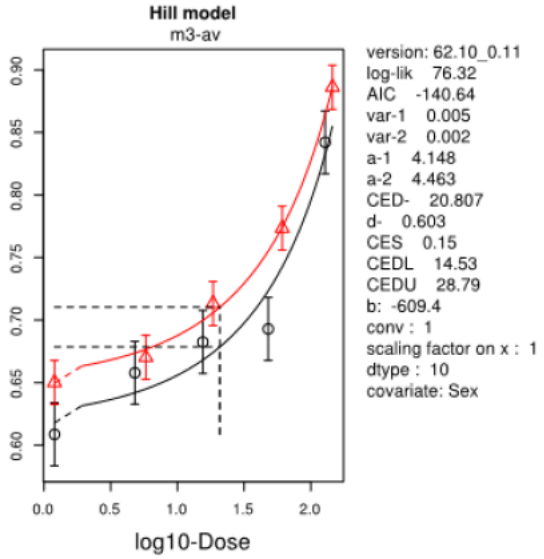
Analysis name: 90d mouse liver
This report was generated by **Anonymous** on **12/20/2016 1:04:01 PM (CET)**. PROAST version 62.10_0.11

Input values

Type of response data
Continuous, summary data
Dose column
Dose
Response column
Rellivwt
Litter effect
No
Dispersion measure column
Rellivwt sd
Relating to
Standard deviation
Group size column
Grpsize
Covariate column
Sex
Benchmark response
0.15
AIC criterion
2

Fitted Models





Exponential Model

model	converged	loglik	npar	AIC
full	1	140.56	11	-259.12
full-v	1	140.87	12	-257.74
m1-	1	8.23	2	-12.46
m1-a	1	8.28	3	-10.56
m2-ab	1	123.65	5	-237.30
m3-a	1	127.67	5	-245.34
m3-b	1	133.83	5	-257.66
m3-ab	1	136.55	6	-261.10
m5-a	1	129.52	6	-247.04
m5-b	1	134.17	6	-256.34
m5-ab	1	137.04	7	-260.08

The chosen Exponential model was **m3-ab**

Hill Model

model	converged	loglik	npar	AIC
m3-a	1	125.96	5	-241.92
m3-b	1	132.97	5	-255.94
m3-ab	1	135.49	6	-258.98
m5-a	1	129.33	6	-246.66
m5-b	1	134.17	6	-256.34
m5-ab	1	137.04	7	-260.08

The chosen Hill model was **m5-ab**

BMD confidence interval

Sex	Lowest BMDL	Highest BMDU
1	13.665	26.896
2	20.614	40.947

DOG 90-DAY STUDY

DOSE	RELLIVER	SEX
0	2.23	M
0	3.034	M
0	3.001	M
0	2.93	M
0	2.799	M
15	2.415	M
15	2.809	M
15	2.802	M
15	3.03	M
15	2.639	M
90	2.882	M
90	3.763	M
90	3.982	M
90	3.214	M
90	3.341	M
180	3.344	M
180	3.717	M
180	3.227	M
180	3.488	M
180	3.078	M
0	2.955	F
0	3.11	F
0	3.438	F
0	3.384	F
0	2.67	F
15	2.371	F
15	3.194	F
15	3.133	F
15	2.479	F
15	2.948	F
90	2.882	F
90	3.45	F
90	3.351	F
90	2.959	F
90	3.289	F
180	3.368	F

180	3.685	F
180	3.945	F
180	4.016	F
180	3.363	F

DOSE	BWGAINRATIO	SEX
0	1.069	M
0	1.108	M
0	1.1	M
0	1.179	M
0	1.224	M
15	1.129	M
15	1.096	M
15	1.108	M
15	1.181	M
15	1.18	M
90	1.096	M
90	1.15	M
90	1.139	M
90	1.18	M
90	1.107	M
180	1.059	M
180	1.071	M
180	1.048	M
180	1.075	M
180	1.089	M
0	1.103	F
0	1.086	F
0	1.039	F
0	1.112	F
0	1.189	F
15	1.082	F
15	1.094	F
15	1.067	F
15	1.067	F
15	1.117	F
90	1.124	F
90	1.011	F
90	1.055	F
90	1.065	F
90	1.041	F
180	1.108	F
180	1	F
180	1.026	F

180	1.03	F
180	1.043	F

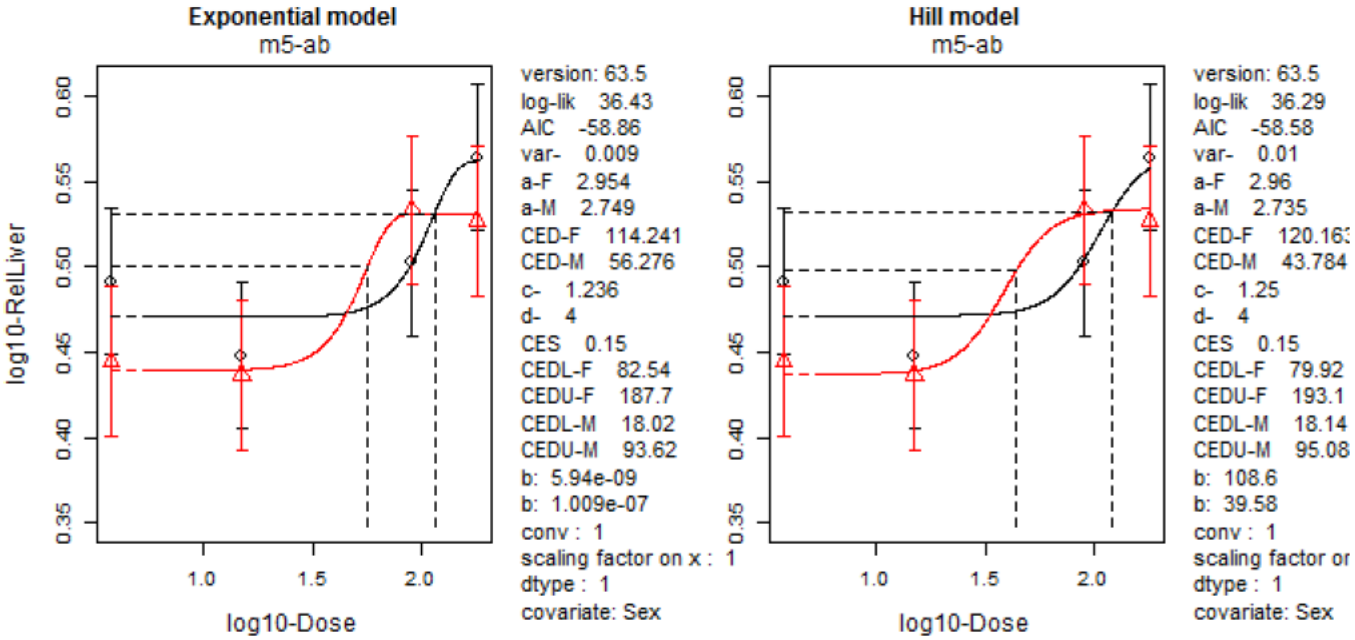
This report was generated by on `2017-03-06` using PROAST version `63.5` and `R version 3.3.2 (2016-10-31)`.

Dose
The dose variable was `'Dose'`.

Response
The response variable was `'RelLiver'`.

Covariate
The covariate was 'Sex' with levels 'F', 'M'.

Fitted Models



Exponential model

model	converged	loglik	npar	AIC
full	1	37.89	9	-57.78
full-v	1	37.90	10	-55.80
m1-	1	22.41	2	-40.82
m1-a	1	22.77	3	-39.54
m2-ab	1	33.21	5	-56.42
m3-	1	32.60	4	-57.20
m3-a	1	33.21	5	-56.42
m3-b	1	32.83	5	-55.66
m3-ab	1	33.22	6	-54.44
m5-a	1	34.47	6	-56.94
m5-b	1	34.42	6	-56.84
m5-ab	1	36.43	7	-58.86

The chosen exponential model was `m5-ab`.

Hill model

model	converged	loglik	npar	AIC

m3-	1	32.52	4	-57.04
m3-a	1	33.13	5	-56.26
m3-b	1	32.78	5	-55.56
m3-ab	1	33.13	6	-54.26
m5-a	1	34.47	6	-56.94
m5-b	1	34.23	6	-56.46
m5-ab	1	36.29	7	-58.58

The chosen Hill model was `m5-ab`.

Benchmark dose

	Covariate	Lowest BMDL	Highest BMDU
1	F	79.919	193.070
2	M	18.016	95.081

DOG ONE-YEAR STUDY

DOSE	SEX	GRP	LIVERREL	LIVER_SD	BW	BW SD	LYMP	LYMPH_SD	ALP	ALP_SD
0	M	5	2.469	0.204	15.6	0.791	3.95	0.87	0.86	0.23
10	M	5	2.689	0.309	15.74	0.73	3.26	0.13	1.23	0.63
30	M	5	2.742	0.393	16	0.889	3.21	0.3	1.14	0.53
150	M	5	3.294	0.255	14.7	1.098	2.81	0.38	4.01	1.47
0	F	5	2.733	0.637	12.64	0.792	3.69	1.02	1.17	0.39
10	F	5	2.564	0.358	12.74	1.281	3.55	0.61	1.09	0.18
30	F	5	3.237	0.793	11.98	1.003	3.35	0.8	1.56	0.74
150	F	5	3.583	0.346	11.24	1.09	2.67	0.69	4.19	3.52

This report was generated by on `2017-03-06` using PROAST version `63.5` and `R version 3.3.2 (2016-10-31)`.

Dose

The dose variable was `'Dose'`.

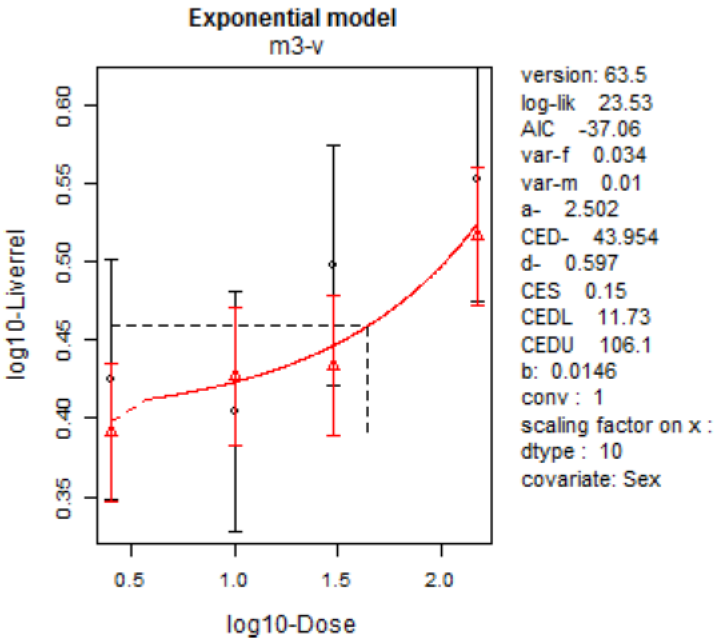
Response

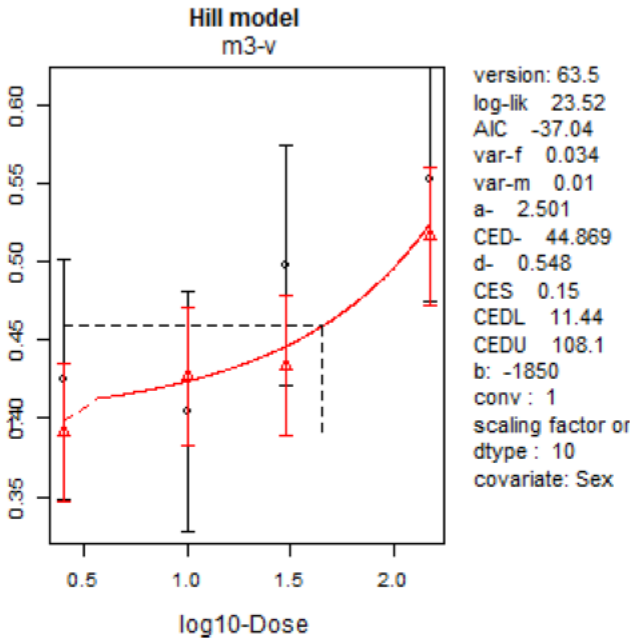
The response variable was `'Liverrel'`.

Covariate

The covariate was 'Sex' with levels 'f', 'm'.

Fitted Models





Exponential model

model	converged	loglik	npar	AIC
full	1	22.93	9	-27.86
full-v	1	25.85	10	-31.70
m1-v	1	12.28	3	-18.56
m1-av	1	12.89	4	-17.78
m2-ab	1	20.66	5	-31.32
m3-v	1	23.53	5	-37.06
m3-av	1	24.51	6	-37.02
m3-bv	1	24.35	6	-36.70
m3-abv	1	24.59	7	-35.18
m5-av	1	24.52	7	-35.04
m5-bv	1	24.70	7	-35.40
m5-abv	1	24.73	8	-33.46

The chosen exponential model was `m3-v`.

Hill model

model	converged	loglik	npar	AIC
m3-v	1	23.52	5	-37.04
m3-av	1	24.50	6	-37.00
m3-bv	1	24.31	6	-36.62
m3-abv	1	24.58	7	-35.16
m5-av	1	24.52	7	-35.04
m5-bv	1	24.68	7	-35.36
m5-abv	1	24.71	8	-33.42

The chosen Hill model was `m3-v`.

Benchmark dose

	Covariate	Lowest BMDL	Highest BMDU
1	All subgroups	11.436	108.08

RAT CHRONIC / CARCINOGENICITY STUDY

DOSE	UREA	SEX
0	3.53	M
0	3.8	M
0	4.08	M
0	4.19	M
0	4.62	M
0	4.01	M
0	3.7	M
0	4.91	M
0	3.7	M
0	3.96	M
5	4.27	M
5	4.91	M
5	4.11	M
5	3.73	M
5	4.3	M
5	4.56	M
5	4.91	M
5	4.77	M
5	3.49	M
5	4.34	M
31	3.95	M
31	4.71	M
31	4.72	M
31	5.52	M
31	4.38	M
31	4.4	M
31	4.47	M
31	4.85	M
31	5.46	M
31	4.72	M
191	5.45	M
191	4.97	M
191	5.66	M
191	4.98	M
191	6.34	M
191	4.87	M
191	4.75	M
191	4.78	M

191	4.9	M
191	4.82	M
0	5.95	F
0	5.47	F
0	6.75	F
0	5.64	F
0	6.24	F
0	6.83	F
0	6.05	F
0	7.03	F
0	5.22	F
0	5.01	F
7	6.74	F
7	4.39	F
7	5.73	F
7	7.25	F
7	5.36	F
7	5.9	F
7	5.73	F
7	7.46	F
7	5.36	F
7	6.21	F
7	4.86	F
41	5.23	F
41	5.35	F
41	5.91	F
41	5.68	F
41	7.52	F
41	6.57	F
41	4.83	F
41	5.56	F
41	5.73	F
300	7.81	F
300	6.82	F
300	6.96	F
300	6.51	F
300	9.21	F
300	6.28	F
300	5.69	F
300	5.54	F
300	6.63	F
300	6.31	F

This report was generated by on `2017-03-06` using PROAST version `63.5` and `R version 3.3.2 (2016-10-31)`.

Dose

The dose variable was `'Dose'`.

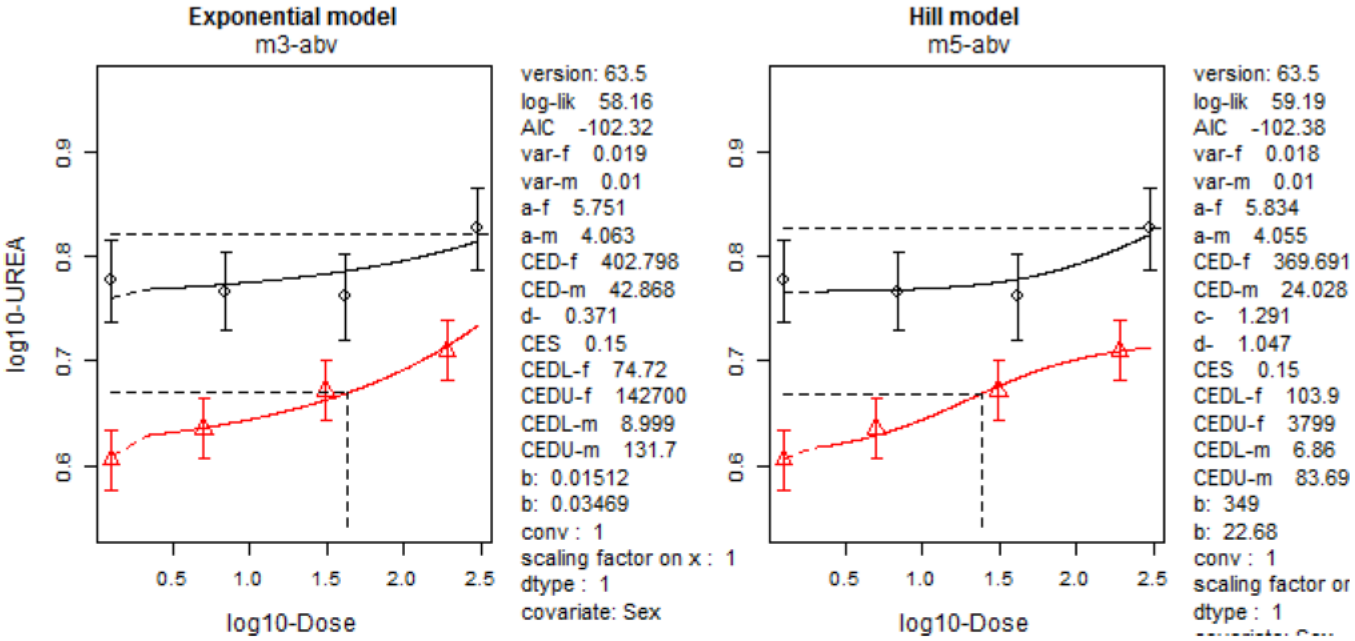
Response

The response variable was `'UREA'`.

Covariate

The covariate was 'Sex' with levels 'f', 'm'.

Fitted Models



Exponential model

model	converged	loglik	npar	AIC
full	1	57.98	9	-97.96
full-v	1	59.94	10	-99.88
m1-v	1	15.05	3	-24.10
m1-av	1	43.95	4	-79.90
m2-ab	1	54.86	5	-99.72
m3-v	1	25.95	5	-41.90
m3-av	1	56.04	6	-100.08
m3-abv	1	58.16	7	-102.32
m5-av	1	56.21	7	-98.42
m5-abv	1	59.14	8	-102.28

The chosen exponential model was `m3-abv`.

Hill model

model	converged	loglik	npar	AIC
m3-av	1	56.01	6	-100.02
m3-abv	1	58.08	7	-102.16
m5-av	1	56.18	7	-98.36
m5-abv	1	59.19	8	-102.38

The chosen Hill model was `m5-abv`.

Benchmark dose

	Covariate	Lowest BMDL	Highest BMDU
1	f	74.7200	142730.00
2	m	6.8598	

MOUSE 18-MONTH CHRONIC STUDY

DOSE	RELLIVERWEIGHT	SEX
0	3.51	M
0	3.7	M
0	4.32	M
0	3.56	M
0	3.64	M
0	3.58	M
0	3.47	M
0	3.29	M
0	3.7	M
0	3.4	M
0	3.9	M
0	3.48	M
0	3.47	M
0	3.24	M
0	3.6	M
0	3.57	M
0	3.53	M
0	3.45	M
0	3.91	M
0	3.91	M
0	8.49	M
0	3.43	M
0	3.07	M
0	3.4	M
0	3.75	M
0	3.94	M
0	4.15	M
0	3.43	M
0	3.85	M
0	3.61	M
0	3.88	M
0	3.76	M
0	3.6	M
0	3.32	M
0	3.04	M
0	3.26	M

0	3.5	M
0	4.25	M
0	3.4	M
0	3.84	M
0	3.55	M
0	5.18	M
0	3.62	M
0	3.69	M
0	3.41	M
0	3.79	M
0	4.18	M
0	3.24	M
0	2.95	M
3.5	4.56	M
3.5	4.24	M
3.5	3.97	M
3.5	4.21	M
3.5	4.18	M
3.5	3.77	M
3.5	3.72	M
3.5	4	M
3.5	4.38	M
3.5	5.22	M
3.5	4.13	M
3.5	3.59	M
3.5	4.07	M
3.5	4.08	M
3.5	3.84	M
3.5	4.01	M
3.5	3.77	M
3.5	4.3	M
3.5	5.19	M
3.5	3.61	M
3.5	4.08	M
3.5	3.97	M
3.5	3.81	M
3.5	7.27	M
3.5	4.26	M
3.5	4.5	M
3.5	4.09	M
3.5	4.29	M
3.5	3.63	M
3.5	3.49	M
3.5	3.46	M

3.5	3.89	M
3.5	4.15	M
3.5	3.74	M
3.5	3.86	M
3.5	5.26	M
3.5	3.83	M
3.5	6.84	M
3.5	4.02	M
3.5	3.99	M
3.5	3.85	M
3.5	4	M
3.5	3.6	M
3.5	4.01	M
3.5	4.03	M
3.5	4.29	M
3.5	3.94	M
3.5	4.21	M
9.1	4.61	M
9.1	4.25	M
9.1	4.6	M
9.1	4.15	M
9.1	4.5	M
9.1	3.89	M
9.1	3.95	M
9.1	4.45	M
9.1	4.42	M
9.1	4.43	M
9.1	4.27	M
9.1	5.11	M
9.1	4.51	M
9.1	4.05	M
9.1	4.69	M
9.1	4.72	M
9.1	4.28	M
9.1	4.98	M
9.1	4.09	M
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9.1	4.24	M
9.1	4.53	M
9.1	4.32	M
9.1	4.04	M
9.1	4.63	M
9.1	4.09	M

9.1	4.28	M
9.1	4.92	M
9.1	4.45	M
9.1	4.39	M
9.1	4.39	M
9.1	4.5	M
9.1	3.98	M
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9.1	4.61	M
9.1	4.9	M
9.1	4.57	M
9.1	4.6	M
9.1	4.7	M
9.1	4.51	M
9.1	4.42	M
9.1	4.14	M
9.1	4.23	M
9.1	4.31	M
9.1	4.06	M
9.1	4.43	M
9.1	4.44	M
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36	5.68	M
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36	5.5	M
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36	5.03	M
36	5.16	M
36	5.08	M
36	4.98	M
36	5.4	M
36	4.95	M
36	5.47	M

36	4.76	M
36	5.17	M
36	6.33	M
36	5.35	M
36	5.07	M
36	4.9	M
36	4.95	M
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36	5.47	M
36	4.97	M
36	4.75	M
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36	5.05	M
36	4.12	M
36	5.23	M
36	5.74	M
36	4.49	M
36	5.39	M
36	5.29	M
36	5.82	M
36	6.4	M
36	4.49	M
36	5.03	M
36	4.82	M
0	3.85	F
0	3.69	F
0	3.95	F
0	3.64	F
0	3.35	F
0	4.23	F
0	4.84	F
0	4.09	F
0	3.77	F
0	3.79	F
0	3.81	F
0	3.55	F
0	3.59	F
0	3.96	F
0	4.25	F

0	3.6	F
0	5.2	F
0	3.34	F
0	3.62	F
0	3.51	F
0	3.89	F
0	3.33	F
0	3.31	F
0	3.81	F
0	3.41	F
0	10.68	F
0	3.61	F
0	4.23	F
0	3.65	F
0	4.12	F
0	4.52	F
0	5.78	F
0	3.71	F
0	3.35	F
0	3.48	F
0	3.26	F
0	5.28	F
0	6.23	F
0	5.14	F
0	4.58	F
0	3.57	F
0	3.54	F
0	4.75	F
0	4.69	F
0	4.34	F
0	4.08	F
4.9	6.8	F
4.9	4.69	F
4.9	3.73	F
4.9	3.93	F
4.9	3.91	F
4.9	5.26	F
4.9	4.66	F
4.9	5.89	F
4.9	3.91	F
4.9	5.52	F
4.9	3.72	F
4.9	3.86	F
4.9	3.37	F

4.9	4.43	F
4.9	5.01	F
4.9	3.98	F
4.9	4.35	F
4.9	3.82	F
4.9	3.77	F
4.9	5.12	F
4.9	3.15	F
4.9	4.34	F
4.9	4.05	F
4.9	4.73	F
4.9	4.22	F
4.9	4.44	F
4.9	5.01	F
4.9	4.38	F
4.9	4.66	F
4.9	3.64	F
4.9	3.69	F
4.9	4.01	F
4.9	4.08	F
4.9	4.34	F
4.9	4.61	F
4.9	5.12	F
4.9	4.07	F
4.9	3.99	F
4.9	3.81	F
4.9	4.78	F
4.9	6.35	F
4.9	3.75	F
4.9	3.89	F
4.9	4.09	F
4.9	4.69	F
4.9	4.45	F
12.6	5.83	F
12.6	4.39	F
12.6	4.72	F
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12.6	4.28	F

12.6	4.48	F
12.6	5.39	F
12.6	4.85	F
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12.6	4.31	F
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12.6	4.11	F
12.6	3.88	F
12.6	5.5	F
12.6	4.43	F
12.6	4.22	F
12.6	4.63	F
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12.6	4.92	F
12.6	3.91	F
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61.5	6.55	F
61.5	6.3	F
61.5	6.33	F
61.5	3.8	F
61.5	6.33	F
61.5	6.54	F
61.5	6.9	F
61.5	7.35	F
61.5	6.34	F
61.5	6.51	F
61.5	6.04	F
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61.5	6.53	F
61.5	7.51	F

61.5	6.31	F
61.5	6.37	F
61.5	7	F
61.5	6.61	F
61.5	6.83	F
61.5	6.96	F
61.5	7.07	F
61.5	5.92	F
61.5	6.71	F
61.5	5.14	F
61.5	7.39	F
61.5	5.52	F
61.5	7.82	F
61.5	7.73	F
61.5	5.9	F
61.5	3.97	F
61.5	6.69	F
61.5	6.53	F
61.5	6.14	F
61.5	6.78	F
61.5	6.76	F
61.5	7.96	F
61.5	6.47	F
61.5	6.36	F
61.5	6.97	F
61.5	8.08	F
61.5	6.58	F

This report was generated by on `2017-03-06` using PROAST version `63.5` and `R version 3.3.2 (2016-10-31)`.

Dose

The dose variable was `'Dose'`.

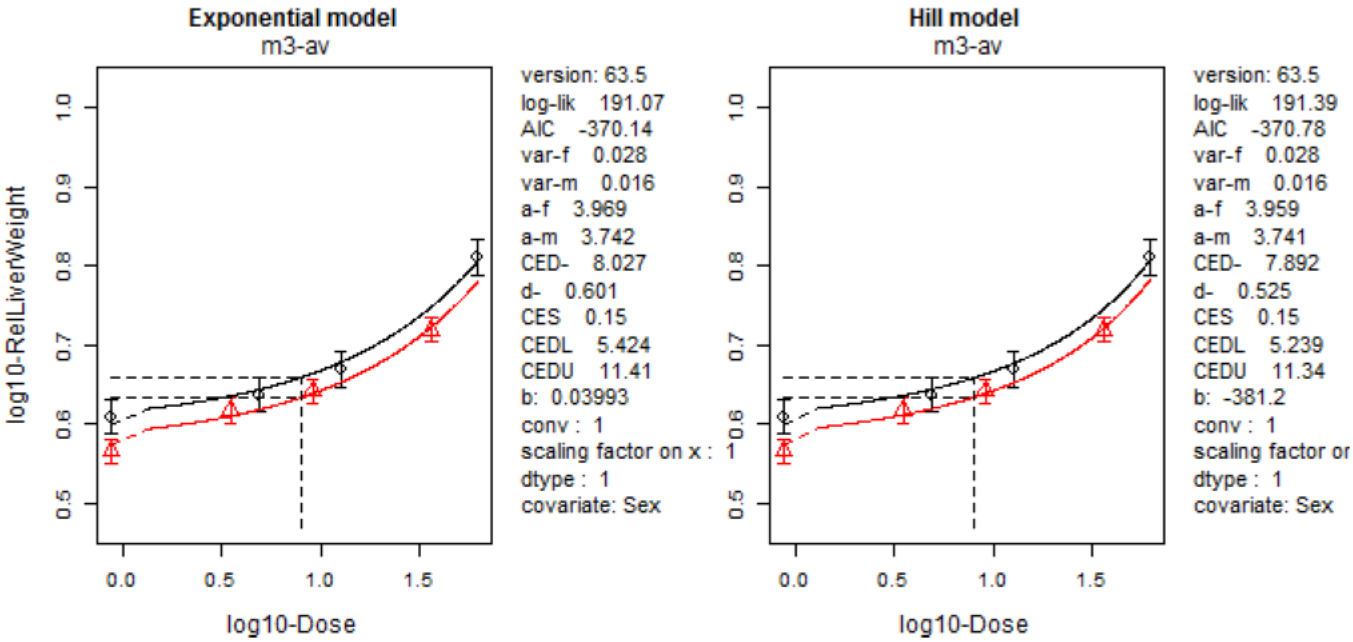
Response

The response variable was `'RelLiverWeight'`.

Covariate

The covariate was 'Sex' with levels 'f', 'm'.

Fitted Models



Exponential model

model	converged	loglik	npar	AIC
full	1	185.63	9	-353.26
full-v	1	193.30	10	-366.60
m1-v	1	48.42	3	-90.84
m1-av	1	57.43	4	-106.86
m2-ab	1	177.68	5	-345.36
m3-v	1	184.46	5	-358.92
m3-av	1	191.07	6	-370.14
m3-abv	1	191.07	7	-368.14
m5-av	1	190.66	7	-367.32
m5-abv	0	190.67	8	-365.34

The chosen exponential model was `m3-av`.

Hill model

model	converged	loglik	npar	AIC
m3-av	1	191.39	6	-370.78
m3-abv	1	191.41	7	-368.82
m5-av	1	190.66	7	-367.32
m5-abv	1	190.67	8	-365.34

The chosen Hill model was `m3-av`.

Benchmark dose

	Covariate	Lowest BMDL	Highest BMDU
1	All subgroups	5.2389	11.4

RAT TWO-GENERATION STUDY

Dose	Sex	Grpsize	Liver	Liver_sd	BW	BW_sd	BWG	BWsd	ALP	ALP_sd	pupbw7	Pupbw7	pupbwg	Pupbwg

												sd		sd
0	m0	25	2.303	0.131					1.23	0.25	16.6	1.5	47	3.2
25	m0	25	2.342	0.113					1.45	0.27	16.9	1.6	46.2	3.6
75	m0	25	2.417	0.123					2.03	0.29	16.7	1.7	47.2	3.8
200	m0	25	2.567	0.095					2.26	0.31	14.7	2.1	42.2	3.9
0	f0	25	3.198	0.395	328.8	26.3	112.1	15.4	0.77	0.17	16.1	1.6	45.7	3.2
25	f0	25	3.197	0.321	322.1	21	103.5	13.7	0.74	0.15	16.3	1.6	44.3	3.5
75	f0	25	3.595	0.614	326.7	21.6	107.3	10.6	0.96	0.26	16.1	1.7	45.6	3.8
200	f0	25	3.772	0.433	299.6	21.2	95.41	14.1	1.15	0.31	14.1	2	40.7	3.9
0	m1	25	2.387	0.16					1.24	0.23	16.9	1.5	47.7	3.2
25	m1	25	2.465	0.154					1.55	0.44	16.6	1.2	44.5	3.4
75	m1	25	2.521	0.156					1.95	0.51	16.2	1.1	44.8	3
200	m1	25	2.689	0.169					2.36	0.82	15.2	1.2	40.6	3.5
0	f1	25	3.317	0.247	329.4	28.3	107.6	17.6	0.89	0.18	16.3	1.3	45.9	2.6
25	f1	25	3.303	0.322	312.1	25.6	98.64	13.4	0.86	0.23	15.9	1.2	42.6	3.1
75	f1	25	3.509	0.279	317.4	27.4	103	14.4	0.84	0.13	15.7	0.9	43.4	2.9
200	f1	25	3.633	0.561	277.7	25.6	79.83	17.3	1.26	0.31	14.7	1.2	38.8	3.3

Analysis name: 2gen ALP
This report was generated by Anonymous on 12/20/2016 1:38:41 PM (CET). PROAST version 62.10_0.11

Input values

Type of response data
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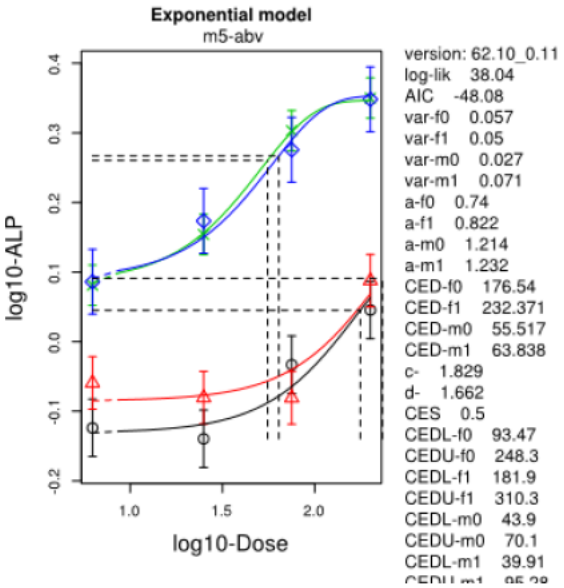
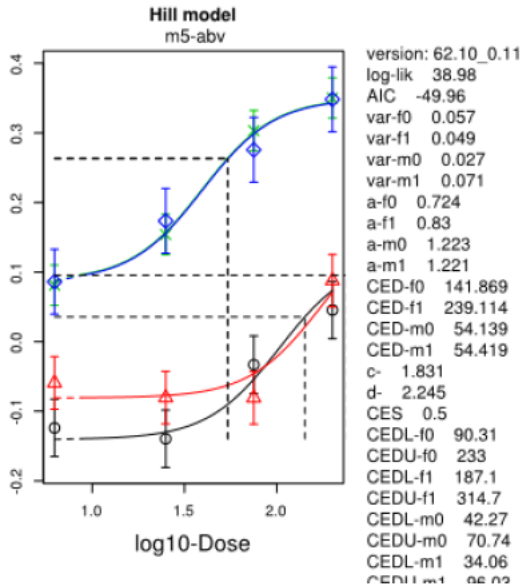
Dose column
    Dose
Response column
    ALP
Litter effect
    No
Dispersion measure column
    ALP sd
Relating to
    Standard deviation
Group size column
    Grpsize
Covariate column
    Sex
Benchmark response
    0.5
AIC criterion
    2

Fitted Models





Exponential Model

model	converged	loglik	npar	AIC
full	1	33.51	17	-33.02
full-v	1	44.69	20	-49.38
m1-v	1	-226.25	5	462.50
m1-av	1	-87.20	8	190.40
m2-ab	1	7.05	9	3.90
m3-av	1	12.89	10	-5.78
m3-bv	0	-39.00	10	98.00
m3-abv	1	21.26	13	-16.52
m5-av	1	16.73	11	-11.46
m5-bv	1	-21.13	11	64.26
m5-abv	1	38.04	14	-48.08

The chosen Exponential model was m5-abv

Hill Model

model	converged	loglik	npar	AIC
m3-av	1	12.13	10	-4.26
m3-bv	0	-39.88	10	99.76
m3-abv	1	18.77	13	-11.54
m5-av	1	16.73	11	-11.46
m5-bv	1	-21.13	11	64.26
m5-abv	1	38.98	14	-49.96

The chosen Hill model was m5-abv

BMD confidence interval

Sex	Lowest BMDL	Highest BMDU
f0	90.314	248.290
f1	181.920	314.690
m0	42.266	70.738
m1	34.062	96.032

ANALYSIS NAME: 2GEN PUP BWG
THIS REPORT WAS GENERATED BY ANONYMOUS ON 12/20/2016 3:36:55 PM (CET). PROAST VERSION 62.10_0.11

INPUT VALUES
TYPE OF RESPONSE DATA
CONTINUOUS, SUMMARY DATA
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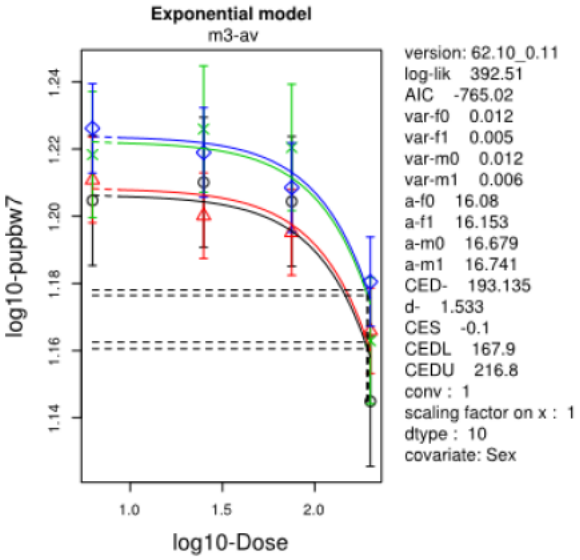
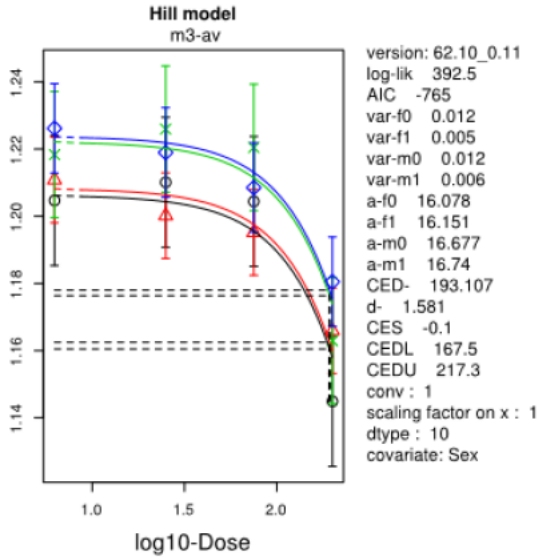
DOSE
RESPONSE COLUMN
PUPBWG
LITTER EFFECT
NO
DISPERSION MEASURE COLUMN
PUPBWG SD
RELATING TO
STANDARD DEVIATION
GROUP SIZE COLUMN
GRPSIZE
COVARIATE COLUMN
SEX
BENCHMARK RESPONSE
0.1
AIC CRITERION
2

FITTED MODELS





EXPONENTIAL MODEL

MODEL	CONVERGED	LOGLIK	NPAR	AIC
FULL	1	465.56	17	-897.12
FULL-V	1	466.93	20	-893.86
M1-	1	370.89	2	-737.78
M1-A	1	383.90	5	-757.80
M2-AB	1	450.62	9	-883.24
M3-A	1	450.77	7	-887.54
M3-B	1	444.34	7	-874.68
M3-AB	1	451.47	10	-882.94
M5-A	1	450.77	8	-885.54
M5-B	1	444.34	8	-872.68
M5-AB	1	451.47	11	-880.94

THE CHOSEN EXPONENTIAL MODEL WAS M3-A

HILL MODEL

MODEL	CONVERGED	LOGLIK	NPAR	AIC
M3-A	1	450.75	7	-887.50
M3-B	1	444.29	7	-874.58
M3-AB	1	451.44	10	-882.88
M5-A	1	450.75	8	-885.50
M5-B	1	444.29	8	-872.58
M5-AB	1	451.44	11	-880.88

THE CHOSEN HILL MODEL WAS M3-A

BMD CONFIDENCE INTERVAL

SEX	LOWEST BMDL	HIGHEST BMDU
F0	163.15	202.86

Sex	Lowest BMDL	Highest BMDU
F1	163.15	202.86
M0	163.15	202.86
M1	163.15	202.86

RAT DEVELOPMENTAL TOXICITY STUDY

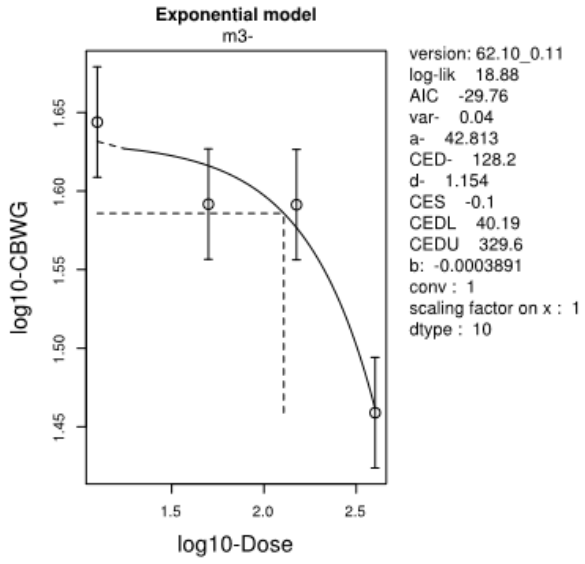
DOSE	GRP	CARCASS	CARCASS_SD	CBWG	CBWG_SD
0	24	243.2	15.03	44.4	5.63
50	24	236.4	17.17	40.1	9.33
150	24	242.7	20.15	40.1	9.49
400	24	226.8	10.52	29.3	5.64

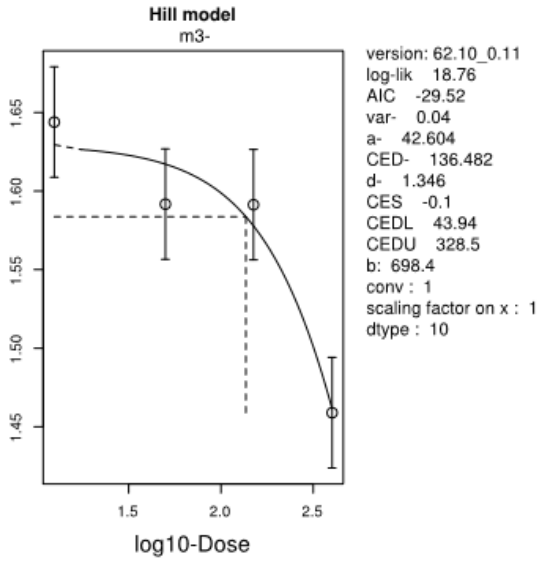
This report was generated by Anonymous on 12/20/2016 1:50:18 PM (CET). PROAST version 62.10_0.11

Input values

Type of response data
Continuous, summary data
Dose column
Dose
Response column
CBWG
Litter effect
No
Dispersion measure column
CBWG sd
Relating to
Standard deviation
Group size column
Grp
Covariate column
Benchmark response
0.1
AIC criterion
2

Fitted Models





Exponential Model

model	converged	loglik	npar	AIC
full	1	20.47	5	-30.94
m1-	1	-3.53	2	11.06
m2	1	18.83	3	-31.66
m3-	1	18.88	4	-29.76
m5-	1	18.88	5	-27.76

The chosen Exponential model was m3-

Hill Model

model	converged	loglik	npar	AIC
m3-	1	18.76	4	-29.52
m5-	1	18.76	5	-27.52

The chosen Hill model was m3-

BMD confidence interval

Lowest BMDL	Highest BMDU
40.187	329.57

RAT NEUROTOXICITY STUDY

DOSE	SEX	GRP	GAIT	MOTOR	MOTOR_SD	FORELIMB	FORELIMB_SD	FOOT_SPLAY	FOOTSPLAY_SD
0	M	10	0	3019	807	5.1	0.8	9.3	1
200	M	10	0	2990	505	6.2	0.8	10	1.4
600	M	10	0	2454	533	4.9	1.5	11.6	1.6
2000	M	10	5	1436	446	4	0.8	12.5	1.1
0	F	10	0	4510	784	6.4	0.7	10	1
200	F	10	0	4240	1360	6.5	0.9	10.2	1.5
600	F	10	0	3480	523	6.5	1	10.9	2.2
2000	F	10	3	2233	639	6.8	0.8	12	1.7

This report was generated by Anonymous on 12/20/2016 1:52:15 PM (CET). PROAST version 62.10_0.11

Input values

Type of response data

Continuous, summary data

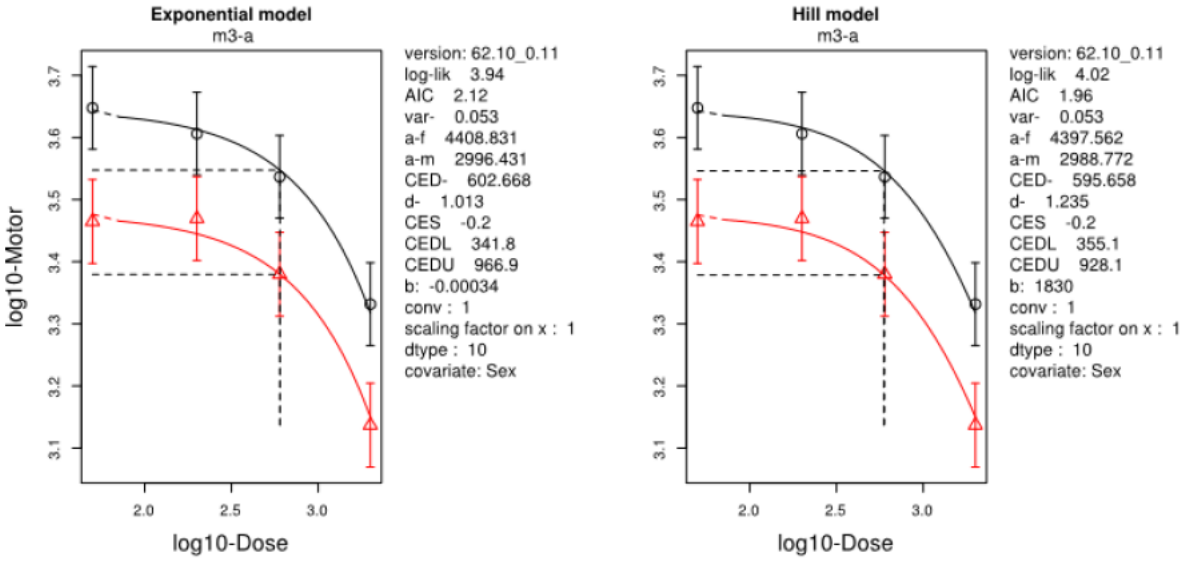
Dose column

Dose

Response column

Motor
Litter effect
No
Dispersion measure column
motor sd
Relating to
Standard deviation
Group size column
Grp
Covariate column
Sex
Benchmark response
0.2
AIC criterion
2

Fitted Models



Exponential Model

model	converged	loglik	npar	AIC
full	1	4.59	9	8.82
full-v	1	4.59	10	10.82
m1-	1	-44.25	2	92.50
m1-a	1	-34.78	3	75.56
m2-ab	1	4.11	5	1.78
m3-a	1	3.94	5	2.12
m3-b	1	-6.90	5	23.80
m3-ab	1	4.11	6	3.78
m5-a	1	4.07	6	3.86
m5-b	1	-5.41	6	22.82
m5-ab	1	4.16	7	5.68

The chosen Exponential model was m3-a

Hill Model

model	converged	loglik	npar	AIC
m3-a	1	4.02	5	1.96
m3-b	1	-6.16	5	22.32
m3-ab	1	4.17	6	3.66
m5-a	1	4.07	6	3.86
m5-b	1	-5.34	6	22.68
m5-ab	1	4.18	7	5.64

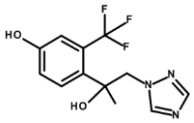
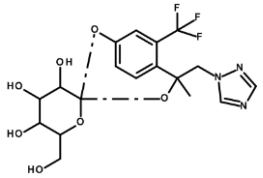
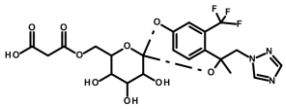
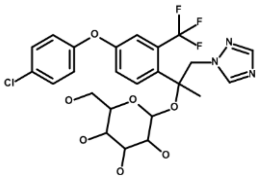
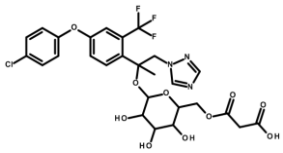
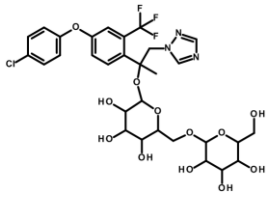
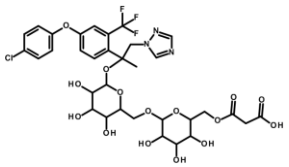
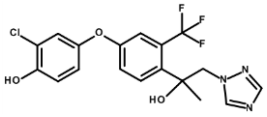
The chosen Hill model was m3-a

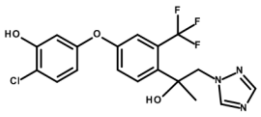
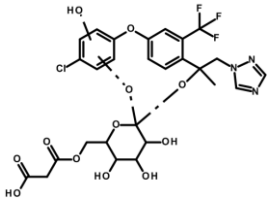
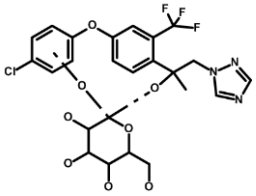
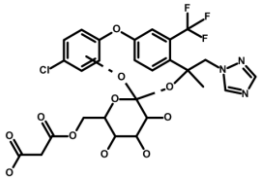
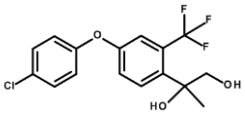
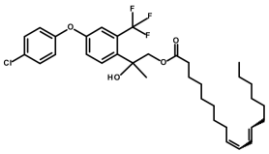
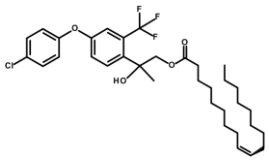
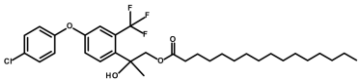
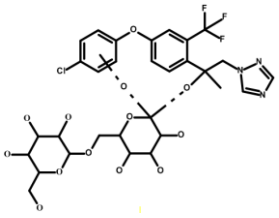
BMD confidence interval

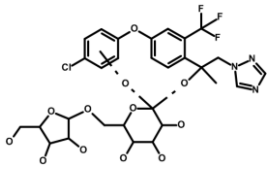
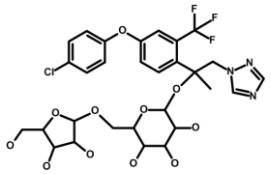
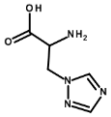
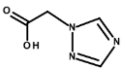
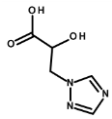
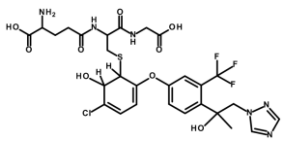
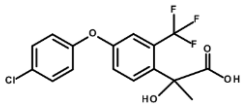
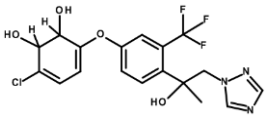
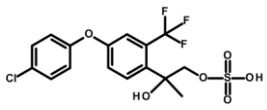
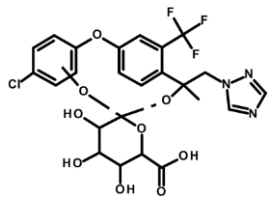
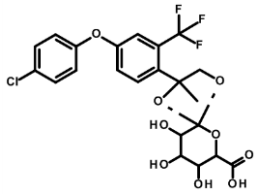
Sex	Lowest BMDL	Highest BMDU
f	341.81	966.9
m	341.81	966.9

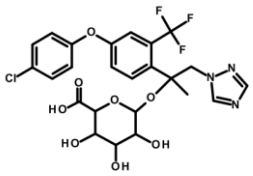
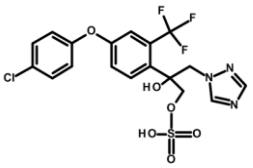
B.6.13. ANNEX III: SUMMARY OF TOXICOLOGICAL RELEVANCE ASSESSMENT OF METABOLITES

Table B6.13.1: BAS 750 F metabolites in food of plant or animal origin

Code	Molecular structure	Conjugation type	Livestock / Plant	≥ 10% TRR (Y / N)	rat metabolism coverage [% AD]
M750F001 (1,2,4-T)		Non-conjugated	Plant	N	up to 20%
			Livestock	Y	
M750F003		Non-conjugated	Livestock	N	> 10%
M750F009		Sugar	Plant	N	See M750F003
M750F010		Sugar	Plant	N	See M750F003
M750F011		Sugar	Plant	N	See BAS 750 F
M750F012		Sugar	Plant	N	See BAS 750 F
M750F013		Sugar	Plant	N	See BAS 750 F
M750F014		Sugar	Plant	N	See BAS 750 F
M750F015		Non-conjugated	Livestock	N	>50%

M750F016		Non-conjugated	Livestock	Y	>50%
M750F018		Sugar	Plant	Y	See M750F016
M750F019		Sugar	Plant	Y	See M750F016
M750F020		Sugar	Plant	Y	See M750F016
M750F022		Non-conjugated	Livestock	Y	<<10%
M750F023		Fatty acid	Livestock	Y	See M750F022
M750F024		Fatty acid	Livestock	Y	See M750F022
M750F025		Fatty acid	Livestock	Y	See M750F022
M750F026		Sugar	Plant	Y	See M750F016

M750F027		Sugar	Plant	Y	See M750F016
M750F028		Sugar	Plant	N	See BAS 750 F
M750F029 (TA)		Non-conjugated	Plant	Y	Not found
M750F030 (TAA)		Non-conjugated	Plant	Y	Not found
M750F031 (TLA)		Non-conjugated	Plant	N	Not found
M750F034		Glutathione	Livestock	Y	See M750F016
M750F038		Non-conjugated	Livestock	Y	See M750F022
M750F041		Non-conjugated	Livestock	N	At least 12%
M750F043		Sulfate	Livestock	Y	See M750F022
M750F063		Glucuronide	Livestock	N	See M750F016
M750F064		Glucuronide	Livestock	Y	See M750F022

M750F068		Glucuronide	Livestock	Y	See BAS 750 F
M750F072		Sulfate	Livestock	N	See BAS 750 F

B.6.14. ANNEX IV: PHOTOGRAPHIC DOCUMENTATION OF SKELETAL VARIATION (FUSED STERNEBRAE; UNCHANGED CARTILAGE) IN RABBIT DEVELOPMENTAL TOXICITY STUDY