Renewal Assessment Report

Dimethenamid-P

Volume 3 – B.9 Ecotoxicology data

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Rapporteur Member State: Germany

Co-Rapporteur Member State: Bulgaria

Version history

When	What
10 August 2016	First version submitted to EFSA

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B Summary of the data and information

B.9 Ecotoxicology data

B.9.1 Effects on birds and other terrestrial vertebrates

B.9.1.1 Effects on birds

Studies were already submitted and accepted in the Monograph of dimethenamid-P, prepared in the context of the inclusion of the active substance in Annex I of the Council Directive 91/414/EEC, 1999 and the EU Review Report Dimethenamid-P, July 2003, (SANCO/1402/2001). They are still valid for AIR 3 according to EFSA/2009/1438.

No new studies were required or submitted for the renewal assessment. To increase the transparency and comprehensibility of the overall assessment, summaries of the studies assessed with the initial evaluation of dimethenamid-P have been added by the RMS.

B.9.1.1.1 Acute oral toxicity to birds

KCA 8.1.1.1/1 [redacted], 1996 (study evaluated in the initial monograph, 2000)

Author:	[redacted].
Title:	SAN 1289H technical: an acute oral toxicity study with the northern bobwhite
Date:	03.06.1996
Doc ID:	131-187; AVS1999-58; BASF RegDoc #1996/5419
Guidelines:	EPA 850.2100, 71-1
GLP:	Yes
Validity:	Acceptable

Material and Methods

The acute oral toxicity of technical dimethenamid-P (91.1 % purity) was tested in bobwhite quail according to EPA guideline 71-1. The test material was applied by intubation at dose levels of 0/292/486/810/1350/2250 mg/kg body weight. Five males and five females, 36 weeks old, were treated per dose level.

Results and Discussion

Signs of intoxication were observed at dose levels of 486 mg/kg and higher; the lowest lethal dose was 810 mg/kg (2 out of 10 birds); body weight was affected at all treatment levels; food consumptions was reduced at dose levels of 486 mg/kg and higher.

Conclusion

The study is acceptable. LD_{50} = 1068 (845-1356) mg/kg bw; NOED < 292 mg/kg bw (sublethal effects).

KCA 8.1.1.1/2 [redacted], 1988a (study evaluated in the initial monograph, 2000)

Author:	[redacted]
Title:	SAN 582 H an acute oral toxicity study with the bobwhite
Date:	01.08.1988

Doc ID: 131-124A; AVS9600045; BASF RegDoc # 1988/11373
Guidelines: EPA 850.2100, 71-1
GLP: Yes
Validity: Acceptable, but test reported only as additional information

Summary from the initial dossier

Material and Methods

The acute oral toxicity of technical dimethenamid (91.4 % purity) was tested in bobwhite quail according to EPA guideline 71-1. The test material was applied by gelatine capsule at dose levels of 0/292/486/810/1350/2250 mg/kg body weight. Five males and five females, 21 weeks old, were treated per dose level.

Results and Discussion

Signs of intoxication, reduced body weight and reduced feed consumption were observed at all treatment levels; the lowest lethal dose was 1350 mg/kg (3 out of 10 birds).

Conclusion

The study is acceptable. LD_{50} = 1908 (1486-3229) mg/kg bw; NOED < 292 mg/kg bw. The test was performed with the racemate of dimethenamid. As there is also a study with the active substance available which in addition shows a lower LD_{50}, the results will not be considered further in the risk assessment.

B.9.1.1.2 Short-term dietary toxicity to birds

No new studies were required or submitted for the renewal assessment. According to current guidance, a specific risk assessment for short-term risks from dietary uptake is not triggered for dimethenamid-P, since there are no indications for delayed action or accumulation of the compound leading to mortality on a short-term time scale.
Therefore, only the study assessment from the initial dimethenamid-P monograph is quoted below for information, but no new evaluation was performed.

KCA 8.1.1.2/1 [redacted] 1996a (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: SAN 1289H Technical: A dietary LC_{50} study with the mallard
Date: 30.07.1996
Doc ID: 131-186; AVS1999-61; BASF RegDoc#1996/5410
Guidelines: OECD 205
GLP: Yes
Validity: Acceptable, but test reported only as additional information

Material and Methods

The 5-day-dietary toxicity of technical dimethenamid-P (91.1 % purity) was tested in mallard duck according to OECD guideline 205 and EPA guideline 71-2. The test material was mixed into the feed with corn oil and acetone at nominal concentrations of 0/0/0/562/1000/1780/3160/5620 ppm. Analysis of the feed showed deviations from nominal concentrations being -4 to -1 %; homogeneity and stability were proven to be sufficient. At each concentration level a group of ten 10-day-old birds of undetermined sex was used.

Results and Discussion

No mortalities or signs of intoxication were observed. Body weight was affected at concentrations of 3160 ppm and above; feed consumption was reduced at 5620 ppm.

Conclusion

The study is acceptable (LC_{50} > 5620 ppm; NOEC = 1780 ppm). Since there are no indications for delayed action or accumulation of the compound leading to mortality on a short-term time scale, a specific risk assessment for short term risks from dietary uptake is not triggered for dimethenamid-P. Hence, the results will not be considered further in the risk assessment.

KCA 8.1.1.2/2 [redacted], 1996b (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: SAN 1289H Technical: A dietary LC_{50} study with the northern bobwhite
Date: 30.07.1996
Doc ID: 131-185; AVS1999-59; BASF RegDoc # BASF 1996/5412
Guidelines: OECD 205
GLP: Yes
Validity: Acceptable; but test reported only as additional information

Material and Methods

The 5-day-dietary toxicity of technical dimethenamid-P (91.1 % purity) was tested in bobwhite quail according to OECD guideline 205 and EPA guideline 71-2. The test material was mixed into the feed with corn oil and acetone at nominal concentrations of 0/0/0/562/1000/1780/3160/5620 ppm. Analysis of the feed showed deviations from nominal concentrations being -8 to +3 %; homogeneity and stability were proven to be sufficient. At each concentration level a group of ten 10-day-old birds of undetermined sex was used.

Results and Discussion

One mortality at 562 ppm occurred that obviously was not related to the treatment. Apart from that no mortalities or signs of intoxication were observed. Body weight was affected at concentrations of 3160 ppm and above.

Conclusion

The study is acceptable showing a LC_{50} > 5620 ppm (> 1737 mg/kg bw/d; daily dose calculated based on study data for food consumption and body weight) and a NOEC of 1780 ppm. Since there are no indications for delayed action or accumulation of the compound leading to mortality on a short-term time scale, a specific risk assessment for short-term risks from dietary uptake is not triggered for dimethenamid-P. Hence, the results will not be considered further in the risk assessment.

KCA 8.1.1.2/3 [redacted] (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: SAN 582H: A dietary LC_{50} study with bobwhite
Date: 09.06.1988
Doc ID: 131-122; AVS9600042; BASF RegDoc#1988/11370
Guidelines: EPA 850.2200, 71-2
GLP: Yes

Validity: Acceptable, but test reported only as additional information

Material and Methods

The 5-day-dietary toxicity of technical dimethenamid (91.4 % purity) was tested in bobwhite quail according to EPA guideline 71-2. The test material was mixed into the feed with corn oil at nominal concentrations of 0/0/0/562/1000/1780/3160/5620 ppm. Analysis of the feed showed deviations from nominal concentrations being -14 to +3 %; homogeneity and stability were proven to be sufficient. At each concentration level a group of ten 10-day-old birds of undetermined sex was used.

Results and Discussion

No mortalities or signs of intoxication were observed. Body weight was affected at concentrations of 3160 ppm and above.

Conclusions

The study is acceptable (LC_{50} > 5620 ppm; NOEC = 1780 ppm). The test was performed with the racemate of dimethenamid. There are also studies with the active substance available. This study with the racemate does not show lower LD_{50}. Since there are no indications for delayed action or accumulation of the compound leading to mortality on a short-term time scale, a specific risk assessment for short-term risks from dietary uptake is not triggered for dimethenamid-P. Hence, the results will not be considered further in the risk assessment.

KCA 8.1.1.2/4 [redacted] (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: SAN 582H: A dietary LC_{50} study with the mallard
Date: 09.06.1988
Doc ID: 131-123; AVS9600043; BASF RegDoc#1988/11369
Guidelines: EPA 850.2200, 71-2
GLP: Yes
Validity: Acceptable, test reported only as additional information

Material and Methods

The 5-day-dietary toxicity of technical dimethenamid (91.4 % purity) was tested in mallard duck according to EPA guideline 71-2. The test material was mixed into the feed with corn oil at nominal concentrations of 0/0/0/562/1000/1780/3160/5620 ppm. Analysis of the feed showed deviations from nominal concentrations being -17 to +7 %; homogeneity and stability were proven to be sufficient. At each concentration level a group of ten 10-day-old birds of undetermined sex was used.

Results and Discussion

No mortalities or signs of intoxication were observed. Body weight was affected at concentrations of 1000 ppm and above.

Conclusions

The study is acceptable (LC_{50} > 5620 ppm; NOEC 562 ppm). The test was performed with the racemate of dimethenamid. There are also studies with the active substance available. This study with the racemate does not show lower LD_{50}. Since there are no indications for delayed action or accumulation of the compound leading to mortality on a short-term time scale, a specific risk assessment for short-term risks from dietary uptake is not triggered for dimethenamid-P. Hence, the results will not be considered further in the risk assessment.

B.9.1.1.3 Sub-chronic toxicity and reproduction to birds

KCA 8.1.1.3/1 [redacted] (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: SAN 582H Technical: A reproduction study with the mallard
Date: 06.05.1994
Doc ID: 131-178; AVS9600047; BASF RegDoc # 1994/11899
Guidelines: OECD 206
GLP: Yes
Validity: Acceptable

Material and Methods

A one-generation reproduction study with mallard duck was conducted according to EPA guideline 71-4. Technical dimethenamid (97.0 % purity) was mixed into the feed without a solvent for an exposure period of 20 weeks at nominal concentrations of 0/360/900/1800 ppm. Analysis of the feed showed deviation from nominal concentration being -6 to +7 %; homogeneity and stability were proven to be sufficient. At each concentration level 16 pairs were tested. The birds were about 22 weeks old at the onset of exposure.

Results and Discussion

There were no treatment related mortalities at any concentration tested. There were no apparent treatment related effects and no significant differences in adult body weight, adult feed consumption, egg shell thickness or offspring body weights between control group and treatment groups. At all concentration levels the hatch rate was reduced (73 % compared to 85 % at the control). However, the differences were not statistically significant, there was no increase of the effect with increasing concentration, and the data at the treatment levels were within the normal range of historical controls; therefore the differences were considered as unrelated to treatment.

Table B.9.1-1: Effect of dimethenamid-racemate on the reproduction of mallard duck

Test concentration ppm	**0**	**360**	**900**	**1800**
number of eggs laid per female	45	45	46	47
% viable embryos of eggs set initially	90	97	83	85
% live 3-week embryos of viable embryos	99	99	99	98
% normal hatchlings of live 3-week embryos	85	73	73	73
% surviving of normal hatchlings	99	99	97	97
number of 14-day survivors per hen	31	27	25	24
egg shell thickness (mm)	0.398	0.407	0.405	0.399

Conclusion

The study is acceptable (NOEC = 1800 ppm). The test was performed with the racemate of dimethenamid. As there are no studies containing only the active substance available, the results will be used further in the risk assessment.

KCA 8.1.1.3/2 [redacted] (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: SAN 582H Technical: a reproduction study with the northern bobwhite
Date: 06.05.1994;
Doc ID: 131-177; AVS9600046; BASF report #1994/11900
Guidelines: OECD 206
GLP: Yes
Validity: Acceptable

Material and Methods

A one-generation reproduction study with bobwhite quail was conducted according to EPA guideline 71-4. Technical dimethenamid (97.0 % purity) was mixed into the feed without a solvent for an exposure period of 20 weeks at nominal concentrations of 0/360/900/1800 ppm. Analysis of the feed showed deviation from nominal concentration being -6 to +7 %; homogeneity and stability were proven to be sufficient. At each concentration level 16 pairs were tested. The birds were about 20 weeks old at the onset of exposure.

Results and Discussion

There were no treatment related mortalities at any concentration tested. One incidental mortality occurred in the 900 ppm as treatment group but no mortalities occurred in the control group or in the 360 or 1800 ppm as treatment group. There were no apparent treatment related effects and no significant differences in adult body weight, egg shell thickness or offspring body weights between control group ant treatment groups. At 360 ppm the adult feed consumption, the survival rate of the chicken and the chick weight at day 14 was reduced; however, these deviations were not considered related to treatment as these endpoints were not affected at higher concentrations. At 1800 ppm egg shell thickness was reduced.

Table B.9.1-2: Effect of dimethenamid on the reproduction of bobwhite quail

Test concentration [ppm]	**0**	**360**	**900**	**1000**
number of eggs laid per female	48	44	46	45
% viable embryos of eggs set initially	93	93	96	91
% live 3-week embryos of viable embryos	99	99	99	99
% normal hatchlings of live 3-week embryos	93	94	94	94
% surviving of normal hatchlings	84	70	82	80
number of 14-day survivors per hen	31	25	30	26
egg shell thickness (mm)	0.228	0.226	0.230	0.215

Conclusion

The study is acceptable (NOEC = 900 ppm). Recalculation of concentration in food to daily dose resulted in a value of NOED = 114 mg/kg bw/d for reproduction toxicity (bobwhite quail, 900 ppm group) based on the following parameters derived from the study: mean body weight = 206 g, mean food consumption = 26 g/d. The test was performed with the racemate of dimethenamid. As there are no studies containing only the active substance available, the results will be used further in the risk assessment.

B.9.1.2 Effects on terrestrial vertebrates other than birds

The risk assessment for terrestrial vertebrates, namely small mammals is based on the same toxicity data that is used for assessing the risk to humans – see chapter B.6 of this RAR.

B.9.1.2.1 Acute oral toxicity to mammals

No new studies were required or submitted for the renewal assessment.

B.9.1.2.2 Long-term and reproduction toxicity to mammals

No new studies were required or submitted for the renewal assessment.

B.9.1.3 Active substance bioconcentration in prey of birds and mammals

No studies submitted, not required.

B.9.1.4 Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No studies submitted.

B.9.1.5 Potential for endocrine disruption

No studies submitted, not required.

B.9.2 Effects on aquatic organisms

B.9.2.1 Acute toxicity to fish

KCA 8.2.1/1 (study evaluated in the initial monograph, 2000)

Author:	[redacted]
Title:	SAN 1289H Technical: A 96-hour flow-through acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*)
Date:	04.06.1996
Doc ID:	131A-163; BASF RegDoc# 96/5417
Guidelines:	EPA 850.1075, 72-1
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item: Dimethenamid-P (SAN 1289H Technical; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.

Test species: Rainbow trout (*Oncorhynchus mykiss*), juveniles, <4 months old at test start (fish were held 113 days prior to testing); body length 5.1 cm (4.2 - 6.0 cm); body weight 1.9 g (0.99 - 3.0 g).

Test design: Flow-through system (96 hours); 5 test item concentrations, a solvent control and a negative (water) control, 10 fish per aquarium (loading 1.3 g fish/L) and per concentration; 2 replicates per treatment and control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, solvent control (0.10 mL dimethylformamide/L), 1.3, 2.2, 3.6, 6.0 and 10.0 mg dimethenamid-P/L (nominal); corresponding to mean measured concentrations of 0, 0, 1.4, 2.3, 3.7, 6.5 and 11.0 mg as/L.

Test conditions: 25-L Teflon-lined polyethylene aquaria, test volume: 15 L in a temperature-controlled water bath; temperature: 12±1 °C; pH 8.2 - 8.3; dissolved oxygen concentration exceeded 81 % of saturation throughout the test; photoperiod:

16 h light : 8 h dark; light intensity: approx. 420 lux at test initiation; flow-rate: approx. 6 volume additions/day; no feeding.

Analytics: Analytical verification of the test item was conducted using gas chromatography with electron capture detection (GC-ECD).

Statistics: Descriptive statistics; binomial method for calculation of the LC_{50}; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration (measured as total dimethenamid) was conducted in each concentration at the beginning of the test, after approximately 48 hours and, except for the highest concentration at the end of the test. The analysed contents of dimethenamid-P ranged from 103 % to 112 % of nominal at test initiation and from 99 % to 114 % of nominal at test termination.

Biological results: Rainbow trouts in the negative control, solvent control, 1.4, 2.3, 3.7 mg/L treatment group appeared normal and healthy throughout the test. After 96 hours of exposure, mortality in the 6.5 and 11 mg SAN 1289H Technical/L (total dimethenamid-P) treatments was 55 % and 100 %, respectively. The LC_{50} value with 95 % confidence limits at 96 hours was calculated from the mortality data and the results are shown in Table B.9.2-1.

Table B.9.2-1: Acute toxicity (96 h) of dimethenamid-P on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**1.3**	**2.2**	**3.6**	**6.0**	**10.0**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**1.4**	**2.3**	**3.7**	**6.5**	**11.0**
Mortality [%]	0	0	0	0	0	55	100
Symptoms	none	none	none	none	none	none	n.d.
Endpoints [mg dimethenamid-P/L] (mean measured)							
LC_{50} (96 h)	6.3 (95 % confidence limits: 3.7 - 11)						
NOEC (96 h)	3.7						

n.d. = not determined; all fish dead

Conclusions

In a flow-through acute toxicity study with rainbow trout the LC_{50} (96 h) of dimethenamid-P was 6.3 mg as/L based on mean measured concentrations. The NOEC (96 h) was determined to be 3.7 mg as/L (mean measured).

KCA 8.2.1/2 (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: SAN 1289H Technical: A 96-hour flow-through acute toxicity test with the bluegill (*Lepomis macrochirus*)
Date: 04.06.1996
Doc ID: 131A-162; BASF RegDoc# 96/5414
Guidelines: EPA 850.1075, 72-1
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (SAN 1289H Technical; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.

Test species: Bluegill (*Lepomis macrochirus*), juveniles. The fish were held for approximately two months prior to testing. The fish were acclimated to test conditions for approximately 51 hours prior to test initiation; average body length at the end of the test: 2.3 cm (1.9 - 2.7 cm); average wet weight: 0.29 g (0.17 - 0.42 g).

Test design: Flow-through system (96 hours); 5 test item concentrations, a solvent control and a negative (water) control, 10 fish per aquarium (loading 0.19 g fish/L) and per concentration; 2 replicates per treatment and control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, solvent control (0.10 mL dimethylformamide/L), 2.6, 4.3, 7.2, 12.0 and 20.0 mg dimethenamid-P/L (nominal); corresponding to mean measured concentrations of 0, 0, 2.6, 4.1, 7.5, 12 and 20 mg as/L.

Test conditions: 25-L Teflon-lined polyethylene aquaria, test volume: 15 L in a temperature-controlled water bath; temperature: 22±1 °C; pH 8.2 - 8.4; dissolved oxygen concentrations exceeded 87 % of saturation throughout the test.; photoperiod: 16 h light : 8 h dark; light intensity: approx. 320 lux at test initiation; flow-rate: approx. 6 volume additions/day; no feeding.

Analytics: Analytical verification of the test item was conducted using gas chromatography with electron capture detection (GC-ECD).

Statistics: Descriptive statistics; binomial method for calculation of the LC_{50}; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration (measured as total dimethenamid) was conducted in each concentration at the beginning of the test, after approximately 48 hours and at the end of the test. The analysed contents of dimethenamid-P ranged from 97 % to 103 % of nominal at test initiation and from 93 % to 104 % of nominal at test termination.

Biological results: No mortality occurred in the negative control, solvent control, 2.6 and 4.1 mg dimethenamid-P/L (total dimethenamid) groups, and all fish appeared healthy and normal throughout the test. No mortality occurred in the 7.5 mg dimethenamid-P/L (total dimethenamid) treatment, although the clinical signs of lethargy and dark discoloration were observed among the fish at the 72 and 96 hour observation periods. After 96 hours of exposure, mortality in the 12 and 20 mg dimethenamid-P/L (total dimethenamid) treatments was 80 and 100 %, respectively. LC_{50} values and 95 % confidence limits at 24, 48, 72 and 96 hours were estimated or calculated from the mortality data and shown in Table B.9.2-2.

Conclusions

Table B.9.2-2: Acute toxicity (96 h) of dimethenamid-P on bluegill (*Lepomis macrochirus*)

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**2.6**	**4.3**	**7.2**	**12**	**20**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**2.6**	**4.1**	**7.5**	**12**	**20**
Mortality [%]	0	0	0	0	0	80	100
Symptoms *	none	none	none	none	C, D	C, D	n.d.
Endpoints [mg dimethenamid-P/L] (mean measured)							
LC_{50} (96 h)	10 (95 % confidence limits: 7.5 - 12)						
NOEC (96 h)	4.1						

n.d. = not determined; all fish dead
* Symptoms after 96 hours: C = lethargy; D = discoloration, darker than the negative controls

Conclusions

In a flow-through acute toxicity study with bluegills the LC_{50} (96 h) of dimethenamid-P was 10 mg as/L based on mean measured concentrations. The NOEC (96 h) was determined to be 4.1 mg as/L (mean measured).

KCA 8.2.1/3 (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: Acute toxicity of SAN-582-H to bluegill sunfish (*Lepomis macrochirus*)
Date: 09.06.1988
Doc ID: 36655; BASF RegDoc# 88/11368
Guidelines: EPA 850.1075, 72-1
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid racemate (SAN-582-H); Lot #8605; purity: 91.4 %.

Test species: Bluegill (*Lepomis macrochirus*), juveniles. All test fish were held in culture tanks on a 16-hour daylight photoperiod with a 30 minute transition period between light and dark and observed for at least fourteen days prior to testing. Average body length: 25 mm (±1.2 mm); average wet weight: 0.38 g (±0.074 g).

Test design: Static system (96 hours); 5 test item concentrations, a solvent control and a negative (water) control, 10 fish per aquarium (loading 0.25 g fish/L) and per concentration; no replicates; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, solvent control (0.10 mL dimethylformamide/L), 1.8, 3.3, 5.6, 10 and 18 mg as/L (nominal); corresponding to mean measured concentrations of 1.8, 3.3, 5.6, 10 and 18 mg as/L.

Test conditions: Five gallon glass vessels containing 15 litres of soft reconstituted water; temperature: 22±1 °C; pH 7.2 - 7.6; dissolved oxygen concentration: 3.9 -

8.2 mg/L; photoperiod: 16 h light : 8 h dark; no feeding.

Analytics: The samples were analysed for SAN-582-H by using a Varian 3700 gas-liquid chromatograph (GLC) equipped with an electron capture detector (ECD).

Statistics: Descriptive statistics; binomial method for calculation of the LC_{50}; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration (measured as total dimethenamid) was conducted in each concentration at the beginning and at the end of the test. The analysed contents of SAN-582-H ranged from 97 % to 103 % of nominal at test initiation and from 97 % to 106 % of nominal at test termination.

Biological results: No mortality occurred in the negative control, solvent control, 1.8 and 3.3 mg SAN-582-H groups. The abnormal effects of morta1ity, loss of equi1ibrium, dark disco1oration, fish on the bottom of test chamber, quiescence, 1abored respiration and/or excitability were observed in the 3.3, 5.6, 10 and 18 mg/L measured test concentrations during the 96-hour exposure period. After 96 hours of exposure, mortality in the 5.6, 10 and 18 mg SAN-582-H treatments was 30, 100 and 100 %, respectively. LC_{50} values and 95 % confidence limits at 96 hours was estimated or calculated from the mortality data and shown in the following table.

Conclusions

Table B.9.2-3: Acute toxicity (96 h) of SAN-582-H (racemate of dimethenamid) on bluegill (*Lepomis macrochirus*)

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**1.8**	**3.3**	**5.6**	**10**	**18**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**1.8**	**3.3**	**5.6**	**10**	**18**
Mortality [%]	0	0	0	0	30	100	100
Symptoms *	none	none	none	4EX	1LR/EX/ LOE; 2LR/EX; 4EX	n.d.	n.d.
Endpoints [mg/L] (mean measured)							
LC_{50} (96 h)	6.4 (95 % confidence limits: 3.3 - 10)						
NOEC (96 h)	1.8						

n.d. = not determined; all fish dead
* Symptoms after 96 hours: EX = excitability; LOE = loss of equilibrium; D = dark discoloration; OB = fish on the bottom of test chamber, Q = quiescence, LR = laboured respiration

Conclusions

The test was performed with the racemate of dimethenamid.
In a static acute toxicity study with bluegills the LC_{50} (96 h) of SAN-582-H (racemate of dimethenamid) was 6.4 mg as/L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.8 mg as/L (mean measured).

KCA 8.2.1/3 (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: Acute toxicity of SAN-582-H to rainbow trout (*Salmo gairdneri*)
Date: 09.06.1988

Doc ID: 36656; BASF RegDoc# 88/11366
Guidelines: EPA 850.1075, 72-1
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid racemate (SAN-582-H; (Lot #8605); purity: 91.4 %.

Test species: Rainbow trout (*Oncorhynchus mykiss*; formerly *Salmo gairdneri*); juveniles. All test fish were held in culture tanks on a 16-hour daylight photoperiod with a 30 minute transition period between light and dark and observed for at least fourteen days prior to testing. Average body length: 34 mm (±4.0 mm); average wet weight: 0.54 g (±0.22 g).

Test design: Static system (96 hours); 5 test item concentrations, a solvent control and a negative (water) control, 10 fish per aquarium (loading 0.36 g fish/L) and per concentration; no replicates; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, solvent control (0.10 mL dimethylformamide/L), 1.0, 1.8, 3.2, 5.6 and 10 mg as/L (nominal); corresponding to mean measured concentrations of control, solvent, 1.0, 1.7, 3.1, 5.8 and 10 mg as/L.

Test conditions: Five gallon glass vessels containing 15 litres of soft reconstituted water; temperature: 22±1 °C; pH 7.2 - 7.6; dissolved oxygen concentration: 9.2 mg/L; photoperiod: 16 h light : 8 h dark; no feeding.

Analytics: The samples were analysed for SAN-582-H by using a Varian 3700 gas-liquid chromatograph (GLC) equipped with an electron capture detector (ECD).

Statistics: Descriptive statistics; binomial method for calculation of the LC_{50}; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration (measured as total dimethenamid) was conducted in each concentration at the beginning and at the end of the test. The analysed contents of SAN-582-H ranged from 100 % to 110 % of nominal at test initiation and from 89 % to 100 % of nominal at test termination.

Biological results: No mortality occurred in the negative control, solvent control, 1.0 and 1.8 mg SAN-582-H groups. The abnormal effects of morta1ity, 1abored respiration and/or excitability were observed in the 3.2, 5.6 and 10 mg/L measured test concentrations during the 96-hour exposure period. After 96 hours of exposure, mortality in the 3.2, 5.6 and 10 mg SAN-582-H treatments was 80, 100 and 100 %, respectively. LC_{50} values and 95 % confidence limits at 96 hours was estimated or calculated from the mortality data and shown in the following table.

Conclusions

Table B.9.2-4: Acute toxicity (96 h) of SAN-582-H (racemate of dimethenamid) on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Mortality [%]	0	0	0	0	80	100	100
Symptoms *	none	none	none	none	2LR/EX	n.d.	n.d.
Endpoints [mg/L] (mean measured)							
LC_{50} (96 h)	2.6 (95 % confidence limits: 1.7 – 5.8)						
NOEC (96 h)	1.8						

n.d. = not determined; all fish dead
* Symptoms after 96 hours: EX = excitability; LR = labored respiration

Conclusions

The test was performed with the racemate of dimethenamid.
In a static acute toxicity study with rainbow trouts the LC_{50} (96 h) of SAN-582-H (racemate of dimethenamid) was 2.6 mg as/L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.8 mg as/L (mean measured).

KCA 8.2.1/4 (new study, submitted with renewal dossier)

Author: [redacted]
Title: SAN 1289H Technical: A 96-Hour Flow-Through Acute Toxicity Test With the Sheepshead Minnow (*Cyprinodon variegatus*)
Date: 03.09.1996
Doc ID: 131A-174; BASF RegDoc# 1996/5416
Guidelines: EPA 850.1075, 72-3
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (SAN 1289H; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.

Test species: Sheepshead minnow (*Cyprinodon variegatus*); juveniles, mean body length 2.1 cm (1.8 - 2.6 cm); mean body weight 0.31 g (0.20 - 0.50 g); collected from in-house culture.

Test design: Flow-through system (96 hours); 5 test item concentrations, a solvent control and a negative (saltwater) control, 10 fish per aquarium (loading 0.21 g fish/L) and per concentration; 2 replicates per treatment and control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, solvent control (0.10 mL dimethylformamide/L), 3.2, 5.4, 9.0, 15.0 and 25.0 mg dimethenamid-P/L (nominal); corresponding to mean measured concentrations of 0, 0, 3.4, 5.3, 9.2, 16.0 and 27.0 mg as/L.

Test conditions: 25-L Teflon-lined polyethylene aquaria, test volume: 15 L, filtered natural seawater, diluted to a salinity of 20 ‰ with well water; temperature: 22.1 °C - 22.9 °C; pH 8.3 - 8.4; oxygen content: 5.0 - 6.6 mg/L; photoperiod: 16 h light : 8 h dark; light intensity: approx. 502 lux at test initiation; flow-rate: approx. 6 volume additions/day; no feeding, no aeration.

Analytics: Analytical verification of the test item was conducted using an HPLC with an ECD-detection (total dimethenamid) and HPLC with UV-detection (s-dimethenamid).

Statistics: Descriptive statistics; binomial method for calculation of the LC_{50}; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration (measured as total dimethenamid) was conducted in each concentration at the beginning of the test, after approximately 48 hours and, except for the highest concentration at the end of the test. The analysed contents of dimethenamid-P ranged from 94 % to 102 % of nominal at test initiation and from 98 % to 112 % of nominal at test termination. Additionally, one sample from the low and high treatments was also analysed concurrently for s-dimethenamid. Measured values for s-dimethenamid collected from the low and the high treatment groups at test initiation ranged from 110 % to 118 % and from 107 % to 108 % of nominal, respectively. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the controls and at mean measured concentrations of up to and including 9.2 mg dimethenamid-P/L, whereas 100 % mortality was observed at the two highest test concentrations of 16.0 and 27.0 mg/L. Signs of toxicity such as discoloration (darker than control fish) was found in the test item concentration of 9.2 mg as/L. The results are summarised in Table B.9.2-5.

Table B.9.2-5: Acute toxicity (96 h) of dimethenamid-P on sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**3.2**	**5.4**	**9.0**	**15.0**	**25.0**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**3.4**	**5.3**	**9.2**	**16.0**	**27.0**
Mortality [%]	0	0	0	0	0	100	100
Symptoms *	none	none	none	none	D	n.d.	n.d.
Endpoints [mg dimethenamid-P/L] (mean measured)							
LC_{50} (96 h)	12 (95 % confidence limits: 9.2 - 16)						
NOEC (96 h)	5.3						

n.d. = not determined; all fish dead
* Symptoms after 96 hours: D = discoloration, darker than control fish

Conclusions

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of dimethenamid-P was 12 mg as/L based on mean measured concentrations. The NOEC (96 h) was determined to be 5.3 mg as/L (mean measured).

KCA 8.2.1/5 (new study, submitted with renewal dossier)

Author: [redacted]
Title: [redacted] - Acute toxicity study in the rainbow trout (*Oncorhynchus mykiss*)
Date: 26.11.2010
Doc ID: 12F0715/085066; BASF RegDoc# 2010/1123696
Guidelines: OECD 203, EPA 850.1075, 72-1
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: [redacted]; batch no. B1210B01KE; purity: 91.6 area-%.

Test species: Rainbow trout (*Oncorhynchus mykiss*), approx. 3 months old; body length 4.7 cm (4.5 - 4.9 cm); body weight 0.77 g (0.63 - 0.91 g); supplied by 'Forellenzucht Trostadt GbR,' Troststadt, Germany.

Test design: Semi-static system (96 hours); water exchange every 24 hours, one replicate with 7 fish per test group (loading: 0.54 g fish/L) and the control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, 0.46, 1.0, 2.2, 4.6 and 10.0 mg/L (nominal, based on test substance mass without correction for purity or composition).

Test conditions: Glass flasks, test volume: 10 L, non-chlorinated charcoal filtered tap water (Frankenthal, Germany) mixed with deionised water and aerated; temperature: 13 °C; pH 8.0 - 8.4; oxygen content: 7.1 - 10.4 mg/L; total hardness: 1 mmol/L; conductivity: 250 µS/cm; photoperiod 16 h light : 8 h dark; approx. 75 – 446 lux; no aeration, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a gas chromatographic procedure with MS-detection.

Statistics: Descriptive statistics, probit analysis for determination of the LC_{50} value.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in fresh solutions at test initiation and after 72 h in samples of the control and all test item concentrations and in old test solutions for the test item treatments with concentrations ≥ 2.2 mg as/L. Measured concentrations could only be determined in the three highest concentrations of ≥ 2.2 mg as/L, since concentrations in the lower test groups were below the limit of quantification. In the ≥ 2.2 mg as/L treatments the measured concentrations ranged from 32 % to 55 % of the nominal concentrations in fresh test solutions. At the end of the 24 hour renewal interval, measured concentrations ranged from 45 % to 115 % of the initial measured values. Since the concentration of test substance in test media could not be verified analytically, due to the high degree of uncertainty associated with the quantitative analytical results, the effect concentration is expressed relative to the nominal concentration or loading rate. Due to the instability of the test substance, the results should be

considered as the effect of the parent test substance and all degradation products. Thus, the following biological results are based on nominal concentration.

Biological results: After 96 hours of exposure, no mortality occurred in the control and at the test item concentrations of up to and including 4.6 mg/L, whereas 86 % mortality was observed at the highest tested concentration of 10 mg/L. At the two highest test item concentrations of 4.6 and 10 mg/L, proptosis and apathy were observed, respectively. No additional adverse effects or abnormal behaviour were observed in any of the test treatments. The results are summarised in Table B.9.2-6.

Table B.9.2-6: Acute toxicity (96 h) of [redacted] on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**control**	**0.46**	**1.0**	**2.2**	**4.6**	**10.0**
Mortality [%]	0	0	0	0	0	86
Symptoms *	none	none	none	none	E	A
Endpoints [mg/L] (nominal)						
LC_{50} (96 h)	9.0 +					
NOEC (96 h)	2.2					

+ 95 % confidence limits could not be calculated
* Symptoms after 96 hours: E = exophthalmos (proptosis); A = apathy

Conclusions

In a semi-static acute toxicity study with rainbow trout, the LC_{50} (96 h) of [redacted] was determined to be 9.0 mg/L based on nominal concentrations. The NOEC was 2.2 mg/L (nominal).

KCA 8.2.1/6 (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: Dimethenamid metabolite (M3): 96-hour acute toxicity study in the rainbow trout
Date: 09.01.1997
Doc ID: RCC 628986; WAT1999-483; BASF RegDoc# 97/10271
Guidelines: OECD 203, EPA 850.1075, 72-1
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid metabolite M3 (batch: PCHB: 1070); purity: 98.5 %.

Test species: Rainbow trout (*Oncorhynchus mykiss*); juveniles, average body length: 5.3 cm (±0.6 cm); average wet weight: 1.2 g (±0.4 g).

Test design: Static system (96 hours); 5 test item concentrations and a negative (water) control, 10 fish per aquarium (loading 0.7-0.8 g fish/L) and per concentration; no replicates; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, 1.6, 3.4, 7.5, 16.5, 36.4 and 80.0 mg dimethenamid metabolite M3/L (nominal); corresponding to mean measured concentrations of 1.6, 3.3, 7.6, 16.7, 37.2 and 81.4 mg dimethenamid metabolite M3/L.

Test conditions: Glass tanks, test volume: 15 L, reconstituted water according to the OECD Guideline No. 203 and the EEC Directive 92/69 No. C.1, except a five times lower concentration of all constituents, therefore, the total hardness of the water will be 50 mg $CaCO_3$/L instead of 250 mg $CaCO_3$ mg/L, temperature ranged from 16.0 to 16.5 °C; pH ranged from 7.5 to 8.2; oxygen concentration ranged from 8.4 to 9.5 mg/L (corresponding to 85-96 %) of the air saturation value; photoperiod: 16 h light : 8 h dark; light intensity: 500-1500 lux; no feeding, no aeration.

Analytics: Analytical verification of the test item was conducted using an HPLC with UV-detection (234 nm).

Statistics: Descriptive statistics; moving average interpolation for calculation of the LC_{50}; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning the test and at the end of the test. Measured test item concentrations ranged from 94.8 % to 104.5 % of nominal.

Biological results: After 96 hours of exposure 10 and 80 % mortality was observed in 1.6 mg/L and 80 mg/L treatments. No clinical signs were observed in the control and in the 1.6, 3.3 and 7.6 mg/I test concentrations. At the next higher test concentrations of 16.7, 37.2 and 81.4 mg/L, clinical signs such as remaining at the top or bottom of the tank, loss of equilibrium, enhanced pigmentation, tail and head dominant swimming and laying on its side with nothing but gill activity were observed between 3 and 96 hours of exposure.

Table B.9.2-7: Acute toxicity (96 h) of dimethenamid metabolite M3 on Rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Negative control**	**1.6**	**3.4**	**7.5**	**16.5**	**36.4**	**80.0**
Concentration [mg/L] (mean measured)	**Negative control**	**1.6**	**3.3**	**7.6**	**16.7**	**37.2**	**81.4**
Mortality [%]	0	10	0	0	0	0	80
Symptoms *	none	none	none	none	LOE/D /OB	LOE/D /OB	LOE/D /OB
Endpoints [mg/L] (mean measured)							
LC_{50} (96 h)	60.8 (95 % confidence limits: 49.2 – 75.1)						
NOEC (96 h)	7.6						

n.d. = not determined; all fish dead
* Symptoms after 96 hours: LOE = loss of equilibrium; D = dark discoloration; OB = fish on the bottom of test chamber

Conclusions

In a static acute toxicity study with rainbow trouts the LC_{50} (96 h) of dimethenamid metabolite M3 was 60.8 mg/L based on mean measured concentrations. The NOEC (96 h) was determined to be 7.6 mg/L (mean measured).

KCA 8.2.1/7 (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: Dimethenamid oxalamide (M23): 96-hour static acute toxicity with the rainbow trout (*Oncorhynchus mykiss*)
Date: 16.01.1995
Doc ID: 94-003-1018; BASF RegDoc# 95/11318
Guidelines: OECD 203, EPA 850.1075, 72-1
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid oxalamide (M23), Lot # RS-5820XA-121593; purity: 99.95 %.

Test species: Rainbow trout (*Oncorhynchus mykiss*); juveniles, average body length: 4.6 cm (range 3.7 to 5.2 cm); average wet weight: 1.3 g range 0.53 to 1.99 g).

Test design: Static system (96 hours); limit-test, 10 fish per aquarium (loading 1.3 g fish/L) and per concentration; no replicates; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, 100 mg/L (nominal); corresponding to a mean measured concentration of 87 mg/L.

Test conditions: 18.5-L glass aquaria containing 15 litres of test solution, soft reconstituted water, temperature ranged from 13.0 to 15.0 °C; pH ranged from 7.5 to 8.2; oxygen concentration ranged from 8.4 to 9.5 mg/L (corresponding to 85-96 %) of the air saturation value; photoperiod: 16 h light : 8 h dark; light intensity: 500-1500 lux; no feeding, no aeration.

Analytics: Analytical verification of the test item was conducted using an HPLC with UV-detection (234 nm).

Statistics: The definitive test was performed as a limit test at a test concentration of 100 mg/L. No statistics were therefore used in this study; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted at the beginning and at the end of the test. The mean measured concentrations for M23 was 87 %. Analyses of the Quality Control samples resulted in measured concentrations which were consistent with the predetermined recovery range and averaged 91 ± 5 % of the nominal fortified levels for M23.

Biological results: No mortality occurred in the test aquarium containing 100 mg/L of M23 or in the control aquarium and no sub-lethal effects were observed.

Table B.9.2-8: Acute toxicity (96 h) of dimethenamid oxalamide (M23) on Rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Negative control**	**100**
Mortality [%]	0	0
Symptoms *	none	none
	Endpoints [mg/L] (nominal)	
LC_{50} (96 h)	>100 (> 87 mg/L, based on measured concentrations)	
NOEC (96 h)	≥100 (≥ 87 mg/L, based on measured concentrations)	

* Symptoms after 96 hours: None of the fish showed any symptoms during the assay.

Conclusions

In a static acute toxicity study with rainbow trouts performed as a limit test the LC_{50} (96 h) of dimethenamid oxalamide (M23) was >100 mg/L (> 87 mg/L based on measured concentrations). The NOEC (96 h) was determined to be ≥100 mg/L (≥ 87 mg/L based on measured concentrations).

KCA 8.2.1/8 (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: Dimethenamid sulfonate sodium salt (M27): 96-hour static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*)
Date: 16.01.1995
Doc ID: 94-006-1018, BASF RegDoc# 95/11330
Guidelines: OECD 203, EPA 850.1075, 72-1
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid sulfonate sodium salt (M27), Lot # RS-582SSS-010494; purity: 97.51 %.

Test species: Rainbow trout (*Oncorhynchus mykiss*); juveniles, average body length: 4.6 cm (range 3.7 to 5.2 cm); average wet weight: 1.3 g range 0.53 to 1.99 g).

Test design: Static system (96 hours); limit-test, 10 fish per aquarium (loading 1.3 g fish/L) and per concentration; no replicates; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, 100 mg/L (nominal); corresponding to a mean measured concentration of 87 mg/L.

Test conditions: 18.5-L glass aquaria containing 15 litres of test solution, soft reconstituted water, temperature ranged from 13.0 to 15.0 °C; pH ranged from 7.5 to 8.2; oxygen concentration ranged from 8.4 to 9.5 mg/L (corresponding to 85-96 %) of the air saturation value; photoperiod: 16 h light : 8 h dark; light intensity: 500-1500 lux; no feeding, no aeration.

Analytics: Analytical verification of the test item was conducted using an HPLC with UV-detection (234 nm).

Statistics: The definitive test was performed as a limit test at a test concentration of 100 mg/L. No statistics were therefore used in this study; determination of NOEC by visual interpretation of mortality and clinical observation data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted at the beginning and at the end of the test. The mean measured concentrations for M27 was 100 %. Analyses of the Quality Control samples resulted in measured concentrations which were consistent with the predetermined recovery range and averaged 101 ± 3 % of the nominal fortified levels for M27.

Biological results: No mortality occurred in the test aquarium containing 100 mg/L of M27 or in the control aquarium and no sub-lethal effects were observed.

Table B.9.2-9: Acute toxicity (96 h) of dimethenamid sulfonate sodium salt (M27) on Rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Negative control**	**100**
Mortality [%]	0	0
Symptoms *	none	none
	Endpoints [mg/L] (nominal)	
LC_{50} (96 h)	>100 (> 100 mg/L, based on measured concentrations)	
NOEC (96 h)	≥100 (≥ 100 mg/L, based on measured concentrations)	

* Symptoms after 96 hours: None of the fish showed any symptoms during the assay.

Conclusions

In a static acute toxicity study with rainbow trouts performed as a limit test the LC_{50} (96 h) of dimethenamid sulfonate sodium salt (M27) was >100 mg/L (nominal, measured concentrations). The NOEC (96 h) was determined to be ≥100 (nominal, measured concentrations)

B.9.2.2 Long-term and chronic toxicity to fish

KCA 8.2.2/1

Author: [redacted]
Title: DOZ 300H (SAN 582H): 21-day rainbow trout toxicity study under flow-through exposure conditions
Date: 05.08.1991
Doc ID: 91/SAS047/0409; WAT95-00668; BASF RegDoc# 91/11906
Guidelines: OECD 204
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid racemate (SAN-582-H); batch 8710; purity: not stated.

Test species: Rainbow trout (*Oncorhynchus mykiss*). Each day during the holding period, the fish were fed with proprietary trout pellets, an amount equivalent to between 1

	to 4 % of the total wet-weight of the fish in the holding tank. The mean wet-weight of the fish used in the test, based on a sample of ten fish taken at random from the holding tank on the day the test started (24 April 1991) was 2.2 g. The mean fork length of these fish was 5.6 cm.
Test design:	Flow-through system (21 days); 6 test item concentrations and a negative (water) control, 10 fish per aquarium; four replicates per treatment; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects (wet weights and fork lengths).
Test concentrations:	Negative control, 0.16, 0.31, 0.63, 1.25, 2.5 and 5 mg as/L (nominal); corresponding to mean measured concentrations of 0.173, 0.319, 0.580, 1.04, 2.19 and 4.73 mg as/L.
Test conditions:	Glass tanks, test volume: 15 L, filtered tap water, temperature ranged from 15±2 °C; pH not controlled; oxygen concentration ranged from 8.4 to 9.5 mg/L (corresponding to 85-96 %) of the air saturation value; photoperiod: 16 h light : 8 h dark; light intensity not stated; feeding: Each day of the test, fish were given an amount of proprietary trout pellets (BP Nutrition Ltd., Mainstream Trout Fry 02), equivalent to 2 % of the total wet-weight of the fish in each vessel. Uneaten food and faeces were removed from each tank by suction at least one hour after feeding. Observations of feeding behaviour were made at feeding times; no aeration
Analytics:	Analytical verification of the test item was conducted using an HPLC with UV-detection (234 nm).
Statistics:	Descriptive statistics; calculation of the LC_{50} using the binomial method; determination of NOEC using Dunnett's multicomparison test.

Results and Discussion

Analytical measurements: The overall means of measured concentrations ranged between 83 and 108 % of their nominal values.

Biological results: Mortalities (100 %) were only observed at the highest exposure concentration (5 mg/L). Therefore, the highest nominal concentration at which no mortalities occurred was 2.5 mg as/L, corresponding to a mean measured concentration of 2.19 mg as/L.
At 1.25 mg/L one fish exhibited darkened pigmentation between Days 18 and 21, and at 2.5 mg/L one fish was lethargic from Day 17 onwards. At 5 mg/L, five fish were affected by treatment (or died) during the first two days of the test, thereafter the surviving fish were all affected, exhibiting darkened pigmentation, nervous behaviour or loss of coordination, before death. Thus the no-effect concentration based on nominal concentration was 0.63 mg/L (mean measured concentration 0.58 mg/L) with a lowest effect concentration of 1.25 mg/L (1.04 mg/L) based on pigmentation and loss of coordination.
At the end of the test, individual wet weights and fork lengths of fish which survived exposure to DOZ 300 H (SAN 582 H) for 21 days were compared with the weights of control fish; no significant differences were found.

Table B.9.2-10: Biological results: Mortality, mean wet weight and fork length measurements after 21 days (*Oncorhynchus mykiss*)

Concentration [mg as/L] (nominal)	**Negative control**	**0.16**	**0.31**	**0.63**	**1.25**	**2.5**	**5.0**
Concentration [mg as/L] (mean measured)	**Negative control**	**0.173**	**0.319**	**0.580**	**1.04**	**2.19**	**4.73**
Mortality [%]	0	0	0	0	0	0	100
Mean wet weight (g)	3.97	3.25	3.23	3.72	3.62	3.79	n.d.
Fork length (cm)	6.83	6.34	6.36	6.83	6.61	6.74	n.d.
Symptoms	none	none	none	none	yes 1)	yes 1)	n.d.
Endpoints [mg SAN-582H/L] (mean measured)							
LC_{50} (21 d)	3.54 mg/L (nominal), 3.22 mg/L (measured)						
NOEC (21 d)	0.63 mg/L (nominal), 0.58 mg/L (measured)						
LOEC (21 d)	1.25 mg/L (nominal), 1.04 mg/L (measured)						

n.d. = not determined; all fish dead
1) symptoms: pigmentation changes and loss of coordination

Conclusions

Under flow-through exposure conditions the 21-day LC_{50} values of SAN 582 H to the rainbow trout, calculated using nominal and mean measured concentrations, were 3.54 mg/L and 3.22 mg/L, respectively. The no observed effect concentration (NOEC) based on nominal values was found to be 0.63 mg/L (mean measured concentration 0.58 mg/L) with a lowest observed effect concentration (LOEC) of 1.25 mg/L (1.04 mg/L) based on pigmentation changes and loss of coordination.

B.9.2.2.1 Fish early life stage toxicity test

KCA 8.2.2/2

Author: [redacted]
Title: SAN 582H Technical (K/E): An early life-stage toxicity test with the rainbow trout (*Oncorhynchus mykiss*)
Date: 12.05.1992
Doc ID: 131A-130A; BASF RegDoc.# 92/12456
Guidelines: EPA FIFRA Subdivision E Series 72-4
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid racemate (SAN-582-H TECHNICAL (K/E)); Lot # 9024; purity: 97 %.

Test species: Rainbow trout (*Oncorhynchus mykiss*), unfertilised eggs and sperm were obtained from Mt. Lassen Trout Farm, Rt. 5, Box 36, Red Bluff, California 96080. Gametes from three females and four males were used in the test. The eggs were fertilised at [redacted]. On November 27, 1991 and the test was initiated within approximate1y 3 hours of fertilisation.

Test design: Flow-through system (90 days); 5 test item concentrations, a solvent control and a negative (water) control. The entire 90 day exposure period included a

30-day period for viable embryos to hatch, and a 60-day post hatch exposure period. At test initiation, groups of newly-fertilised eggs were placed in incubation cups and exposed to concentrations of SAN 582H TECHNICAL (K/E) in the test water. Four replicate test chambers were maintained in each treatment and control group, with two incubation cups in each test chamber. Each incubation cup contained IS newly-fertilised embryos resulting in a total of 30 embryos per replicate or 120 embryos per control or treatment group. Four additional cups, each containing 30 embryos, were held in control test chambers and sacrificed on Day 12 to evaluate egg viability (percent fertilisation); loading (the total wet weight of the fish per litre of test water) was measured on the last day of exposure in one negative control replicate and was determined to be 0.22 g of fish per litre of test water that passed through the test chamber in 24 hours. Instantaneous loading was 2.6 g of fish per litre of test water in the test chamber at any given time.

Endpoints: Visual observations, hatching success, time to swim up, survival of larvae, mean length, mean wet and dry weight of fish larvae were measured in each treatment and control group. Data were evaluated to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC).

Test concentrations: Negative control, solvent control (≤0.054 mL dimethylformamide/L), 0.0625, 0.125, 0.25, 0.5 and 1.0 mg active substance (as)/L (nominal); corresponding to mean measured concentrations of 0.06, 0.12, 0.24, 0.48, and 0.95 mg as/ mg/L.

Test conditions: 9-L glass aquaria, test volume: 7.5 L in a temperature-controlled environmental chamber; temperature: 12±1 °C; pH 8.2 - 8.3; dissolved oxygen concentration exceeded 81 % of saturation throughout the test; photoperiod: 16 h light : 8 h dark; light intensity: approx. 420 lux at test initiation; flow-rate: approx. 6 volume additions/day; feeding: Once larvae reached the swim up stage, feeding began using Salmonstarter mash. Swim up larvae were fed 3 times per day during the first 7 days post hatch. Thereafter, they were fed Salmon-starter mash 3 times daily on weekdays and 2 times daily on weekends until the test was terminated.

Analytics: Analytical verification of the test item was conducted using gas chromatography (GC) with nitrogen–phosphorus detector (NPD).

Statistics: Data collected during this test were either continuous-variable data (e.g., weight and length) or discrete-variable data (e.g. mortality proportions). Discrete-variable data were first transformed using the arc-si ne-square root transformation. All continuous-variable data were evaluated for normality using a chi-square test and for homogeneity of variance using the Bartlett's test. Negative control and solvent control groups were compared using the Student's t-test. If no differences were detected between the two control groups, then the control groups were pooled and used to assess treatment level effects. If differences were detected, comparisons were made with the solvent control. For data passing both homogeneity of variance and normality tests, the Dunnett's test was used to evaluate differences between treatment and control group means. If the data set failed to pass the tests for normality and homogeneity, then the Kruskal-Wallis test was used to evaluate differences between the treatment and control groups. The results of the statistical analyses were used to aid in the determination of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC).

Results and Discussion

Analytical measurements: The method validation results yielded a mean and standard deviation of 101 % ± 12 (n=11). The concentrations of SAN-582H in water samples ranged from 79 % of nominal to 123 % of nominal. The procedural recoveries yielded a mean and standard deviation of 95 % ± 7.3 (n=36).

Biological results:
Observations: There were no apparent treatment related signs of toxicity at the 0.06, 0.12, 0.24, and 0.48 mg as/L test concentrations. Fish exposed at the 0.95 mg as/L test concentration were smaller, darker and more lethargic than the controls. Fish in this treatment group were also observed to develop more slowly than fish in the negative control group and appeared to exhibit a higher incidence of mortality. The observations noted at the 0.95 mg as/L test concentration were considered to be treatment related.
Hatching Success: There were no apparent treatment related effects upon hatching success at the 0.06 and 0.12 mg a. i./L test concentrations. Although there was an apparent increase in hatching success at the 0.24 and 0.48 mg as/L test concentrations, when compared to the controls, the cause of the difference could not be determined. In the 0.95 mg as/L treatment group, there was an apparent treatment related reduction in hatching success that was statistically different from the solvent control ($p<0.05$).
Time to Swim Up: Rainbow trout larvae began swimming up from the bottom of the test chambers 10 days post hatch (Day 40). By Day 15 post hatch (Day 45) all rainbow trout larvae except one each in the solvent and negative control groups were swimming. In one each of the negative and solvent control replicates, one larvae had a yolk sac present and therefore was unable to swim up. There were no apparent treatment related effects upon swim up at test concentrations of 0.06, 0.12, 0.24, and 0.48 mg as/L from the time that swim up commenced on Day 10 post hatch until it was completed on Day 15. The differences in the percentage of larvae swimming up at these concentrations were not significantly different ($p<0.05$) from the pooled control groups. However, at the 0.95 mg a. i./L test concentration, there was a lower percentage of larvae swimming up from the bottom of the test chamber when compared to the pooled control groups. The difference was both treatment related and statistically significant ($p<0.05$) on Day 10 and on Day 15 post hatch.
Survival: There were no apparent treatment related effects upon survival during the entire post hatch exposure period at the 0.06, 0.12, 0.24, and 0.48 mg as/L test concentrations. At the 0.95 mg as/L test concentration there appeared to be a treatment related reduction in survival before thinning.
However, this was not confirmed by the statistical analysis. Survival of fish exposed to 0.95 mg as/L during the period from Day 7 (after thinning) to the end of the test was significantly ($p<0.05$) lower than the survival within the pooled control groups.
Growth: There were no apparent treatment-related effects upon growth during the study at the 0.06 and 0.12 mg as/L test concentrations. There appeared to be treatment related reductions in growth in the 0.24, 0.48, and 0.95 mg as/L treatment groups. The effects upon growth were first apparent on Day 31 post hatch as treatment related reductions in mean length at the 0.24, 0.48, and 0.95 mg as/L test concentrations. The differences in mean length from the pooled control group were statistically significant ($p<0.05$). Although effects upon length and wet weight measurements at the 0.24 and 0.48 mg as/L test concentrations were not statistically significant ($p<0.05$) on Day 60 post hatch, dry weight measurements in the 0.48 mg as/L treatment group were statistically significant ($p<0.05$) when compared to the pooled control groups and treatment-related effects upon growth in the 0.24 and 0.48 mg a. i./L treatment related effects upon growth at test termination could not be precluded. However, length and weight measurements of the fish in the 0.95 mg as/L treatment group and dry weight measurements in the 0.48 mg as/L group were treatment related and statistically different ($p<0.05$) from the pooled controls on Day 60 post hatch.

Table B.9.2-11: Biological results from an early life stage toxicity test with the rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**0.0625**	**0.125**	**0.25**	**0.5**	**1.0**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**0.06**	**0.12**	**0.24**	**0.48**	**0.95**
Hatching succes [%]	79	89	78	87	94	97	50 1)
Cumulative no. swimming up / no. exposed on day 15	98	98	97	97	98	100	49 2)
Survival of larvae on day 60 post-hatch [%]	97	98	100	98	98	98	62 2)
Length [mm] on day 31 post-hatch; Mean (±SD)	35.0 (2.1)	34.5 (2.5)	34.4 (2.2)	34.7 (2.7)	33.4 (2.0)2)	33.0 (2.7)2)	29.9 (3.5)2)
Length [mm] on day 60 post-hatch; Mean (±SD)	41.5 (2.8)	40.7 (3.7)	41.2 (4.0)	41.6 (5.0)	41.9 (3.8)	41.5 (4.5)	35.3 (5.9)2)
Wet weight [g] on day 60 post-hatch; Mean (±SD)	1.25 (0.22)	1.18 (0.27)	1.24 (0.31)	1.21 (0.38)	1.19 (0.28)	1.13 (0.32)	0.76 (0.33)2)
Dry weight [g] on day 60 post-hatch; Mean (±SD)	0.25 (0.05)	0.24 (0.06)	0.25 (0.07)	0.25 (0.09)	0.24 (0.06)	0.22 (0.07) 2)	0.14 (0.07) 2)
Symptoms	none	none	none	none	none	none	yes 3)
Endpoints [mg SAN-582H/L] (mean measured)							
NOEC (60 d)	0.12 based on fish length on day 31 post-hatch						
LOEC (60 d)	0.24 based on fish length on day 31 post-hatch						

1) significantly different from the solvent control at $p<0.05$
2) significantly different from the pooled control groups at $p<0.05$
3) symptoms: lethargy, dark coloration, slowed development

Conclusions

Based on the reduction in growth, the no observed effect concentration (NOEC) was 0.12 mg as/L, while the lowest observed effect concentration (LOEC) was 0.24 mg as/L.

B.9.2.2.2 Fish full life cycle test

No studies were required or submitted for the renewal assessment.

B.9.2.2.3 Bioconcentration in fish

No new studies were required or submitted for the renewal assessment. To increase the transparency and comprehensibility of the overall assessment, selected summaries of the studies assessed with the initial evaluation of dimethenamid-P were provided by the applicant and have been added by the RMS.

KCA 8.2.2.3/1 (study evaluated in the initial monograph, 2000)

Author: [redacted]
Title: Accumulation of (^{14}C) SAN-582H in bluegill sunfish
Date: 01.07.1988
Doc ID: N0958-2500, WAT95-00663

Guidelines: US EPA Subdivision N, Environmental Fate, Series 165-4
GLP: yes
Validity: Acceptable

Material and Methods

Test item: Radiolabelled SAN-582H; Approximately 2.0 mCi of (^{14}C)SAN-582H was supplied by Sandoz Crop Pretection for the study. The specific activity was 43.2 mCi/mmole and the radiochemical purity was >93 %. The radiolabelled material was diluted with unlabelled SAN-582H (technical grade; 91.4 % purity; batch no. 8605 supplied by Sandoz Crop Pretection) to the appropriate specific activity prior to use in the accumulation study.

Test species: Bluegill sunfish (*Lepomis macrochirus*) were used in this study as test organisms. The fish were obtained from Fattig Fish Hatchery, Brady, Nebraska. The fish arrived at Battelle's Environmental Research Laboratory on October 8, 1987. On October 9, all fish were treated with 180 mg/L formalin for one hour. The fish were then reared under controlled laboratory conditions in dilution water until they were placed into test chambers on November 4, 1987. The mean weight of fish used in this study was 2.612 ± 1.084 g (mean ± sd) with a range of 0.679 - 7.225 g. These fish had a mean length of 57.0 ±6.8 mm (mean ± sd) with a range of 40 - 78 mm. The longest fish was less than twice the length of the shortest used in the study. Fish were fed both during acclimation and the duration of the study once daily with a quantity of frozen brine shrimp that equalled an amount approximately 3 % of their body weight. During the acclimation period, the brine shrimp meal was periodically supplemented with Purina Trout Chow and/or Tetra Min Flakes.

Test design: The accumulation of (^{14}C)SAN 582H in bluegill sunfish study consisted of a 28-day uptake phase and a 14-day depuration phase. The ^{14}C-1abelled test material was delivered continuously via a flow-through diluter system (in dilution water consisting of reverse osmosis/treated well water mix) to the test chambers containing the bluegill sunfish. Two groups of fish were used for study. In the uptake phase, one group was exposed to test material and the other group was a dilution water control. In the depuration phase, both groups of fish were subjected to dilution water only. Water and fish samples were taken during the study and analysed radiometrically for test material concentrations. Additional fish samples were taken during the latter stages of the uptake period for residue identification.
The diluter system used in this study consists of a proportional flow-through diluter in a temperature controlled water bath which can deliver five concentrations of test material plus a dilution water control to duplicate 15-litre test chambers (water depth approx. 40 cm) at a rate of 500 mL per chamber per cycle. For the accumulation study, the diluter system was adjusted to deliver the same concentration of test material to all exposure tanks. A second diluter system, which is housed in the same water bath as the first, was used in the accumulation study for exposing control fish to dilution water only.

Endpoints: Water and tissue residue concentrations, bioconcentration factors (BCFs), and elimination halfslives were determined during the 42-day study.

Test concentrations: The specific activity of the working stock solution delivered during the 28-day uptake phase of the accumulation study was 1.08 dpm/ng. Mean flow rates of dilution water in exposed and control conditions during the 42-day study averaged 6.4 tank volumes per day (96 L/day).

Test conditions: Sixteen test chambers, each holding 15 L of dilution water or test solution, were used in the study. Eight test chambers were used as dilution water controls and eight were used in (^{14}C)SAN-582H exposure. A random numbers table was used to assign the test organisms to appropriate test chambers. At the beginning of the accumulation study, 18 bluegill sunfish each were transferred to the 16 test chambers. These fish were used for sampling during the subsequent uptake and depuration phases of the accumulation study. The loading rate during the study averaged 0.3 g of fish/L/day (range = 0.1 to 0.6 g/L/day) for exposed fish and averaged 0.3 g/L/day (range a 0.1 to 0.5 g/L/day) for control fish. Temperature: 21.7±0.8 °C; pH 6.99±0.14; dissolved oxygen concentrations 7.7±0.7 mg/L; hardness: 118±5 mg/L as $CaCO_3$; photoperiod: 16 h light : 8 h dark; light intensity: approx. 320 lux at test initiation; flow-rate: approx. 6 volume additions/day; feeding: Fish were fed both during acclimation and the duration of the study once daily with a quantity of frozen brine shrimp that equalled an amount approximately 3 % of their body weight. During the acclimation period, the brine shrimp meal was periodically supplemented with Purina Traut Chow; and/or Tetra Min Flakes; the feeding and sampling schedule will be coordinated, such that fish and water samples are taken at least four hours after fish are fed.

Analytics: Each group of tissue samples (whole body, edible, and non-edible) was separately pooled and homogenised. Therefore, one sample from each tissue group was obtained for each sample period. Duplicate samples of each homogenate were combusted with a Harvey Biological Oxidiser® and analysed for radioactivity using a Beckman Model 3801 liquid Scintillation Counter. The combustion efficiency of the oxidiser was 103.6 ± 2.8 %. Five millilitre aliquots of the water samples collected from each test condition during each fish sampling period were radioassayed in duplicate using Aquasol-2® (New England Nuclear) as a scintillation fluor. The water remaining from each sample period after radiometric determinations was stored frozen for later shipment to Sandoz Crop Protection.

Statistics: Nonlinear regression analysis was used to estimate the parameters k_u and k_d.

Results and Discussion

Accumulation: No adverse toxicological effects were observed during the duration of the study.
Radioactive residues accumulated in the sunfish to a limited extent. The bioconcentration factor for whole fish, based upon an accumulation model which uses non-linear regression analysis of the test-data to estimate the rate constants for uptake and depuration, was 58.
14C-residues of SAN-582H accumulated in whole bluegill sunfish to a limited extent. Plateau of the uptake curve was evidenced as early as day 14 of the 28-day uptake phase. Whole body concentrations determined on day 14 and day 21 were 89.4 % and 92.4 %, respectively, of that observed on day 28 in test fish.
Radiolabelled residue concentrations in non-edible tissue appeared to reach a plateau by day 7 of the uptake phase. Residues in edible tissue were approaching equilibrium on day 28 of the uptake phase. Generally, greater than 80 % of the sum total radioactivity between the edible and non-edible tissue was found in non-edible tissue during the uptake phase.
The actual BCFs for whole body, edible, and non-edible tissues for each sample period were determined by dividing the ^{14}C-residues concentration in the respective tissue by the concentration in the water at each sample period. The average of the actual BCFs determined for the three sample periods at or near steady-state (days 14, 21, and 28) were 57, 20, and 100 for the whole body, edible, and non-edible tissues, respectively. The actual BCF value for whole body, 57, compares favorably to that calculated using the regression model, which was 58.

Elimination Half-Life: Whole bluegill sunfish demonstrated one-compartment elimination kinetics. The elimination half-life for whole body is 10.7 days. Less than 20 % of the ^{14}C-residues remained in whole bluegills at the end of the 14-day depuration period.

Conclusions

The bioconcentration factor (BCF) for whole fish was 58 (≈60).

B.9.2.3 Potential for endocrine disruption

No studies submitted, not required.

B.9.2.4 Acute toxicity to aquatic invertebrates

B.9.2.4.1 Acute toxicity to *Daphnia magna*

KCA 8.2.4.1/1

Author:	Graves, W., Swigert, J.
Title:	SAN 1289 H Technical: A 48-hour flow-through acute toxicity test with the cladoceran (*Daphnia magna*)
Date:	04.06.1996
Doc ID:	131A-164; WAT1999-487; BASF RegDoc# 96/5415
Guidelines:	EPA 850.1010, 72-2
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item:	Dimethenamid-P (SAN 1289H Technical; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.
Test species:	Water flea (*Daphnia magna*), daphnid neonates used in the test were less than 24-hours old and were obtained from cultures maintained by Wildlife International Ltd., Easton, Maryland.
Test design:	Flow-through system (48 hours), 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Negative control, solvent control (0.10 mL dimethylformamide/L), 3.2, 5.4, 9.0, 15 and 25 mg dimethenamid-P/L (nominal); corresponding to mean measured concentrations of 0, 0, 3.4, 5.2, 9.0, 14 and 26 mg as/L.
Test conditions:	Two replicate test chambers (glass beakers approximately 6.5 cm in diameter and 12 cm in height) were maintained in each treatment and control group, with 10 daphnids in each test chamber. Nytex® screen was attached to an opening on each side of the test compartments to allow water to flow in and out of the test compartments. The beakers were suspended in 8-L stainless steel test chambers filled with approximately 6.5 L oftest water. The depth of the test water in a representative test chamber was approximate!y 17.9 cm, whereas the depth of the test water in a representative test compartment was 7.0 cm. Test

chambers were impartially positioned in a temperature-controlled water bath designed to maintain a temperature of 20±1 °C. The water bath was enclosed in a plexiglass ventilation hood in order to minimise potential for cross-contamination. Water temperatures were within the 20±1 °C range established for the test. Dissolved oxygen concentrations exceeded 60 % of saturation throughout the test. Measurements of pH ranged from 8.1 to 8.3.

Analytics: Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).

Statistics: Descriptive statistics; binomial method for calculation of the EC_{50}; determination of NOEC by visual interpretation of observation data.

Results and Discussion

Analytical measurements: Samples collected at 0 Hours had measured values that ranged from 104 to 118 % of nominal values, while measured values for samples taken at 48 hours ranged from 81 to 108 %. When measured concentrations of samples collected at initiation and at test termination were averaged, the mean measured concentrations for the study were 3.4, 5.2, 9.0, 14, and 26 mg SAN 1289H Technical/L (total dimethenamid). Mean measured concentrations were used in the calculations of EC_{50} values.

Biological results: Daphnids in the negative control and solvent control groups appeared healthy and normal throughout the test. Daphnids in the 3.4 mg SAN 1289H Technical/L (total dimethenamid) treatment group also appeared normal with no mortalities or overt signs of toxicity. In the 5.2 and 9.0 mg SAN 1289H Technical/L (total dimethenamid) treatment groups all daphnids appeared normal through 24 hours of exposure. At test termination, one daphnid in the 5.2 mg SAN 1289H Technical (total dimethenamid) treatment group appeared lethargic and one daphnid in the 9.0 mg SAN 1289H Technical/L (total dimethenamid) treatment group was immobile and one daphnid was lethargic. A few daphnids in the 14 and 26 mg SAN 1289H Technical/L (total dimethenamid) treatment groups were lethargic when observed at 4 hours, and by test termination, mortality in these treatment groups had reached 90 and 100 %, respectively. EC_{50} values and 95 % confidence limits 48 hours were calculated or estimated from the mortality/immobility data, and are shown in Table B.9.2-12.

Table B.9.2-12: Effect of SAN 1289H Technical (dimethenamid-P) on *Daphnia magna* immobility

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**3.2**	**5.4**	**9.0**	**15**	**25**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**3.4**	**5.2**	**9.0**	**14**	**26**
Immobility (48 h) [%]	0	0	0	0	5	90	100
Symptoms (48 h)	none	none	none	1C	1C	1C	n.d.
Endpoints [mg dimethenamid-P/L] (mean measured)							
EC_{50} (48 h)	12 (95 % confidence limits: 10 - 13)						
NOEC (48 h)	3.4						

n.d. = not determined (all daphnia dead); C = lethargy

Conclusions

The 48-hour EC_{50} value for daphnids exposed to SAN 1289H Technical was 12 mg/L (mean measured). The 95 % confidence limits were 10 and 13 mg/L.

KCA 8.2.4.1/2 (study evaluated in the initial monograph, 2000)

Author:	Frazier, S.
Title:	Acute toxicity of SAN-582-H to *Daphnia magna*
Date:	04.05.1988
Doc ID:	36657; WAT95-00680 BASF RegDoc# 88/11367
Guidelines:	EPA 850.1010, 72-2
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item:	Dimethenamid racemate SAN-582-H (Lot #8605); purity: 91.4 %.
Test species:	Water flea (*Daphnia magna*), daphnid neonates used in the test were less than 24-hours old.
Test design:	Flow-through system (48 hours), 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Negative control, solvent control (<0.5 mL acetone/L), 6.5, 13, 25, 50 and 100 mg SAN-582-H/L (nominal); corresponding to mean measured concentrations of 5.8, 12, 22, 46 and 90 mg SAN-582-H/L.
Test conditions:	Two replicate test chambers, 250-mL glass beakers, containing 200 mL of daphnid culture/test water were kept at 20 (±1.0) °C in a temperature controlled water bath. The dissolved oxygen concentrations ranged between 7.6 and 9.0 mg/L. These values represented 87 and 103 % saturation at 20 °C, respectively. The pH values of the test solutions ranged from 7.8 to 8.4 and were consistent with the control.
Analytics:	Analytical verification of the test item was conducted using a Varian 3700 gas-liquid chromatograph (GLC) equipped with an electron capture detector (ECD).
Statistics:	Descriptive statistics; binomial method for calculation of the EC_{50}; determination of NOEC by visual interpretation of observation data.

Results and Discussion

Analytical measurements: Measured recovery values represent 90±1.8 % of the nominal concentrations. Mean measured concentrations were used in the calculations of EC_{50} values.

Biological results: EC_{50} values and 95 % confidence limits at 48 hours were calculated or estimated from the mortality/immobility data, and are shown in Table B.9.2.13.

Table B.9.2.13: Acute toxicity (48 h) of SAN-582-H on *Daphnia magna* immobility.

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**6.3**	**13**	**25**	**50**	**100**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**5.8**	**12**	**22**	**46**	**90**
Immobility [%] at 48 h	0	- #	5	0	100	100	100
Symptoms	none	- #	none	none	n.d.	n.d.	n.d.
Endpoints [mg dimethenamid-P/L] (mean measured)							
EC_{50} (48 h)	16 (95 % confidence limits: 12 - 22)						
NOEC (48 h)	12						

# no data presented in the original study report
n.d. = not determined (all daphnia dead)

Conclusions

The 48-hour EC_{50} value for daphnids exposed to SAN 582H was 16 mg/L. The 95 % confidence limits were 12 and 22 mg/L.

KCA 8.2.4.1/3 (new study, submitted with renewal dossier)

Author: Janson, G.-M.
Title: Acute toxicity of Reg.No. 360 712 (metabolite of BAS 656 H) to *Daphnia magna* STRAUS in a 48-hour static test
Date: 12.08.2008
Doc ID: 2008/1042207
Guidelines: OECD 202
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M656H031 (M31, Reg. No. 360 712); metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. RS-582TAS-050495, purity: 99.4 %.

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from the Institute National de Recherche Chimique Appliquee, France), > 2 < 24 hours old at test initiation.

Test design: Static system (48 hours), 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Control, 6.25, 12.5, 25, 50 and 100 mg M656H031/L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water: “M4” (Elendt medium); temperature: 19.3 °C - 21.6 °C; pH 7.87 - 8.04; oxygen content: 8.1 mg/L - 8.9 mg/L; total hardness: 2.27 mmol/L at test initiation; conductivity: 620 µS/cm at test initiation; photoperiod: 16 hours light : 8 hours dark; light intensity: 220 lux - 685 lux; no feeding and no aeration..

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. Measured values for M656H031 ranged from 96.4 % to 104.3 % of nominal concentrations at test initiation and from 97.2 % to 99.7 % of nominal at test termination. As the analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No immobility of daphnids was observed in the control or any of the test item treatment groups after 24 hour of exposure. After 48 hours of exposure, 5 % immobility was observed at the three highest tested concentrations of 25, 50 and 100 mg M656H031/L. The results are summarised in Table B.9.2-14.

Table B.9.2-14: Effect of M656H031 (metabolite of dimethenamid-P) on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Immobility (24 h) [%]	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	5	5	5
Endpoints [mg M656H031/L] (nominal)						
EC_{50} (48 h)	> 100					
NOEC (48 h)	100					

Conclusions

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of M656H031 (metabolite of dimethenamid-P) was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

KCA 8.2.4.1/4 (study evaluated in the initial monograph, 2000)

Author: Gruetzner, I.
Title: Dimethenamid metabolite M3: 48-hour acute toxicity to *Daphnia magna*
Date: 09.01.1997
Doc ID: RCC 628964; WAT1999-488; BASF RegDoc# 97/10272
Guidelines: EPA 850.1010, 72-2
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid metabolite M3 (batch: PCHB: 1070); purity: 98.5 %.

Test species: Water flea (*Daphnia magna*); neonates, ≤ 24 hours old.

Test design: Static system (48 hours); 5 test item concentrations and a negative (water) control, 20 daphnids per test vessel; no replicates

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Negative control, 4.3, 9.4, 20.7, 45.5, and 100 mg dimethenamid metabolite M3/L (nominal); corresponding to mean measured concentrations of 4.1, 9.2, 20.6, 45.0, 101.6 mg dimethenamid metabolite M3/L.

Test conditions: Glass vessels; temperature: the ambient (room) temperature was recorded

continuously throughout the entire test and ranged fram 21 - 22 °C; pH: pH values were at 7.9 at the start of the exposure period and ranged from 7.8 to 7.9 at the end of the exposure period; Oxygen: Values were at 8.4 mg O_2/L at the start of the exposure period and ranged from 8.3 to 8.4 mg O_2/L at the end of the exposure period.

Analytics: Analytical verification of the test item was conducted using an HPLC with UV-detection (234 nm).

Statistics: Descriptive statistics; EC_{50}; determination of NOEC by visual interpretation

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning the test and at the end of the test. Measured test item concentrations ranged from 95.8 % to 103.6 % of nominal.

Biological results: No immobility of daphnids was observed in the control or any of the test item treatment groups after 48 hours of exposure. The results are summarised in Table B.9.2-15.

Table B.9.2-15: Effect of dimethenamid metabolite M3 on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Negative control**	**4.3**	**9.4**	**20.7**	**45.5**	**100**
Concentration [mg/L] (mean measured)	**Negative control**	**4.1**	**9.2**	**20.6**	**45.0**	**101.6**
Immobility (24 h) [%]	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	0	0	0
Symptoms	none	none	none	none	yes[1)]	yes[1)]
Endpoints [mg/L] (mean measured)						
EC_{50} (48 h)	> 101.6					
NOEC (48 h)	20.6					

1) All ten daphnids were trapped at the surface of the test medium. After the 24-hour assessment, the animals were gently released from their trap.

Conclusions

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of metabolite M3 (metabolite of dimethenamid-P) was determined to be > 101.6 mg/L based on mean measured concentrations. The NOEC was 20.6 mg/L.

KCA 8.2.4.1/5 (study evaluated in the initial monograph, 2000)

Author: van der Kolk, J.
Title: Dimethenamid oxalamide (M23): 48-hour static acute immobilisation toxicity test with daphnids (*Daphnia magna*)
Date: 16.01.1995
Doc ID: 94-004-1018; WAT95-00671
BASF RegDoc# 95/11319
Guidelines: OECD 202; EPA 850.1010, 72-2
GLP: Yes
Validity: Acceptable

Material and Methods

Test item:	Dimethenamid oxalamide (M23) (Lot # RS-5820XA- 121593); purity: 99.95 %.
Test species:	Water flea (*Daphnia magna*); neonates, ≤ 24 hours old.
Test design:	Static system (48 hours); limit-test, 4 replicates of 5 daphnids each were used per treatment group.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	Negative control and 100 mg dimethenamid oxalamide (M23)/L (nominal); corresponding to mean measured concentration of 100 mg dimethenamid oxalamide (M23)/L.
Test conditions:	The toxicity test was conducted in 250-mL glass beakers containing 200 mL of test solution; the ambient (room) temperature was recorded continuously throughout the entire test and was 20±2 °C; pH values ranged from 7.66 to 7.71 (controls) and 6.49-7.41 (100 mg/L test solution); oxygen values ranged between 7.70 and 8.33 mg O_2/L and ranged (controls) and from 7.61 to 8.25 mg O_2/L (100 mg/L test solution).
Analytics:	Analytical verification of the test item was conducted using an HPLC with UV-detection (234 nm).
Statistics:	Descriptive statistics; EC_{50}; determination of NOEC by visual interpretation

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning the test and at the end of the test. Measured test item concentrations ranged from 94-95 % of nominal.

Biological results: No immobilisation were observed among daphnids exposed to the concentration tested (100 mg/L), or the control. Two daphnids were caught on the surface of the test solution in replicate C of the 100 mg/L after 24 hours of exposure. In replicate A of the 100 mg/L test concentration, one daphnid was lethargie and one was caught on the surface of the test solution after 48 hours. No effects were observed in replicates B and D of the 100 mg/L test concentration nor in the control at any observation time.

Table B.9.2-16: Acute toxicity (48 h) of dimethenamid oxalamide (M23) on *Daphnia magna*

Concentration [mg/L] (nominal)	**Negative control**	**100**
Immobility [%]	0	0
Symptoms *	none	yes
Endpoints [mg/L] (nominal)		
EC_{50} (48 h)	>100 (> 95 mg/L, based on measured concentrations)	
NOEC (48 h)	≥100 (≥ 95 mg/L, based on measured concentrations)	

* Symptoms: Two daphnids were caught on the surface of the test solution in replicate C of the 100 mg/L after 24 hours of exposure. In replicate A of the 100 mg/l test concentration, one daphnid was lethargie and one was caught on the surface of the test solution after 48 hours

Conclusions

In a static acute toxicity study with *Daphnia magna* performed as a limit test the EC_{50} (48 h) of dimethenamid oxalamide (M23) was >100 mg/L (> 95 mg/L based on measured concentrations). The NOEC (48 h) was determined to be ≥100 mg/L (≥ 87 mg/L based on measured concentrations).

KCA 8.2.4.1/6 (study evaluated in the initial monograph, 2000)

Author: van der Kolk, J.
Title: Dimethenamid sulfonate sodium salt (M27): 48-hour static acute immobilisation toxicity test with daphnids (*Daphnia magna*)
Date: 16.01.1995
Doc ID: 94-007-1018; WAT95-00672; BASF RegDoc.# 95/11331
Guidelines: OECD 202; EPA 850.1010, 72-2
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid sulfonate sodium salt (M27) (Lot #RS-582SSS-010494); purity: 97.51 %.

Test species: Water flea (*Daphnia magna*); neonates, ≤ 24 hours old.

Test design: Static system (48 hours); limit-test, 4 replicates of 5 daphnids each were used per treatment group.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Negative control and 100 mg dimethenamid oxalamide (M23)/L (nominal); corresponding to mean measured concentration of 100 mg dimethenamid oxalamide (M23)/L.

Test conditions: The toxicity test was conducted in 250-mL glass beakers containing 200 mL of test solution; the ambient (room) temperature was recorded continuously throughout the entire test and was 20±2 °C; pH values ranged from 7.66 to 7.70 (controls) and 7.68-7.74 (100 mg/L test solution); oxygen values ranged between 7.70 and 8.33 mg O_2/L and ranged (controls) and from 7.61 to 8.31 mg O_2/L (100 mg/L test solution).

Analytics: Analytical verification of the test item was conducted using an HPLC with UV-detection (234 nm).

Statistics: Descriptive statistics; EC_{50}; determination of NOEC by visual interpretation

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning the test and at the end of the test. The measured test item concentration was 100 % of nominal.

Biological results: No immobilisation were observed among daphnids exposed to the concentration tested (100 mg/L), or in the control. In one replicate C at 100 mg/L, four daphnids were caught on the surface of the test solution after 24 hours of exposure. At 48-hour three of them were still caught on the surface of the test solution. No observations were made in the other replicates.

Table B.9.2-17: Acute toxicity (48 h) of dimethenamid sulfonate sodium salt (M27) on *Daphnia magna*

Concentration [mg/L] (nominal)	**Negative control**	**100**
Immobility [%]	0	0
Symptoms *	none	yes
Endpoints [mg/L] (nominal)		
EC_{50} (48 h)	>100 (>100 mg/L, based on measured concentrations)	
NOEC (48 h)	≥100 (≥ 100 mg/L, based on measured concentrations)	

* Symptoms: In one replicate C at 100 mg/L, four daphnids were caught on the surface of the test solution after 24 hours of exposure. At 48-hour three of them were still caught on the surface of the test solution.

Conclusions

In a static acute toxicity study with *Daphnia magna* performed as a limit test the EC_{50} (48 h) of dimethenamid sulfonate sodium salt (M27) was >100 mg/L (> 100 mg/L based on measured concentrations). The NOEC (48 h) was determined to be ≥100 mg/L (≥ 100 mg/L based on measured concentrations).

KCA 8.2.4.1/7 (new study, submitted with renewal dossier)

Author: Salinas, E.
Title: [redacted] - Acute toxicity (immobilisation) study in the water flea Daphnia magna STRAUS
Date: 13.12.2010
Doc ID: BASF RegDoc# 2010/1212802
Guidelines: OECD 202 (2004)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: [redacted], batch no. B1210B01KE; purity: 91.0 area-%.

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France), less than 24 hours old at test initiation.

Test design: Semi-static system (48 hours); water exchange after 24 hours, 6 test item concentrations plus control, 4 replicates with 5 daphnids in each; assessment

	of immobility after 24 and 48 hours.
Endpoints:	EC_0 and EC_{50} based on immobility of daphnids.
Test concentrations:	Control, 0.22, 0.46, 1.0, 2.2, 4.6 and 10.0 mg/L (nominal).
Test conditions:	Test tubes (glass) sealed with gas impermeable Teflon caps, test volume 23 mL, dilution water: "M4" (Elendt medium); temperature: 19.5 °C - 19.8 °C; pH 8.1 - 8.3; oxygen content: 8.5 mg/L - 9.0 mg/L; total hardness: 2.20 - 3.20 mmol/L; conductivity: 550 - 650 µS/cm; photoperiod: 16 hours light : 8 hours dark; light intensity: about 149 lux - 640 lux; no feeding and no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using a gas chromatographic procedure with MS detection.
Statistics:	Descriptive statistics, probit analysis for determination of the EC_{50} value.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in fresh solutions at test initiation in samples of the control and all test item concentrations.

In old test solutions measurements were conducted at the end of each exposure interval in all test item treatments ≥ 1.0 mg as/L with mobile daphnids and the lowest concentration with 100 % immobilisation. Measured concentrations could only be determined in the four highest concentrations of ≥ 1.0 mg as/L, since concentrations in the lower test groups were below the limit of quantification. In the ≥ 1.0 mg as/L treatments the measured concentrations ranged from 69 % to 86 % of the nominal concentrations in fresh test solutions. At the end of the 24 hour renewal interval, measured concentrations ranged from 46 % to 51 % of the initial measured values. Mean measured concentrations were between 55 % and 77 % of the nominal concentrations. Since the concentration of test substance in test media could not be verified analytically, due to the high degree of uncertainty associated with the quantitative analytical results, the effect concentration can be expressed relative to the nominal concentration or loading rate. Due to the instability of the test substance, the results should be considered as the effect of the parent test substance and all degradation products. Thus, the following biological results are based on nominal concentration.

Biological results: After 48 hours of exposure, no immobility of daphnids was observed in the control and at the three lowest tested concentrations, whereas 5 % and 35 % of the daphnids were immobile at 2.2 mg/L and 4.6 mg/L, respectively. At the highest test item concentration of 10 mg/L all daphnids were immobile after 24 and 48 hours of exposure. No additional adverse effects or abnormal behaviour were observed in any of the test groups. For results see Table B.9.2-18.

Table B.9.2-18: Effect [redacted] on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	Control	0.22	0.46	1.0	2.2	4.6	10.0
Immobility (24 h) [%]	0	0	0	0	0	10	100
Immobility (48 h) [%]	0	0	0	0	5	35	100
Endpoints [mg/L] (nominal)							
EC_{50} (48 h)	4.87 (95 % confidence limits: 4.04 - 6.00)						
EC_0 (48 h)	1.0						

Conclusions

In a 48-hour semi-static acute toxicity study with *Daphnia magna* the EC_{50} of [redacted] was determined to be 4.87 mg/L based on nominal concentrations. The EC0 was 1.0 mg/L (nominal).

B.9.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute toxicity study on the saltwater mysid *Americamysis bahia* (former name: *Mysidopsis bahia*) performed with the active substance dimethenamid-P was conducted due to U.S. data requirements and has not been evaluated previously on EU level. However, the study can principally be used for registration in the EU.

KCA 8.2.4.2/1 (new study, submitted with renewal dossier)

Author: Graves, W., Swigert, J.
Title: SAN 1289H Technical: A 96-Hour Flow-Through Acute Toxicity Test With The Saltwater Mysid (*Mysidopsis bahia*)
Date: 03.09.1996
Doc ID: 1996/5413
Guidelines: EPA 850.1035, 72-3
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid (BAS 656 H; Reg. No. 360 720), s-racemate; lot no. 6663-50-1, purity: 91.1 % by weight for s-dimethenamid and 96.3 % by weight for total dimethenamid.

Test species: Saltwater mysid (*Americamysis bahia;* former name: *Mysidopsis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.

Test design: Flow-through system (96 hours); 5 test concentrations plus control, 2 replicate test chambers per aquarium and treatment each containing 10 mysids, giving a total of 20 mysids per aquarium and per treatment; assessment of mortality and symptoms of toxicity approx. 5, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (96 h), LC_{50} (48 h), NOEC (96 h), mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.10 mL/L dimethylformamide) and 1.0, 1.7, 2.9, 4.8 and 8.0 mg dimethenamid/L (nominal), corresponding to mean measured concentrations of 1.2, 1.8, 3.0, 5.5 and 9.2 mg dimethenamid/L.

Test conditions: Polyethylene aquaria (8 L) containing two test chambers, test volume approx. 6.5 L; test chambers: 500 mL glass beakers with nylon mesh screen attached at each side of the beaker; dilution water: filtered and diluted seawater; flow rate: approx. 14 volume additions per 24 hours; salinity: 20 ‰; temperature: 24.8 °C - 25.0 °C; pH 8.3; oxygen content: 5.1 - 6.5 mg/L; photoperiod 16 h light : 8 h dark; light intensity: approx. 227 lux; feeding: juvenile mysids were fed daily with brine shrimps (*Artemia nauplii*).

Analytics: Analytical verification of test item concentrations was conducted using gas chromatography with electron capture detection (total dimethenamid) and an HPLC-method with DAD detection (s-dimethenamid).

Statistics: Descriptive statistics; probit analysis for calculation of the LC_{50}.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, once during the test after 48 hours and at test termination. Measured concentrations for total dimethenamid ranged from 103 % to 113 % of nominal at test initiation, from 101 % to 114 % after 48 hours and from 113 % to 129 % of nominal at test termination. Concentrations of s-dimethenamid were determined in the lowest and highest treatments and were in the range of 99 % - 104 % of nominal at test initiation and between 114 % - 122 % of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and no other toxic effects were observed in the solvent control and at the lowest tested mean measured concentration of 1.2 mg dimethenamid/L. In the dilution water control, 5 % mortality was observed after 96 hours. Mortality rates of 20 %, 45 %, 85 % and 95 % after 96 hours were observed in the 1.8, 3.0, 5.5 and 9.2 mg as/L test item groups, respectively. At concentrations of 1.8 mg/L and above, erratic swimming of the surviving mysids was observed. The results are summarised in Table B.9.2-19.

Table B.9.2.19: Acute toxicity of dimethenamid to saltwater mysids (*Americamysis bahia*)

Concentration [mg/L] (nominal)	**control**	**Solvent control**	**1.0**	**1.7**	**2.9**	**4.8**	**8.0**
Concentration [mg/L] (mean measured)	**control**	**Solvent control**	**1.2**	**1.8**	**3.0**	**5.5**	**9.2**
Mortality after 96 h [%]	5	0	0	20	45	85	95
Symptoms *	none	none	none	E	E	E	E
Endpoints [mg/L] (mean measured)							
LC_{50} (48 h)	>9.2 (95 % confidence limits: n.c.)						
LC_{50} (96 h)	3.2 (95 % confidence limits: 2.7 - 3.9)						
NOEC (96 h)	1.2						

* Symptoms: E = erratically swimming
n.c. = confidence limits could not be calculated

Conclusions

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for dimethenamid was determined to be 3.2 mg/L based on mean measured concentrations. The NOEC (96 h) was 1.2 mg/L (mean measured). The LC_{50} (48 h) for dimethenamid was determined to be

> 9.2 mg/L (mean measured).

B.9.2.5 Long-term and chronic toxicity to aquatic invertebrates

B.9.2.5.1 Reproductive and development toxicity to *Daphnia magna*

KCA 8.2.5.1/1 (study evaluated in the initial monograph, 2000)

Author: Holmes, C., Swigert, J.
Title: SAN 582H: A flow-through life-cycle toxicity test with the cladoceran (*Daphnia magna*)
Date: 09.12.1992
Doc ID: 131A-147A; BASF RegDoc# 92/12455
Guidelines: FIFRA SUBDIVISION E, SERIES 72-2
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid racemate (SAN-582-H); Lot #9024; purity: 97 %

Test species: Water flea (*Daphnia magna*), <24 hours at test initiation, neonates were obtained from cultures maintained by Wildlife International Ltd., Easton, Maryland.

Test design: Flow-through system, 5 test concentrations plus control, 3 replicates with 5 daphnids in each, and 7 replicates with 1 daphnid each; assessment of immobility and reproduction after 2, 4, 7 , 10, 11, 14, 16, 18, 21 days.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, solvent control (0.10 mL dimethylformamide/L), 0.312, 0.625, 1.25, 2.50, and 5.00 mg SAN 582H/L (nominal); corresponding to mean measured concentrations of 0.33, 0.72, 1.36, 2.51, and 4.94 mg SAN 582H/L.

Test conditions: To begin the test, an individual neonate daphnid was impartially distributed to each of seven replicate test chambers assigned to each treatment and control group. These daphnids were observed for survival, reproduction, and growth. In addition, groups of the daphnids were impartially placed in each of three beakers assigned to each treatment and control group. These 15 daphnids were observed for survival and growth.
Test compartments were constructed from 300 mL glass beakers approximate1y 6.5 cm in diameter and 12 cm in height. Nytex® screen was attached to an opening on each side of the beakers to allow water to flow in and out of the test compartments. The beakers were suspended in Teflon® -lined, 8-L test chambers filled with approximately 6.5 L of water. The test chambers were indi scrimi nately positioned in a temperature-controlled water bath to maintain a temperature of 20±1 °C.

The water bath was enclosed in a plexiglass ventilation hood in order to minimise potential for cross-contamination. Water temperatures were within the 20± °C range established for the test. Dissolved oxygen concentrations exceeded 60 % of saturation throughout the test. Measurements of pH ranged from 7.6 to 7.9.

Analytics: Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).

Statistics: Daphnid survival was statistically evaluated using the Kruskal-Wallis test. Reproduction (numbers of young produced per adult daphnid) and growth data (lengths and dry weights) were evaluated for normality by a ChiSquare test and for homogeneity of variances by the Bartlett's test. All reproduction and length data passed the tests for normality and homogeneity of variances. The dry weight data failed to meet the assumptions and therefore a square-root transformation was performed. After the square root transformation, the data passed the tests for normality and homogeneity of variances. Due to the high level of mortality at the 2.51 and 4.94 mg/L test concentrations, reproduction, length, and dry weight data were not included in the Day 21 statistical analyses. All negative and solvent control data for reproduction and growth were compared to each other using a t-test. No statistical differences (alpha = 0.05) between the two control groups was found and the reproduction and growth data for the two control groups were pooled for the analysis of variance (ANOVA).

Results and Discussion

Analytical measurements: Weekly measurements, within each test concentration, showed relatively low variability. Overall mean measured concentrations were 0.33, 0.72, 1.36, 2.51, and 4.94 mg SAN 582H/L for the low through high test concentrations, respectively. These mean measured values correspond to 106, 115, 109, 100, and 99 % of nominal test concentrations, respectively. Measured values for all samples from the solvent and negative controls were less than the lower limit of quantitation (0.21 mg SAN 582H/L). Mean measured concentrations were used in the derivation of NOECs.

Biological results:

Survival: No statistically significant survival effects were apparent among the different treatment levels ($p>0.05$). By day 14, a statistically significant reduction ($p<0.05$) in daphnid survival existed in the 4.94 mg/L treatment group. There were no apparent treatment-related effects upon the survival of first generation daphnids at test concentrations less than or equal to 1.36 mg/L during the 21-day test. Mortality values in the negative and solvent control groups were 9 % and 0 %, respectively, while values in the 0.33, 0.72, and 1.36 mg/L treatment groups were 5 %, 0 %, and 9 %, respectively. Percent mortality values for those three groups were not statistically different ($p<0.05$) from the negative control. However, there was an apparent treatment-related reduction in mean survival in the 2.51 and 4.94 mg/L treatment groups when compared with the negative control group. The 21-day mortality values in those two groups were 68 % and 100 %, respectively, and were both concentration-dependant and statistically significant when compared to the negative control ($p<0.05$).

Reproduction: Statistically significant mortality in the 4.94 mg/L treatment group precluded the inclusion of this treatment level in the 14-day and the 21-day analyses. During the first 14 days of the test, no statistically significant ($p>0.05$) reproductive effects were found at test concentrations less than or equal to 2.51 mg/l. Similarly, when reproduction was evaluated at the end of the test, no apparent treatment related effects were seen at the 0.33, 0.72 and 1.36 mg/L test concentrations. Overall, the concentrations not statistically significant for survival also were not statistically significant for reproduction.

Growth: There were no apparent treatment related effects upon the growth of first generation daphnids in the 0.33, 0.72, and 1.36 mg/L test concentrations during the 21-day study. Any differences in length or dry weight between the pooled control and eaeh of those treatment groups were slight and not statistically significant ($p>0.05$). Although extensive mortality among first generation daphnids in the

2.51 mg/L test concentration precluded a full evaluation of effects upon growth, there appeared to be a reduction in dry weight. However, no effects upon daphnid length were noted at that concentration. Since there was 100 % mortality among first generation daphnids in the 4.94 mg/L treatment group, growth could not be evaluated. Survival, reproduction and growth data are shown in Table B.9.2-20.

Table B.9.2-20: Effects of dimethenamid-P on *Daphnia magna* immobility, reproduction and growth after 21 days

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	0.312	0.625	1.25	2.50	5.00
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	0.33	0.72	1.36	2.51	4.94
Immobility [%]	9	0	5	0	9	68*	100*
Reproduction [mean neonate production]	64	77	91	77	77	78	n.d.
Length; mean ± SD [mm]	3.83 ± 0.42	3.87 ± 0.30	3.98 ± 0.33	4.01 ± 0.25	3.90 ± 0.23	3.74 ± 0.20	n.d.
Dry weight; mean ± SD [mg]	0.45 ± 0.20	0.49 ± 0.22	0.50 ± 0.19	0.55 ± 0.19	0.43± 0.15	0.29 ± 0.09	n.d.
Endpoints [mg dimethenamid-P/L] (mean measured)							
NOEC (21 d)	1.36						

n.d. = not determined (all daphnia dead);
* Statistically significant when compared to the negative control ($p<0.05$)

Conclusions

There were no apparent treatment-related effeets upon the survival, growth, or reproduction of *Daphnia magna* exposed to SAN 582H concentrations of 0.33, 0.72, and 1.36 mg/L. However, there were marked effects upon survival at both the 2.51 and 4.94 mg/L test concentrations. Based on those findings, the no observed effect concentration (NOEC) for the study was 1.36 mg/L, and the lowest observed effect concentration (LOEC) was 2.51 mg/L.

KCA 8.2.5.1/2 (study evaluated in the DAR for dimethenamid, 2003)

Author: Jenkins, C.A.
Title: DOZ 300 H (SAN 582 H): *Daphnia magna* 21 day juvenile production test under semistatic conditions
Date: 11 April 1991
Doc ID: 91/SAS048/0981; BASF RegDoc# 91/11952
Guidelines: OECD 202 (1984)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: DOZ 300 H (SAN 582 H); dimethenamid racemate; Batch #8710; purity: 92.7 %

Test species: Water flea (*Daphnia magna*), <24 hours at test initiation, the strain used in this study was derived from a culture received from the the University of Sheffield, England, which had been shown by an electrophoretic assay to be genetically homogenous.

Test design:	Semi-static (21 days), 5 test concentrations plus control, 4 replicates with 10 daphnids each; daily assessment of immobility; numbers of juvenile (reproduction) were coutned three times each week.
Endpoints:	LC_{50}, EC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Negative control, 0.35, 0.7, 1.4, 2.8 and 5.6 mg DOZ 300 H (SAN 582 H)/L (nominal); corresponding to mean measured concentrations of 0.365, 0.681, 1.27, 2.60 and 5.17 mg (DOZ 300) SAN 582 H/L, respectively.
Test conditions:	500-mL glass beakers, each containing 400 mL control or test medium. Four vessels were employed at each exposure level. During the test they were covered with clear perspex sheets. During the the test, temperature ranged from 19.6 to 21.1; pH ranged in fresh medium from 7.5 to 7.8, in old medium from 8.0 to 8.5; dissolved oxygen levels (air saturation values) were between 97 % and 102 %, total hardness ranged from 208 to 236 mg $CaCO_3$/L in all vessels.
Analytics:	The concentrations of DOZ 300 (SAN 582 H) in the dilute solutions were quantitatively determined by gas liquid chromatography using an electron capture detector.
Statistics:	Descriptive statistics; calculation of the EC_{50} and LC_{50} using the binomial method; determination of NOEC using Dunnett's multicomparison test.

Results and Discussion

Analytical measurements: Weekly measurements resulted in overall mean measured concentrations of 0.365, 0.681, 1.27, 2.60 and 5.17 mg (DOZ 300) SAN 582 H/L, respectively. These mean measured values correspond to 104, 97 %, 91 %, 93 % and 92 % of nominal test concentrations, respectively. Mean measured concentrations were used in the derivation of the NOEC.

Biological results:

Survival: After 21 days, 12.5 % of the parental control daphnia had died. In groups exposed to DOZ 300 H (SAN 582 H), mortality was concentration related, ranging from 12.5 % at the lowest exposure level (0.35 mg/L) to 67.5 % at the highest level (5.6 mg/L).
The median LC_{50} of DOZ 300 (SAN 582 H) to parental daphnia calculated at 21 days was 4.06 mg/L based on nominal concentration, with 95 % confidence limits of 3.20 and 5.27 mg/L.

Statistical comparison (Dunnett's test; α= 0.05) of the numbers of surviving parental daphnia after 21 days showed no significant differences between the control group and and those exposed to DOZ 300 H (SAN 582 H) at 0.35 mg/L, 0.7 and 1.4 mg/L. At 2.8 and 5.6 mg/L the survival rates were significantly lower. The NOEC of DOZ 300 (SAN 582 H) on the survival of the parental daphnia is therefore considered to be 1.4 mg/L.

Reproduction: Gravid daphnia were first observed on day 6 in all of the control vessels and in most of the test vessels. After 21 days the mean cumulative number of juveniles produced per adult was 66.9 in the controls. The 21-day EC_{50} values of DOZ 300 (SAN 582 H) calculated using the total number of juveniles produced at each nominal concentration was found to be 3.91 mg/L, with 95 % confidence limits of 3.35 and 4.75 mg/L.
The results of the Dunnet's multi-comparison test (α = 0.05) using the total number of juveniles that had been produced in each vessel by day 21, showed that there was no significant difference between the control and the exposure concentrations at 0.35 and 0.7 mg/L. At 1.4, 2.8 and 5.6 mg/L significantly fewer juveniles were produced (see table below). At these levels the apparent reduction

in reproduction was caused by the toxicity of DOZ 300 H (SAN 582 H) to the parental daphnia, particularly during the first 16 days of the test. Survival and reproduction data are shown in Table B.9.2-21.

Table B.9.2-21: Effects of DOZ 300 (SAN 582 H) on *Daphnia magna* immobility and reproduction after 21 days

Concentration [mg as/L] (nominal)	**Negative control**	0.35	0.7	1.4	2.8	5.6
Concentration [mg as/L] (mean measured)	**Negative control**	0.365	0.681	1.27	2.60	5.17
Immobility [%]	12.5	12.5	15	17.5	30*	67.5*
Cumulative no. juveniles/vessel	2621	2349	2314	2095*	1919*	694*
Endpoints [mg SAN 582 H/L] (mean measured)						
$NOEC_{survival}$ (21 d)	1.27					
$NOEC_{reproduction}$ (21 d)	0.68					

* Statistically significant when compared to the negative control ($p<0.05$)

Conclusions

The no observed effect concentration (NOEC) for the Daphnia reproduction study was 0.68 mg/L based on mean measured concentrations, the lowest observed effect concentration (LOEC) was 1.27 mg/L.

B.9.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

No study required. Thus, this point is not addressed *via* new toxicity studies.

B.9.2.5.3 Development and emergence in *Chironomus riparius*

No study required. Thus, this point is not addressed *via* new toxicity studies.

B.9.2.5.4 Sediment dwelling organisms

No study required. Thus, this point is not addressed *via* new toxicity studies.

B.9.2.6 Effects on algal growth

B.9.2.6.1 Effects on growth of green algae

KCA 8.2.6.1/1 (new study amendment, submitted with renewal dossier)

Author:	Hoberg, J. (amended by Kubitza, 2004)
Title:	SAN 1289H Technical - toxicity to the freshwater green alga, *Selenastrum capricornutum*
Date:	20.01.1997 (Amendment: 2004)
Doc ID:	96-11-6778; US Reg. Doc. No. 1997/5170 (US and EU submission); BASF RegDoc# 97/10746 (Amendment: 2004/1025684)
Guidelines:	EPA 850.5400, 122-2, 123-2

GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (SAN 1289H; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.

Test species: *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*); source: Springborn culture.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 3 replicates per treatment including controls; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and biomass after exposure over 120 hours.

Test concentrations: Control, 0.0016, 0.0030, 0.0063, 0.013, 0.025 and 0.050 mg as/L (nominal), corresponding to mean measured concentrations of 0.0013,0.0021, 0.0054, 0.0096, 0.021 and 0.044 mg as/L

Test conditions: 250-mL Erlenmeyer flasks; test volume 100 mL; AAP medium was used to prepare the exposure solutions; pH was adjusted to 7.5±0.1; pH 7.3-7.6 at test initiation and pH 7.7- 10.0 at test termination; temperature: 25 °C; initial cell densities 1 x 10^4 cells/mL; continuous light intensity within the range 4000-4500 lux; continuous shaking.

Analytics: Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and biomass.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test.
The measured concentrations of dimethenamid-P ranged from 92 % to 108 % of nominal at test initiation and from 50 % to 76 % of nominal at test termination (day 5). Therefore, the following biological results are based on geometric mean measured concentrations. Mean measured concentrations (days 0-5) ranged from 70 to 87 % of the nominal concentrations. As the initially measured values confirm the correct application of the test item and the overall mean recovery was within 80-120 % of nominal (80 %), the following biological results are based on nominal test concentrations.

Biological results: At test termination, cells exposed to the 0.0016, 0.0030, 0.0063, 0.013 and 0.025 mg as/L treatment levels and the control were observed to be normal, whereas bloated cells were observed at the highest treatment level tested, 0.050 mg as/L. Control cultures averaged 243 x 10^4 cells/mL at test termination. Cell density in the exposure levels (0.0016, 0.0030, 0.0063, 0.013, 0.025 and 0.050 mg as/L) averaged 181, 237, 198, 167, 66 and 1.8 x 10^4 cells/mL, respectively, at test termination. Statistical analysis (Williams' Test) of this data established a significant reduction in cell density in the 0.0063, 0.013, 0.025 and 0.050 mg as/L treatment levels when compared to the performance of the control. Therefore, the 5-day NOEC for cell density was determined to be 0.0030 mg as/L. Inhibitions of cell growth (cell density), NOEC, EC_{50} values and their corresponding 95 % confidence limits are summarised in Table B.9.2-22.

In the study amendment by Kubitza (2004; Doc ID: 2004/1025684), additional endpoints related to

growth rate (r) and biomass (b) after 72, 96 and 120 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). Unlike the EC_{50}s reported in the original study by Hoberg (1997), the endpoints in the amendment were based on nominal concentrations, as the initial measured values confirmed the correct application of the test item.

Table B.9.2-22: Effect of dimethenamid-P on the growth of green alga *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*); endpoints based on nominal concentrations amended by Kubitza (2004)

Concentration [mg as/L] (nominal)	**control**	**0.0016**	**0.0030**	**0.0063**	**0.013**	**0.025**	**0.050**
Concentration [mg as/L] (measured)	**control**	**0.0013**	**0.0021**	**0.0054**	**0.0096**	**0.021**	**0.044**
Inhibition in 72 h (biomass) [%]	--	6.2	-4.9	-2.5	27.1	69.5	99.0
Inhibition in 72 h (growth rate) [%]	--	1.7	-0.7	0.0	7.8	25.8	96.4
Inhibition in 96 h (biomass) [%]	--	26.6	-3.8	7.7	31.5	85.5	99.0
Inhibition in 96 h (growth rate) [%]	--	5.6	-0.7	1.7	8.2	34.1	75.1
Inhibition in 120 h (biomass) [%]	--	24.9	2.3	18.5	31.3	72.9	99.4
Inhibition in 120 h (growth rate) [%]	--	4.3	0.3	3.4	5.8	20.5	73.9
Endpoints [mg as/L] (nominal)							
E_rC_{50} (72 h)	0.0303 (95 % confidence limits: 0.0296 – 0.0310)						
E_rC_{10} (72 h)	0.0156 (95 % confidence limits: 0.0149 – 0.0163)						
E_yC_{50} (72 h) [1)]	0.0185 (95 % confidence limits: 0.0087 – 0.0389)						
E_yC_{10} (72 h) [1)]	0.0093 (95 % confidence limits: 0.0049 – 0.0179)						
E_bC_{50} (72 h)	0.0191 (95 % confidence limits: 0.0186 – 0.0197)						
E_bC_{10} (72 h)	0.0076 (95 % confidence limits: 0.0072 – 0.0080)						
E_rC_{50} (96 h)	0.0339 (95 % confidence limits: 0.0327 – 0.0352)						
E_rC_{10} (96 h)	0.0106 (95 % confidence limits: 0.0099 – 0.0114)						
E_yC_{50} (96 h) [1)]	0.0168 (95 % confidence limits: 0.0096 – 0.0290)						
E_yC_{10} (96 h) [1)]	0.0101 (95 % confidence limits: 0.0063 – 0.0163)						
E_bC_{50} (96 h)	**0.0140 (95 % confidence limits: 0.0135 – 0.0145)**						
E_bC_{10} (96 h)	0.0032 (95 % confidence limits: 0.0029 – 0.0034)						
E_rC_{50} (120 h)	0.0378 (95 % confidence limits: 0.0364 – 0.0392)						
E_rC_{10} (120 h)	0.0128 (95 % confidence limits: 0.0120 – 0.0136)						
E_yC_{50} (120 h) [1)]	0.0188 (95 % confidence limits: 0.0107 – 0.0328)						
E_yC_{10} (120 h) [1)]	0.0094 (95 % confidence limits: 0.0058 – 0.0154)						
E_bC_{50} (120 h)	0.0143 (95 % confidence limits: 0.0137-0.0149)						
E_bC_{10} (120 h)	0.0026 (95 % confidence limits: 0.0024 – 0.0029)						
NOEC (120 h)	0.0030						

[1)] calculated additionally by the RMS

Conclusions

In a 120-hour algae test with *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*), the

lowest E_bC_{50} value for dimethenamid-P was determined to be 0.014 mg as/L at day 4, based on nominal concentrations. The NOEC was 0.0030 mg as/L (nominal).

KCA 8.2.6.1/2 (study evaluated in the initial monograph, 2000)

Author:	Thompson, S., Peters, G.
Title:	SAN 582H: A 5-day toxicity test with the freshwater alga (*Selenastrum capricornutum*)
Date:	25.09.1991
Doc ID:	131A-126; WAT95-00677 BASF RegDoc# 91/11915
Guidelines:	EPA 850.5400, 122-2, 123-2
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item: Dimethenamid racemate (SAN 582 H); Bacth #5083-133; the test substance was characterised by the Sponsor as 79.3 % active substance. Test concentrations were adjusted to 100 % active substance.

Test species: *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*), stock cultures were maintained in culture medium at Wildlife International Ltd. from May 31, 1991 until test initiation. Algae used in tests were in exponential growth phase.

Test design: Static system (120 hours), 5 test concentrations plus control/solvent control, three replicates per treatment; algal growth was monitored at 24-hour intervals during the test; the study was conducted in accordance with the approved protocol with the fol1owing changes: Cell counts were performed using a hemocytometer, rather than an electronic particle counter; The initial cell density was 8.0 x 10^3 cells/mL, rather than 1.0 x 10^4 cells/mL.

Endpoints: EC_{50} and NOEC with respect to growth rate and biomass after exposure over 120 hours.

Test concentrations: Negative control, solvent control (0.0625 mL acetone/L), 0.008, 0.016, 0.031, 0.063 and 0.125 mg SAN 582 H (nominal); corresponding to mean measured concentrations of 0.004, 0.011, 0.024, 0.049 and 0.112 mg SAN 582 H/L.

Test conditions: Test chambers were sterile, 250-mL Erlenmeyer flasks containing 100 mL of test solution (algal medium with vitamins). The test chambers were plugged with sterile cotton and gauze stoppers and held in an environmental chamber throughout the study to maintain the desired test temperature. The test chambers were shaken continuously at approximately 100 rpm on a mechanical shaker. Temperature ranged from 24 to 25 °C. Measurements of pH ranged from 7.2 to 8.6.

Analytics: Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).

Statistics: Descriptive statistics; calculation of EC_{50}; determination of NOEC.

Results and Discussion

Analytical measurements: Samples collected at 0 Hours had measured values that ranged from 75 to 99 % of nominal values, while measured values for samples taken at 120 hours ranged from 38 to

79 %. Mean measured concentrations were used in the calculations of EC_{50} values.

Biological results: Growth in the solvent control replicates was inhibited by 14.4 % when compared with the negative control, indicating that the solvent (acetone) caused a reduction in growth at a concentration of 0.0625 mL/L. Since the acetone concentration was equivalent in all treatment groups, the effects of the test substance were assessed by comparing growth in the treatment groups with growth in the solvent control.
There were no treatment related effects upon growth rate at the 0.004 mg SAN 582 H/L test concentrat ion. Treatment related reductions in growth were observed at the 0.011, 0.024, 0.049, and 0.112 mg as/L test concentrations that were statistically significant (α = 0.05). Mean growth rates at those concentrations were reduced by 18.3 %, 27.2 %, 26.3 %, and 55.5 %, respectively, when compared with the mean growth rate in the solvent control. Based on mean percent inhibition values calculated from estimates of mean growth rates, the *Selenastrum capricornutum* 5-day EC_{50} value for SAN 582H was 0.096 mg as/L (95 % confidence limits were 0.049 and 0.112 mg as/L). The 5-day no observed adverse effect concentration was 0.004 mg as/L, based upon reductions in growth rates at all test concentrations ≥0.011 mg as/L. Based on mean percent inhibition values calculated using mean cell densities, the 5-day ECSO value for SAN S82H was 0.018 mg as/L. The 95 % confidence 1imits were 0.011 and 0.024 mg as/L. However, the endpoints generated using percent inhibition values based on changes in cell density may have been adversely effected by the slight variations in initial cell density, and the endpoints generated using the growth rate data were deemed more appropriate. NOEC and EC_{50} values with 95 % confidence limits at 120 hours are shown in Table B.9.2-23.

Table B.9.2-23: Effect of SAN 582 H on the growth of green alga *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*)

Concentration [mg as/L] (nominal)	**Negative control**	**Solvent control**	**0.008**	**0.016**	**0.031**	**0.063**	**0.125**
Concentration [mg as/L] (mean measured)	**Negative control**	**Solvent control**	**0.004**	**0.011**	**0.024**	**0.049**	**0.112**
%Inhibition in 5 d (cell density)	-172.6 #	-	22.4	29.0*	62.8*	76.1*	95.2*
%Inhibition in 5 d (growth rate)	-14.4 #	-	1.3	18.3*	27.2*	26.3*	55.5*
Endpoints [mg SAN 582 H/L] (mean measured)							
E_rC_{50} (120 h)	0.096 (95 % confidence limits: 0.049- 0.112)						
E_bC_{50} (120 h)	0.018 (95 % confidence limits: 0.0.11- 0.024)						
NOEC (120 h)	0.004						

Negative values indicate stimulated growth

* Statistically significantly different from the solvent control (α = 0.05)

Conclusions

In a 120-hour algae test with *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*), the E_bC_{50} for SAN 582 H was determined to be 0.018 mg as/L and the E_rC_{50} was 0.096 mg as/L, based on mean measured concentrations. The NOEC was 0.004 mg as/L (mean measured).

KCA 8.2.6.1/3 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Pseudokirchneriella subcapitata*
Date: 25.04.2013
Doc ID: 2013/1078075
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.
Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*; (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.
Test design:	Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg as/L (nominal).
Test conditions:	100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.83 - 8.02 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 92 % to 96 % of nominal at test initiation and from 44 % to 63 % of nominal at test termination. Therefore, the following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.100 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-24.

Table B.9.2-24: Effect of dimethenamid-P on the growth of green alga *Pseudokirchneriella subcapitata*

Concentration [mg as/L] (nominal)	**Control**	**0.0031**	**0.00625**	**0.0125**	**0.0250**	**0.0500**	**0.100**
Concentration [mg as/L] (geometric mean measured)	**Control**	**0.0021**	**0.0043**	**0.0089**	**0.0159**	**0.0373**	**0.0765**
Inhibition in 72 h (growth rate) [%] #	--	-1.5	-0.5	1.4	20.4	42.8	48.3
Inhibition in 72 h (yield) [%] #	--	-9.4	-3.3	7.6	68.2	92.2	94.5
Endpoints [mg as/L] (geometric mean measured)							
E_rC_{50} (72 h)	0.0663 (95 % confidence limits: 0.0558 - 0.0787)						
E_rC_{10} (72 h)	0.0094 (95 % confidence limits: 0.0070 - 0.0126)						
E_yC_{50} (72 h)	0.0138 (95 % confidence limits: 0.0130 - 0.01453)						
E_yC_{10} (72 h)	0.0093 (95 % confidence limits: 0.0081 - 0.0106)						
E_bC_{50} (72 h) 1)	0.0138 (95 % confidence limits: 0.0123 - 0.0151)						
E_bC_{10} (72 h) 1)	0.0093 (95 % confidence limits: 0.0064 - 0.0109)						

Negative values indicate stimulated growth.
1) calculated by the RMS additionally

Conclusions

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} for dimethenamid-P was determined to be 0.0873 mg as/L and the E_yC_{50} was 0.0210 mg as/L, based on nominal concentrations.

KCA 8.2.6.1/4 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Pseudokirchneriella subcapitata* after different exposure durations
Date: 20.01.2014
Doc ID: 2013/1299405
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*; (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; exposure phase of 6 h and 24 h with 2 and 3 test concentrations, respectively, each with 5 replicates per treatment plus a control with 10 replicates; at the end of the exposure phase cell densities were determined, alga cell were transferred to untreated test medium and incubated for a 72 h growth phase; assessment of growth after 72 h growth phase

Endpoints:	EC_{50} with respect to growth rate and yield after exposure over two different exposure phases followed by a 72 h growth phase; area under the curve (AUC) values.
Test concentrations:	6 h exposure scenario: control, 1.2 and 2.4 mg as/L (nominal). 24 h exposure scenario: control, 0.3, 0.6 and 1.2 mg as/L (nominal).
Test conditions:	250 mL glass Erlenmeyer dimple flasks; test volume: 100 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation of both exposure phases; pH 7.70 -7.91 and pH 7.69 - 8.00 after 72 h growth phase for the 6 h and 24 h exposure scenario, respectively; temperature: 22 °C ± 1 °C; initial cell densities: 1x 106 cells/mL for the exposure phase and 1x 104 cells/mL for the growth phase; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit and Weibull-analysis for 24 hour exposure scenario data; no statistical analysis was conducted for the 6-hour exposure data as only two concentrations were tested.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration of both exposure scenarios at test initiation and at the end of the exposure phase. For the 6 h exposure scenario, the mean measured values for dimethenamid-P at test initiation were 52 % and 103 % of nominal concentrations in the 2.4 mg/L and 1.2 mg/L treatment, respectively. At the end of the 6 h exposure period, the mean measured values were 46 % of nominal in the 2.4 mg/L treatment and 96 % of nominal in the 1.2 mg/L treatment. For the 24 h exposure scenario, the mean measured values for dimethenamid-P were between 103 % and 107 % of nominal in all treatments at test initiation. At the end of the 24 h exposure phase, the mean measured values were 29 %, 40 % and 58 % of nominal in the 0.3, 0.6 and 1.2 mg as/L treatment, respectively. The initial recovery confirmed the correct application of the test item, except for the concentration of 2.4 mg/L in a 6-hour exposure (recovery about 50 %). Thus, the concentrations in both treatments of the 6-hour exposure scenario were (nearly) identical, *i.e.* 1.2 mg as/L. Therefore, the biological results of the 2.4 mg/L treatment will be reported based on the measured concentration of 1.2 mg/L. For all other treatments the results are based on nominal test item concentrations.

Biological results: No morphological effects on alga cells were observed in the control and at any test item concentration in both exposure scenarios. The AUC allows comparing different peaks (variable concentration and exposure time) and the resulting effects. The calculated AUC of 0.3 mg/L*d results in effects in the same range for yield and growth rate for the concentration of 1.2 mg/L and exposure time of 6 hours as well as for the concentration of 0.3 mg/L and exposure time of 24 hours. The effects on algal growth rate and yield are summarised in Table B.9.2-25.

Table B.9.2-25: Effect of dimethenamid-P on the growth of green alga *Pseudokirchneriella subcapitata*

Exposure scenario	**6 h exposure phase**		**24 h exposure phase**		
Concentration [mg as/L] (nominal)	**1.2**	**1.2 + (nominal: 2.4)**	**0.3**	**0.6**	**1.2**
Inhibition after 72 h growth phase (growth rate) [%]	10.7	9.9	11.1	15.6	30.7
Inhibition after 72 h growth phase (yield) [%]	43.4	40.8	45.9	57.8	84.4
AUC [mg/L*d] #	0.3	0.3	0.3	0.6	1.2
Endpoints [mg dimethenamid-P/L] (mean measured)					
E_rC_{50}	> 1.2 1)		> 1.2 (extrapolated value: 2.485)		
E_yC_{50}	> 1.2 1)		0.388		

AUC = Area under the curve
+ The biological results in this treatment group are based on the mean measured concentration of 1.2 mg/L.
# Calculation of AUC (Area under the curve) values by multiplication of the test item concentration [mg/L] by the exposure time [d].
1) No statistical analysis was conducted for the 6-hour exposure data as only two concentrations were tested.

Conclusions

The results of this study demonstrate that exposure to dimethenamid-P over time periods typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to more long-term constant exposure simulated in the standard studies on *P. subcapitata*. The E_rC_{50} and E_yC_{50} value for dimethenamid-P after exposure over 6 h followed by a 72 h growth phase are determined to be both > 1.2 mg as/L (based on nominal and mean measured concentrations, respectively). Exposure over 24 h followed by a 72 h growth phase results in an E_rC_{50} of > 1.2 mg as/L (extrapolated value: 2.485 mg as/L) and E_yC_{50} of 0.388 mg/L, based on nominal concentrations.

KCA 8.2.6.1/5 (new study, submitted with renewal dossier)

Author: Hoffmann, F.
Title: Effect of Reg.No. 360 712 (M31, metabolite of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata*
Date: 08.08.2008
Doc ID: 2008/1035874
Guidelines: OECD 201
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M656H031 (M31, Reg. No. 360 712); metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. RS-582TAS-050495, purity: 99.4 %.

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 5 test concentrations with 5 replicates for each plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 10, 18, 32, 56, 100 mg M656H031/L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume: 60 mL; nutrient solution (according to OECD 201); pH 8.1 at test initiation and pH 7.64 - 7.73 at test termination; temperature: 22 °C ± 1 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 8000 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656H031 ranged from 99.7 % to 101.9 % of nominal at test initiation and from 99.0 % to 102.4 % of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. The effects on algal growth rate and yield are summarised in Table B.9.2-26.

Table B.9.2-26: Effect of M656H031 (metabolite of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**10**	**18**	**32**	**56**	**100**
Inhibition in 72 h (growth rate) [%] *	--	-1.5	-0.3	-0.3	1.5	3.1
Inhibition in 72 h (yield) [%] *	--	-8.2	-1.7	-1.8	7.7	15.1
Endpoints [mg M656H031/L] (nominal)						
E_rC_{50} (72 h)	> 100					
E_rC_{10} (72 h)	> 100					
E_yC_{50} (72 h)	> 100					
E_yC_{10} (72 h)	76.6 (95 % confidence limits: 72.2 - 81.2)					

* Negative values indicate stimulated growth compared to the control.

Conclusions

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} and the E_yC_{50} of M656H031 (metabolite of dimethenamid-P) were both determined to be > 100 mg/L, based on nominal concentrations.

KCA 8.2.6.1/6 (new study, submitted with renewal dossier)

Author: Salinas, E.
Title: Reg.No. 364 802 [redacted] - Growth inhibition study in unicellular green algae *Pseudokirchneriella subcapitata* KORSHIKOV
Date: 15.03.2011

Doc ID: 2010/1079231
Guidelines: OECD 201 (2006)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Reg. No. 364 802, [redacted] (BAS 656-PH, Reg. No. 363 851), batch no. B1112B01C4, purity: 98.3 corr. area-%; test substance is a mixture.

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; in-house culture stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 5 test item concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 10, 22, 46, 100, 220 mg/L (nominal).

Test conditions: 250 mL Erlenmeyer dimple flasks plugged with gas permeable silicone sponge caps; test volume: 100 mL; nutrient solution according to OECD 201; pH 7.9 - 8.0 at test initiation and pH 7.9 - 8.5 at test termination; temperature: 22.9 °C - 23.3 °C; initial cell densities: 0.5 x 10^4 cells/mL; continuous light at about 5277 lux (± 15 %), continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted by determination of total organic carbon (TOC) using an infrared gas analyser.

Statistics: Descriptive statistics, determination of EC_x values by interpolation, Dunnett's test ($p \leq 0.01$) for calculation of NOEC.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Measured concentrations for the test item ranged from 91 % to 103 % of nominal concentrations at test initiation and from 88 % to 101 % of nominal at test termination. As the analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 72 hours of exposure, no morphological effects on algae were determined in the control group and at the test item concentrations of up to and including 46 mg/L, whereas at 100 mg/L low cell density occurred and at 220 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at the three highest concentrations based on growth rate data and at the four highest concentrations based on yield data (Dunnett`s test, $p \leq 0.01$). The effects on algal growth rate and yield are summarised in Table B.9.2-27.

Table B.9.2-27: Effect of Reg. No. 364 802 [redacted] on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	Control	10	22	46	100	220
Inhibition in 72 h (growth rate) [%] *	--	-0.416	2.16	9.46*	51.6*	95.9**
Inhibition in 72 h (yield) [%] #	--	-14.1	10.0*	30.5*	91.8*	99.8*
Endpoints [mg/L] (nominal)						
E_rC_{50} (72 h)	97.0					
E_rC_{10} (72 h)	46.5					
NOE_rC (72 h)	22.0					
E_yC_{50} (72 h)	58.9					
E_yC_{10} (72 h)	22.0					
NOE_yC (72 h)	10.0					

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (Dunnett`s test, $p \leq 0.01$)

Conclusions

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of Reg. No. 364 802 [redacted] was determined to be 97.0 mg/L and the E_yC_{50} was 58.9 mg/L, based on nominal concentrations.

KCA 8.2.6.1/7 (new study, submitted with renewal dossier)

Author: Salinas, E.
Title: [redacted] - Growth inhibition study in unicellular green algae *Pseudokirchneriella subcapitata* KORSHIKOV
Date: 10.02.2011 (Amendment: 20.10.2011)
Doc ID: 2010/1154437 (Amendment: 2011/1255812)
Guidelines: OECD 201 (2006)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: [redacted], batch no. B1112B01TCK, purity 90.6 corr. area-%; test item is a mixture.

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; in-house culture; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 5 test item concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 1.0, 3.2, 10, 32, 100 mg/L (nominal).

Test conditions: 250 mL Erlenmeyer dimple flasks closed with glass plugs; test volume: 300 mL; nutrient solution (according to OECD 201); pH 7.0 - 7.1 at test initiation and pH 7.0 - 9.6 at test termination; temperature: 23 °C; initial cell densities: 0.3 x 10^4 cells/mL; continuous light at about 5158 lux (± 15 %), continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted by determination of total organic carbon (TOC) using an infrared gas analyser.

Statistics: Descriptive statistics, determination of EC_x values by interpolation, Dunnett's test ($p \leq 0.01$) for calculation of NOEC.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each treatment group ≥ 10 mg/L at the beginning and at the end of the test since concentrations in the lower test groups were below the limit of quantification (*i.e.* LoQ = 2.8 - 5.9 mg/L). Measured concentrations for the test item in the test solutions of test groups ≥ 10 mg/L ranged from 85 % to 99 % of nominal concentrations at test initiation and from 83 % to 98 % of nominal at test termination. As the analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations. Due to the instability of the test substance, the results should be considered as the effect of the parent test substance and all degradation products.

Biological results: After 72 hours of exposure, no morphological effects on algae were observed in the control group and at the test item concentrations of up to and including 3.2 mg/L, whereas at 10 mg/L low cell density occurred and at 32 and 100 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at the three highest concentrations based on growth rate data and at the four highest concentrations based on yield data (Dunnett`s test, $p \leq 0.01$). The effects on algal growth rate and yield are summarised in Table B.9.2-28.

Table B.9.2-28: Effect of [redacted] on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**1.0**	**3.2**	**10**	**32**	**100**
Inhibition in 72 h (growth rate) [%]	--	1.63	2.79	21.4*	72.3*	100*
Inhibition in 72 h (yield) [%] *	--	-0.015	15.1*	73.6*	98.8*	100*
Endpoints [mg/L] (nominal)						
E_rC_{50} (72 h)	19.2					
E_rC_{10} (72 h)	4.97					
NOE_rC (72 h)	3.2					
E_yC_{50} (72 h)	6.32					
E_yC_{10} (72 h)	2.16					
NOE_yC (72 h)	1.0					

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (Dunnett`s test, $p \leq 0.01$)

Conclusions

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] was determined to be 19.2 mg/L based on nominal concentrations and the E_yC_{50} was 6.32 mg/L (nominal).

KCA 8.2.6.1/8 (new study, submitted with renewal dossier)

Author: Salinas, E.
Title: [redacted] - Growth inhibition study in unicellular green algae *Pseudokirchneriella subcapitata* KORSHIKOV
Date: 31.01.2011
Doc ID: 2010/1185631
Guidelines: OECD 201 (2006)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: [redacted] batch no. B1210B01KE, purity 91.6 area%; the test substance is a mixture of chemical components.

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; in-house culture; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 6 test concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.32, 1.0, 3.2, 10, 32 and 100 mg/L (nominal).

Test conditions: 250 mL Erlenmeyer dimple flasks closed with glass plugs; test volume: 300 mL; nutrient solution (according to OECD 201); pH 7.1 - 7.7 at test initiation and pH 7.5 - 10.1 at test termination; temperature: 23 °C; initial cell densities: 0.3 x 10^4 cells/mL; continuous light at about 5153 lux (± 15 %), continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with MS-detection.

Statistics: Descriptive statistics, determination of EC_x values by interpolation, Dunnett's test ($p \leq 0.01$ and $p \leq 0.05$) for calculation of NOEC.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each treatment group at the beginning and at the end of the test. Measured concentrations for the test item in the test solutions of test item groups ≥ 1.0 mg/L ranged from 62 % to 79 % of nominal at test initiation, while the concentration in the lowest test group was below the limit of quantification (LoQ). At test termination, the measured concentrations in the two highest test item groups were both 15 % of nominal, whereas in all lower test item groups the test item concentrations were below the LoQ. Since the concentration of test substance in test media could not be verified analytically, due to the high degree of uncertainty associated with the quantitative analytical results, the effect concentration can be expressed relative to the nominal concentration or loading rate. Due to the instability of the test substance, the results should be considered as the effect of the parent test substance and all degradation products. Thus, the following biological results are based on nominal concentration.

Biological results: After 72 hours of exposure, no morphological effects on algae occurred in the control group and at test item concentrations of up to and including 10 mg/L, whereas in two highest test item concentrations of 32 mg/L and 100 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at 3.2 mg/L and the two highest concentrations based on growth rate data and at the three highest concentrations based on yield data (Dunnett`s test, $p \leq 0.01$ or $p \leq 0.05$; for details see table below). The effects on algal growth rate and yield are summarised in Table B.9.2-29.

Table B.9.2-29: Effect of [redacted] on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**0.32**	**1.0**	**3.2**	**10**	**32**
Inhibition in 72 h (growth rate) [%] #	--	-4.43	-9.72	6.10 *	2.81	70.0 **
Inhibition in 72 h (yield) [%] #	--	-34.8	-47.4	-0.771	2.95 *	99.4 **
Endpoints [mg/L] (nominal)						
E_rC_{50} (72 h)	22.6					
E_rC_{10} (72 h)	11.3					
E_yC_{50} (72 h)	17.6					
E_yC_{10} (72 h)	10.9					
NOE_rC (72 h)	1.0					
NOE_yC (72 h)	3.2					

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (Dunnett`s test, $p \leq 0.05$).
** Statistically significantly different from the control (Dunnett`s test, $p \leq 0.01$).

Conclusions

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] was determined to be 22.6 mg/L based on nominal concentrations and the E_yC_{50} was 17.6 mg/L (geometric mean measured).

KCA 8.2.6.1/9 (new study, submitted with renewal dossier)

Author: Backfisch K.,
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Ankistrodesmus bibraianus*
Date: 06.05.2013
Doc ID: 2012/1246639
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Unicellular fresh water green alga, *Ankistrodesmus bibraianus*; specification: SAG 278-1; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg as/L (nominal); corresponding to geometric mean measured concentrations of 0, 0.0015, 0.0031, 0.0060, 0.0122, 0.0241 and 0.0492 mg as/L.

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.65 - 7.74 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 48 % to 51 % of nominal at test initiation and from 46 % to 50 % of nominal at test termination. Therefore, the following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.0492 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-30.

Table B.9.2-30: Effect of dimethenamid-P on the growth of green alga *Ankistrodesmus bibraianus*

Concentration [mg as/L] (nominal)	**Control**	**0.0031**	**0.00625**	**0.0125**	**0.0250**	**0.0500**	**0.100**
Concentration [mg as/L] (geometric mean measured)	**--**	**0.0015**	**0.0031**	**0.0060**	**0.0122**	**0.0241**	**0.0492**
Inhibition in 72 h (growth rate) [%]	--	0.6	4.9	9.5	36.9	43.6	50.7
Inhibition in 72 h (yield) [%]	--	2.0	14.7	26.5	70.9	77.2	82.5
Endpoints [mg as/L] (geometric mean measured)							
E_rC_{50} (72 h)	0.0370 (95 % confidence limits: 0.0308 - 0.0444)						
E_rC_{10} (72 h)	0.0037 (95 % confidence limits: 0.0027 - 0.0050)						
E_yC_{50} (72 h)	0.0097 (95 % confidence limits: 0.0086 - 0.0109)						
E_yC_{10} (72 h)	0.0023 (95 % confidence limits: 0.0018 - 0.0030)						

Conclusions

In a 72-hour algae test with *Ankistrodesmus bibraianus*, the E_rC_{50} for dimethenamid-P was determined to be 0.0370 mg as/L and the E_yC_{50} was 0.0097 mg as/L, based on geometric mean measured concentrations.

KCA 8.2.6.1/10 (new study, submitted with renewal dossier)

Author:	Backfisch, K.
Title:	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Desmodesmus subspicatus*
Date:	30.04.2013
Doc ID:	2012/1246638
Guidelines:	OECD 201 (2006), EPA 850.5400
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.
Test species:	Unicellular fresh water green alga, *Desmodesmus subspicatus;* specification: SAG 86.81; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.
Test design:	Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg as/L (nominal); corresponding to geocmetric mean measured concentrations of 0.00160, 0.00315, 0.00625, 0.0127, 0.0246 and 0.0509 mg as/L.
Test conditions:	100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.65 - 7.76 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 50 % to 51 % of nominal at test initiation and from 48 % to 50 % of nominal at test termination. Therefore, the following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.0509 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-31.

Table B.9.2-31: Effect of dimethenamid-P on the growth of green alga *Desmodesmus subspicatus*

Concentration [mg as/L] (nominal)	**Control**	**0.0031**	**0.00625**	**0.0125**	**0.0250**	**0.0500**	**0.100**
Concentration [mg as/L] (geometric mean measured)	**--**	**0.00160**	**0.00315**	**0.00625**	**0.0127**	**0.0246**	**0.0509**
Inhibition in 72 h (growth rate) [%] #	--	-1.7	9.4	6.5	9.8	24.6	38.9
Inhibition in 72 h (yield) [%] #	--	-7.8	31.1	21.7	28.3	62.5	79.3
Endpoints [mg as/L] (geometric mean measured)							
E_rC_{50} (72 h)	> 0.0509 extrapolated: 0.0857 (95 % confidence limits: 0.0617 - 0.1190)						
E_rC_{10} (72 h)	0.00927 (95 % confidence limits: 0.00682 - 0.0126)						
E_yC_{50} (72 h)	0.0183 (95 % confidence limits: 0.0132 - 0.0255)						
E_yC_{10} (72 h)	0.0024 (95 % confidence limits: 0.0012 - 0.0049)						

Negative values indicate stimulated growth.

Conclusions

In a 72-hour algae test with *Desmodesmus subspicatus*, the E_rC_{50} for dimethenamid-P was determined to be > 0.0509 mg as/L (extrapolated: 0.0857 mg as/L) and the E_yC_{50} was 0.0183 mg as/L, based on geometric mean measured concentrations.

KCA 8.2.6.1/11 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Neochloris aquatica*
Date: 08.05.2013
Doc ID: 2012/1246637
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Unicellular fresh water green alga, *Neochloris aquatica*; specification: UTEX 138; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over

72 hours.

Test concentrations: Control, 0.01, 0.03, 0.1, 0.3 and 1.0 mg as/L (nominal)

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.89 - 8.03 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 105 % to 109 % of nominal at test initiation and from 105 % to 118 % of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-32.

Table B.9.2-32: Effect of dimethenamid-P on the growth of green alga *Neochloris aquatica*

Concentration [mg as/L] (nominal)	**Control**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	3.4	4.9	10.3	18.5	25.5
Inhibition in 72 h (yield) [%]	--	11.9	17.0	32.1	50.1	61.9
Endpoints [mg as/L] (nominal)						
E_rC_{50} (72 h)	> 1.0 (95 % confidence limits: n.d.)					
E_rC_{10} (72 h)	0.0871 (95 % confidence limits: 0.0689 - 0.1101)					
E_yC_{50} (72 h)	0.3680 (95 % confidence limits: 0.3000 - 0.4510)					
E_yC_{10} (72 h)	0.0091 (95 % confidence limits: 0.0059 - 0.0141)					

n.d. = not determined due to mathematical reasons

Conclusions

In a 72-hour algae test with *Neochloris aquatica*, the E_rC_{50} for dimethenamid-P was determined to be > 1.0 mg as/L and the E_yC_{50} was 0.368 mg as/L, based on nominal concentrations.
The study author calculated a coefficient of variation (c.v.) of 35.8 % (criterion: ≤35 %) for section-by-section specific growth rate in the control. This value was incomprehensible as the RMS' calculation revealed a lower c.v. of 34.2 %. The study is therefore deemed acceptable.

KCA 8.2.6.1/12 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of

the green alga *Monoraphidium griffithii* after different exposure durations

Date: 27.01.2014
Doc ID: 2013/1299407
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Unicellular fresh water green alga, *Monoraphidium griffithii*, SAG 202-13; in-house culture; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; exposure phase of 6 h and 24 h with 2 and 3 test concentrations, respectively, each with 5 replicates per treatment plus a control with 10 replicates; at the end of the exposure phase cell densities were determined, alga cell were transferred to untreated test medium and incubated for a 72 h growth phase; assessment of growth after 72 h growth phase.

Endpoints: EC_{50} with respect to growth rate and yield after exposure over two different exposure phases followed by a 72 h growth phase; area under the curve (AUC) values.

Test concentrations: 6 h exposure scenario: control, 1.2 and 2.4 mg as/L (nominal).
24 h exposure scenario: control, 0.3, 0.6 and 1.2 mg as/L (nominal).

Test conditions: 250 mL glass Erlenmeyer dimple flasks; test volume: 100 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation of both exposure phases; pH 7.48 -7.53 and pH 7.47 - 7.68 after 72 h growth phase for the 6 h and 24 h exposure scenario, respectively; temperature: 22 °C ± 1 °C; initial cell densities: 1x 10^6 cells/mL for the exposure phase and 1x 10^4 cells/mL for the growth phase; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration of both exposure scenarios at test initiation and at the end of the exposure phase. For the 6 h exposure scenario, the mean measured values for dimethenamid-P were 96 % of nominal concentrations in both treatments at test initiation and 92 % and 94 % of nominal in the 1.2 mg/L and 2.4 mg/L treatment, respectively, at the end of the exposure phase. For the 24 h exposure scenario, the mean measured values for dimethenamid-P were between 89 % and 91 % of nominal in all treatments at test initiation and between 23 % and 48 % at the end of the exposure phase. As initially mean measured concentrations confirmed the correct application of the test item in both exposure scenarios, the following biological results are based on nominal test item concentrations.

Biological results: No morphological effects on alga cells were observed in the control and at any test item concentration in both exposure scenarios. The AUC allows comparing different peaks (variable concentration and exposure time) and the resulting effects. The calculated AUC of 0.3 mg/L*d results in effects in the same range for yield and growth rate for the concentration of 1.2 mg/L and exposure

time of 6 hours as well as for the concentration of 0.3 mg/L and exposure time of 24 hours. The same is true for the treatment groups with AUC values of 0.6 mg/L*d. The effects on algal growth rate and yield are summarised in Table B.9.2-33.

Table B.9.2-33: Effect of dimethenamid-P on the growth of green alga *Monoraphidium griffithii* in different exposure scenarios followed by a growth phase of 72 h

Exposure scenario	**6 h exposure phase**		**24 h exposure phase**		
Concentration [mg as/L] (nominal)	**1.2**	**2.4**	**0.3**	**0.6**	**1.2**
Inhibition after 72 h growth phase (growth rate) [%]	1.7	3.7	4.4	4.3	4.5
Inhibition after 72 h growth phase (yield) [%]	7.7	15.9	18.6	18.1	18.9
AUC [mg/L*d] #	0.3	0.6	0.3	0.6	1.2
	Endpoints [mg dimethenamid-P/L] (nominal)				
E_rC_{50} / E_yC_{50}	> 2.4		> 1.2		

AUC = Area under the curve
# Calculation of AUC (Area under the curve) values by multiplication of the test item concentration [mg/L] by the exposure time [d].

Conclusions

The results of this study demonstrate that exposure to dimethenamid-P over time periods typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to more long-term constant exposure simulated in the standard study on *M. griffithii*. The E_rC_{50} and E_yC_{50} values for dimethenamid-P after exposure over 6 h followed by a 72 h growth phase are determined to be both > 2.4 mg as/L, based on nominal concentrations. Exposure over 24 h followed by a 72 h growth phase results in an E_rC_{50} and an E_yC_{50} value of > 1.2 mg/L (nominal).

KCA 8.2.6.1/13 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Chlamydomonas reinhardtii*
Date: 04.07.2013
Doc ID: 2013/1078084
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Unicellular fresh water green alga, *Chlamydomonas reinhardtii;* specification: UTEX 2243; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg as/L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.09 - 7.71 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 98 % to 102 % of nominal at test initiation and from 97 % to 105 % of nominal at test termination. As the analytical measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-34.

Table B.9.2-34: Effect of dimethenamid-P on the growth of green alga *Chlamydomonas reinhardtii*

Concentration [mg as/L] (nominal)	**Control**	**0.003**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	0.7	1.1	3.2	17.0	66.7	85.9
Inhibition in 72 h (yield) [%]	--	3.3	5.0	13.7	54.4	96.1	99.0
Endpoints [mg as/L] (nominal)							
E_rC_{50} (72 h)	0.2245 (95 % confidence limits: 0.2049 - 0.2460)						
E_rC_{10} (72 h)	0.0620 (95 % confidence limits: 0.0511 - 0.0751)						
E_yC_{50} (72 h)	0.0854 (95 % confidence limits: 0.0797 - 0.0916)						
E_yC_{10} (72 h)	0.0273 (95 % confidence limits: 0.0236 - 0.0317)						

Conclusions

In a 72-hour algae test with *Chlamydomonas reinhardtii*, the E_rC_{50} for dimethenamid-P was determined to be 0.2245 mg as/L and the E_yC_{50} was 0.0854 mg as/L, based on nominal concentrations. It was noted that the mean coefficient of variation for section-by-section specific growth rate in the control was 36.6 % (criterion: ≤35 %) and thus slightly exceeded. However, since *Chlamydomonas reinhardtii* does not belong to the standard green algae and is not listed in Annex 2 (recommended species/strains) of OECD 201 and in view of the low deviation from exponential growth in control replicates as well as the absence of a lag phase, the study is deemed acceptable.

KCA 8.2.6.1/14 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of

	the green alga *Pandorina morum*
Date:	21.06.2013
Doc ID:	2013/1078083
Guidelines:	OECD 201, EPA 850.5400
GLP:	Yes
Validity:	Not acceptable (OECD 201 validity criterion not fulfilled)

Material and Methods

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.
Test species:	Unicellular fresh water green alga, *Pandorina morum*; specification: UTEX 18; stock obtained from the "University of Texas", Austin, USA.
Test design:	Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg as/L (nominal).
Test conditions:	100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.25 - 7.88 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 98 % to 104 % of nominal at test initiation and from 98 % to 112 % of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg as/L. However, it can be seen from the raw data that the test algae was not maintained in a state of exponential growth throughout the test period (see figure below). The mean section-by-section specific growth rates for days 0-1, 1-2 and 2-3 were 1.38, 1.76 and 0.62, respectively, indicating deviation from exponential growth during the exposure period.

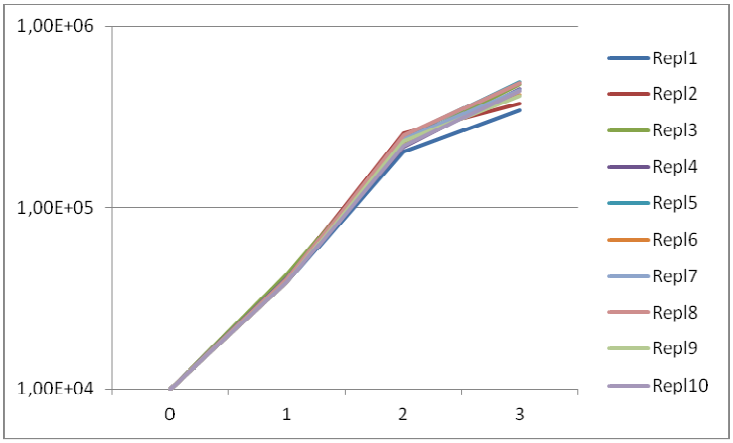

Figure B.9.2-1: Growth of the green alga *Pandorina morum*

The effects on algal growth rate and yield are summarised in Table B.9.2-35.

Table B.9.2-35: Effect of dimethenamid-P on the growth of green alga *Pandorina morum*

Concentration [mg as/L] (nominal)	**Control**	**0.003**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	3.1	3.2	3.8	19.9	40.4	47.0
Inhibition in 72 h (yield) [%]	--	11.9	11.8	14.1	54.3	80.1	85.0
Endpoints [mg as/L] (nominal)							
E_rC_{50} (72 h)	0.9238 (95 % confidence limits: 0.7345 - 1.162)						
E_rC_{10} (72 h)	0.0329 (95 % confidence limits: 0.0230 - 0.0471)						
E_yC_{50} (72 h)	0.0978 (95 % confidence limits: 0.0799 - 0.1196)						
E_yC_{10} (72 h)	0.0120 (95 % confidence limits: 0.0078 - 0.0183)						

Conclusions

The study is not valid as the mean coefficient of variation for section-by-section specific growth rate in the control was 46.2 %, which is considerably above the limit of 35 %. As this criterion was clearly not met for the non-standard green algae, *Pandorina morum* (not listed in Annex 2 of OECD 201), indicating a strong deviation from exponential growth, the study is not deemed acceptable.

KCA 8.2.6.1/15 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Planktosphaeria botryoides*
Date: 22.05.2013
Doc ID: 2013/1078081
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Unicellular fresh water green alga, *Planktosphaeria botryoides*; specification: LB 951; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.01, 0.03, 0.1, 0.3 and 1.0 mg as/L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.43 - 7.59 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 107 % to 110 % of nominal at test initiation and from 85 % to 97 % of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-36.

Table B.9.2-36: Effect of dimethenamid-P on the growth of green alga *Planktosphaeria botryoides*

Concentration [mg as/L] (nominal)	**Control**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	0.6	3.5	14.4	37.2	48.4
Inhibition in 72 h (yield) [%]	--	3.4	14.7	46.7	81.0	88.6
Endpoints [mg as/L] (nominal)						
E_rC_{50} (72 h)	0.9120 (95 % confidence limits: 0.7420 - 1.121)					
E_rC_{10} (72 h)	0.0517 (95 % confidence limits: 0.0370 - 0.0724)					
E_yC_{50} (72 h)	0.1110 (95 % confidence limits: 0.0980 - 0.1250)					
E_yC_{10} (72 h)	0.0203 (95 % confidence limits: 0.0157 - 0.0262)					

Conclusions

In a 72-hour algae test with *Planktosphaeria botryoides*, the E_rC_{50} for dimethenamid-P was determined to be 0.9120 mg as/L and the E_yC_{50} was 0.1110 mg as/L, based on nominal concentrations.

KCA 8.2.6.1/16 (new study, submitted with renewal dossier)

Author:	Backfisch, K.
Title:	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Dictyococcus varians*
Date:	11.06.2013
Doc ID:	2013/1078080
Guidelines:	OECD 201, EPA 850.5400
GLP:	Yes
Validity:	Not acceptable (OECD 201 validity criterion not fulfilled)

Material and Methods

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.
Test species:	Unicellular fresh water green alga, *Dictyococcus varians*; specification: CCALA 331; stock obtained from "Institute of Botany, v.v.i. Academy of Science of the Czech Republic", Trebon, Czech Republic.
Test design:	Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.01, 0.03, 0.1, 0.3 and 1.0 mg as/L (nominal).
Test conditions:	100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.75 - 8.04 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 105 % to 112 % of nominal at test initiation and from 98 % to 109 % of nominal at test termination. As the analytical measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.1 mg as/L. However, it can be seen from the raw data that the test algae was not maintained in a state of exponential growth throughout the test period (see

figure below; cell density expressed as cells/mL). The mean section-by-section specific growth rates for days 0-1, 1-2 and 2-3 were 1.55, 0.60 and 1.48, respectively, indicating deviation from exponential growth during the exposure period. The effects on algal growth rate and yield are summarised in Table B.9.2-37.

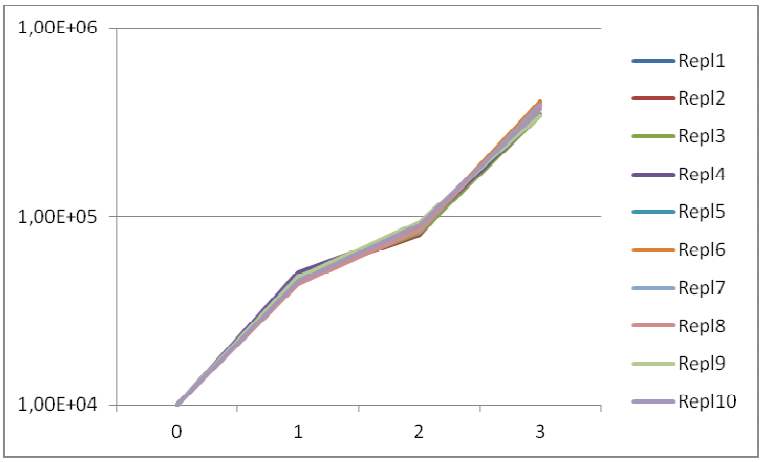

Figure B.9.2-2: Growth of fresh water green alga *Dictyococcus varians*

Table B.9.2-37: Effect of dimethenamid-P on the growth of green alga *Dictyococcus varians*

Concentration [mg as/L] (nominal)	**Control**	**0.001**	**0.003**	**0.01**	**0.03**	**0.1**
Inhibition in 72 h (growth rate) [%]	--	2.6	5.9	17.5	26.3	44.1
Inhibition in 72 h (yield) [%]	--	9.3	19.6	48.0	63.0	81.9
Endpoints [mg as/L] (nominal)						
E_rC_{50} (72 h)	> 0.100 extrapolated: 0.1498 (95 % confidence limits: 0.1213 - 0.1851)					
E_rC_{10} (72 h)	0.0049 (95 % confidence limits: 3.75 - 6.44)					
E_yC_{50} (72 h)	0.0141 (95 % confidence limits: 0.0118 - 0.0168)					
E_yC_{10} (72 h)	0.0010 (95 % confidence limits: 0.0007 - 0.0015)					

Conclusions

The study is not valid as the mean coefficient of variation for section-by-section specific growth rate in the control was 43.7 %, which is above the limit of 35 %. As this criterion was not met for the non-standard green algae, *Dictyococcus varians* (not listed in Annex 2 of OECD 201), indicating a strong deviation from exponential growth, the study is not deemed acceptable.

KCA 8.2.6.1/17 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Monoraphidium griffithii*
Date: 22.05.2013
Doc ID: 2013/1078078
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.
Test species:	Unicellular fresh water green alga, *Monoraphidium griffithii*; specification: SAG 202-13; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.
Test design:	Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.001, 0.003, 0.01, 0.03 and 0.1 mg as/L (nominal).
Test conditions:	100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.78 - 8.15 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 104 % to 110 % of nominal at test initiation and from 55 % to 67 % of nominal at test termination. As the initially measured values confirm the correct application of the test item and the overall mean recovery (0-3 d) of all test concentrations was >80 % (80.1 %), the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.1 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-38.

Table B.9.2-38: Effect of dimethenamid-P on the growth of green alga *Monoraphidium griffithii*

Concentration [mg as/L] (nominal)	**Control**	**0.001**	**0.003**	**0.01**	**0.03**	**0.1**
Inhibition in 72 h (growth rate) [%] #	--	-0.1	2.4	33.3	63.4	66.3
Inhibition in 72 h (yield) [%] #	--	-0.6	9.6	75.9	94.2	95.1
Endpoints [mg as/L] (nominal)						
E_rC_{50} (72 h)	0.0250 (95 % confidence limits: 0.0199 - 0.0313)					
E_rC_{10} (72 h)	0.0026 (95 % confidence limits: 0.016 - 0.0042)					
E_yC_{50} (72 h)	**0.0066 (95 % confidence limits: 0.0061 - 0.0071)**					
E_yC_{10} (72 h)	0.0030 (95 % confidence limits: 0.0026 - 0.0035)					

Negative values indicate stimulated growth.

Conclusions

In a 72-hour algae test with *Monoraphidium griffithii*, the E_rC_{50} for dimethenamid-P was determined to be 0.0250 mg as/L and the E_yC_{50} was 0.0066 mg as/L, based on nominal concentrations.

KCA 8.2.6.1/18 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Schroederia setigera*
Date: 05.07.2013
Doc ID: 2013/1078077
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Unicellular fresh water green alga, Schroederia setigera; specification: LB 2454; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.010, 0.032, 0.10, 0.32 and 1.0 mg as/L (nominal); corresponding to geometric mean measured concentrations of 0, 0.0042, 0.0131, 0.0403, 0.1270 and 0.4055 mg as/L.

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.96 - 7.84 at test

termination; temperature: 22 °C ± 1 °C; initial cell densities 3 x 10^3 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 38 % to 43 % of nominal at test initiation and from 39 % to 42 % of nominal at test termination. Therefore, the following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.4055 mg as/L. The effects on algal growth rate and yield are summarised in Table B.9.2-39.

Table B.9.2-39: Effect of dimethenamid-P on the growth of green alga *Schroederia setigera*

Concentration [mg as/L] (nominal)	**Control**	**0.010**	**0.032**	**0.10**	**0.32**	**1.0**
Concentration [mg as/L] (geometric mean measured)	**--**	**0.0042**	**0.0131**	**0.0403**	**0.1270**	**0.4055**
Inhibition in 72 h (growth rate) [%]	--	1.5	6.0	11.7	22.6	29.5
Inhibition in 72 h (yield) [%]	--	5.6	18.2	32.7	54.2	64.3
Endpoints [mg as/L] (geometric mean measured)						
E_rC_{50} (72 h)	> 0.4055					
E_rC_{10} (72 h)	0.0287 (95 % confidence limits: 0.0200 - 0.0411)					
E_yC_{50} (72 h)	0.1267 (95 % confidence limits: 0.0981 - 0.1635)					
E_yC_{10} (72 h)	0.0051 (95 % confidence limits: 0.0029 - 0.0089)					

Conclusions

In a 72-hour algae test with *Schroederia setigera*, the ErC50 for dimethenamid-P was determined to be > 0.4055 mg as/L and the EyC50 was 0.1267 mg as/L, based on geometric mean measured concentrations.

KCA 8.2.6.1/19 (new study, submitted with renewal dossier)

Author: Backfisch, K.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga *Staurastrum punctulatum*
Date: 12.06.2013
Doc ID: 2013/1078076
Guidelines: OECD 201, EPA 850.5400
GLP: Yes
Validity: Not acceptable (OECD 201 validity criterion not fulfilled)

Material and Methods

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.
Test species:	Unicellular fresh water green alga, *Staurastrum punctulatum*; specification: UTEX 173; stock obtained from the "University of Texas", Austin, USA.
Test design:	Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.010, 0.032, 0.10, 0.32 and 1.0 mg as/L (nominal).
Test conditions:	100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.82 - 7.99 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 99 % to 104 % of nominal at test initiation and from 98 % to 103 % of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg as/L. However, raw data for the cell density indicated a slow growth (lag phase) for the the initial two days followed by a fast growth period showing that the test algae was not maintained in a state of exponential growth throughout the test period (see growth curve data expressed as cells/mL in the figure below). The mean section-by-section specific growth rates for days 0-1, 1-2 and 2-3 were 0.69, 0.82 and 1.81, respectively. The effects on algal growth rate and yield are summarised in Table B.9.2-40.

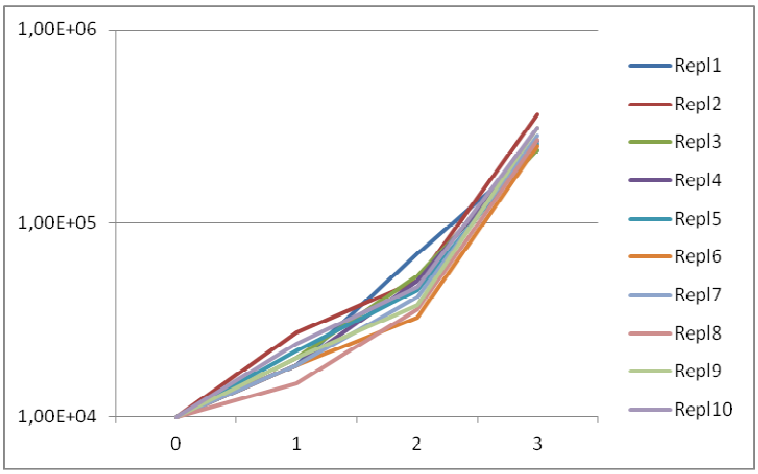

Figure B.9.2-3: Growth of the green alga *Staurastrum punctulatum*

Table B.9.2-40: Effect of dimethenamid-P on the growth of green alga *Staurastrum punctulatum*

Concentration [mg as/L] (nominal)	**Control**	**0.010**	**0.032**	**0.10**	**0.32**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	2.1	7.9	23.7	32.8	34.9
Inhibition in 72 h (yield) [%]	--	7.1	24.2	56.8	69.0	71.3
Endpoints [mg as/L] (nominal)						
E_rC_{50} (72 h)	> 1.0					
E_rC_{10} (72 h)	0.0227 (95 % confidence limits: 0.0125 - 0.0410)					
E_yC_{50} (72 h)	0.1223 (95 % confidence limits: 0.0936 - 0.1597)					
E_yC_{10} (72 h)	0.0055 (95 % confidence limits: 0.0029 - 0.0106)					

Conclusions

The study is not valid as the mean coefficient of variation for section-by-section specific growth rate in the control was 58.3 %, which is considerably above the limit of ≤35 %. As this criterion was clearly not met for the non-standard green algae, *Staurastrum punctulatum* (not listed in Annex 2 of OECD 201), and in view of the fact that a strong deviation from exponential growth was observed, the study is not deemed acceptable.

KCA 8.2.6.1/20 (study evaluated in the initial monograph, 2000)

Author: Gruetzner, I.
Title: Dimethenamid metabolite M3: Acute toxicity to Scenedesmus subspicatus
Date: 20.02.1997
Doc ID: RCC 628975; WAT1999-501
BASF RegDoc.# 97/10274
Guidelines: OECD 201
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid metabolite M3 (batch: PCHB: 1070); purity: 98.5 %.

Test species:	Unicellular fresh water green alga, *Scenedesmus subspicatus* (= *Desmodesmus subspicatus*), strain no. 86.81 SAG
Test design:	Static system; test duration 72 hours; 6 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate (μ) and biomass (AUC) after exposure over 72 hours.
Test concentrations:	Control, 0.32, 1.0, 3.2, 10, 32 and 100 mg as/L (nominal), corresponding to 0.33, 0.99, 3.0, 9.4, 29 and 92.5 mg/L (mean measured concentration).
Test conditions:	50 mL Erlenmeyer dimple flasks; nutrient solution according to OECD 201; pH 8.0 to 8.1 at test initiation and pH 8.8 – 10.2 at test termination; temperature: ranged from 22 °C to 24 °C °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000-9900 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid metabolite M3 ranged from 90.8 % to 99.1 % of nominal at test initiation and from 92.2 % to 104.8 % of nominal at test termination.

Biological results: At five of the six concentrations, a vey flat concentration-response relationship with inhibition rates of 22.3, 31.4, 30.4, 25.8 and 12.9 % was noted for the 0.33, 0.99, 3.0, 9.4, and 29.3 mg/L mean measured test concentrations, respectively. At the highest test concentration of 92.5 mg/L, a major increase to 72.7 % growth inhibition was noted. The observed differences between these inhibition rates and those of the control were evaluated by Dunnett's test. Because of the flat curve between 0.33 and 29.3 mg/I, the statistically significantly different inhibition rates of 31.4, 30.4 and 25.8 % at 0.99, 3.0 and 9.4 mg/L, respectively, versus statistically not significantly different inhibition rates of 22.3 and 12.9 % at 0.33 and 29.3 mg/L are most probably due to random variation rather than to any real differences. Therefore, a better measure of determining the NOEC was the calculation of the EC_{10} by linear regression analysis using the effects of the two highest test concentrations of 29.3 and 92.5 mg/I after 72 hours of exposure. In addition, the EC_{50} was calculated in the same way.

The test is regarded as valid since the cell concentration in the control cultures increased by a factor of >16 within three days. The pH of the control was 8.1 at the start of the test and increased to 10.2 by the end of the test. This increase slightly exceeds the tolerance of 1.5 pH units given by the guidelines, but can be justified by the high cell density (59.2 x 10^4 cells per ml) in the control after 72 hours of exposure which results in an increased CO_2 consumption. Because of this rapid growth a pH increase >1.5 units could not be avoided, although the solutions were continuously stirred at 500 rpm.
The effects on algal growth rate and biomass are summarised in Table B.9.2-41.

Table B.9.2-41: Effect of dimethenamid metabolite M3 on the growth of green alga *Scenedesmus subspicatus* (= *Desmodesmus subspicatus*)

Concentration [mg as/L] (nominal)	**Control**	**0.32**	**1.0**	**3.2**	**10.0**	**32.0**	**100**
Concentration [mg as/L] (m.m.c.)	**Control**	**0.33**	**0.99**	**3.0**	**9.4**	**29.3**	**92.5**
Inhibition in 72 h (growth rate) [%]	--	4.0	7.1	6.0	4.7	3.0	46.6
Inhibition in 72 h (biomass) [%]	--	22.3	31.4	30.4	25.8	12.9	72.7
Endpoints [mg as/L] (nominal)							
E_rC_{50} (72 h)	97.4 (95 % confidence limits: n.d.)						
E_rC_{10} (72 h)	39.4 (95 % confidence limits: n.d.)						
E_bC_{50} (72 h)	68.5 (95 % confidence limits: n.d.)						
E_bC_{10} (72 h)	26.2 (95 % confidence limits: n.d.)						

m.m.c. = mean measured concentrations
n.d.. = not determined

Conclusions

In a 72-hour static algal toxicity study with *Scenedesmus subspicatus (= Desmodesmus subspicatus)* the E_bC_{50} of metabolite M3 (metabolite of dimethenamid-P) was found to be 68.5 mg/L based on mean measured concentrations. The E_rC_{50} was found 97.4 mg/L.

KCA 8.2.6.1/21 (study evaluated in the initial monograph, 2000)

Author: van der Kolk, J.
Title: Dimethenamid oxalamid (M23): 72-hour static acute toxicity test with freshwater alga *Selenastrum capricornutum*
Date: 16.01.1995
Doc ID: 94-005-1018; WAT95-00669
BASF RegDoc.# 95/11320
Guidelines: OECD 201; US-EPA 123-2
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid oxalamide (M23) (Lot # RS-5820XA- 121593); purity: 99.95%.

Test species: *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*); source: Springborn culture

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate (μ) and biomass (AUC) after exposure over 72 hours.

Test concentrations: Control, 100 mg as/L (nominal), corresponding to 94.0 mg/L based on mean measured concentrations.

Test conditions: 100 mL Erlenmeyer flasks; nutrient solution according to OECD 201; pH 6.67

to 7.61 at test initiation and pH 7.76 – 7.32 at test termination; temperature: ranged from 24 °C to 25 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 6100-6700 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and biomass.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid oxalamide (M23) was 93 % of nominal at test initiation and 94 % of nominal at test termination.

Biological results: At test termination, cell density in the control averaged 51 x 10^4 cells/mL. The increase in cell density in the control cultures within 72 hours was more than the minimum factor of 16 prescribed by the guidelines (OECD 1984, EC 1992), which renders this test valid. The cell density in the 100 mg/L test concentration averaged 55 x 10^4 cells/mL, respectively. SublethaI effects (e.g. bloated cells) were seen in the 100 mg/L test concentration. No effects were observed in the control. The biomass was calculated as Area Under the growth Curve (AUC), for each observation interval. The total AUC averaged 42 days*cells/mL in both the control and the 100 mg/L level. The average growth rate in the control was 1.3 day^{-1}. The average growth rate in the 100 mg/L test concentration was 1.4 day^{-1}. The growth rate of the algae was not influenced by 100 mg/L M23.

Conclusions

In a 72-hour static algal toxicity study with *Selenastrum capricornutum (= Pseudokirchneriella subcapitata)* the E_bC_{50} and E_rC_{50} values of metabolite M23 (metabolite of dimethenamid-P) were found to be >94 mg/L based on mean measured concentrations.

KCA 8.2.6.1/22 (study evaluated in the initial monograph, 2000)

Author: van der Kolk, J.
Title: Dimethenamid sulfonate sodium salt (M27): 72-hour static acute toxicity test with the freshwater alga Selenastrum capricornutum
Date: 16.01.1995
Doc ID: 94-008-1018; WAT95-00670
BASF RegDoc.# 95/11332
Guidelines: OECD 201; US-EPA 123-2
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid sulfonate sodium salt (M27), Lot # RS-582SSS-010494; purity: 97.51 %.

Test species: *Selenastrum capricornutum* (= *Pseudokirchneriella subcapitata*); source: Springborn culture

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 3 replicates per treatment plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate (μ) and biomass (AUC) after exposure over 72 hours.

Test concentrations: Control, 13, 25, 50, 100 and 200 mg as/L (nominal), corresponding to 13, 25, 53, 103 and 208 mg/L based on mean measured concentrations.

Test conditions: 100 mL Erlenmeyer flasks; nutrient solution according to OECD 201; pH 7.85 to 7.95 at test initiation and pH 7.99 – 8.16 at test termination; temperature: ranged from 24 °C to 25.5 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 6100-7800lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and biomass.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured concentrations of M27, were 13, 25, 53, 103, and 208 mg/L (99 %, 99 %, 106 %, 103 %, and 104 % of nominal concentrations), respectively.

Biological results: Cell density in the control averaged 70 x 10^4 cells/mL. The increase in cell density in the control cultures within 72 hours was more than the minimum factor of 16 prescribed by the guidelines (OECD 1984, EC 1992), which renders this test valid.
The cell density in the 13, 25, 50, 100, and 200 mg/L test concentration averaged 65, 64, 69, 61, and 64 x 10^4 cells/mL, respectively, and were not significantly different to the control (Dunnett's test). Some bloated cells, cell fragments and misshaped cells were observed in the test cultures containing M27. No effects were observed in the control. Inhibition of biomass (cell density) and the total Area Under the growth Curve (AUC) for day 3 are presented in the table below. In the control the total AUC averaged 58 days·cells/mL. The average total AUC in the 13, 25, 50, 100, and 200 mg/L test concentration were 51, 51, 54, 48 and 49 days·cells/mL, and were statistically not different from the control (Dunnett's test). The effects on algal biomass are summarised in Table B.9.2-42.

Table B.9.2-42: Effect of dimethenamid sulfonate sodium salt (M27) on the growth of green alga *Senesatrum capricornutum (= Desmodesumus capricornutum)*

Concentration [mg as/L] (nominal)	**Control**	**13**	**25**	**50**	**100**	**200**
Concentration [mg as/L] (mean measured)	**--**	**13**	**25**	**53**	**103**	**208**
Inhibition in 72 h (AUC) [%]	--	12	12	7	17	16
Inhibition in 72 h (cell density) [%]	--	7	9	1	13	9
Endpoints [mg as/L] (geometric mean measured)						
E_rC_{50} (72 h)	> 208 (95 % confidence limits: n.d.)					
E_yC_{50} (72 h)	> 208 (95 % confidence limits: n.d.)					
NOEC (72 h)	> 208					

Conclusions

Both the biomass E_bC_{50} (based on AUC) and the growth rate E_rC_{50} are higher than 200 mg/L (> 208 mg/L, based on mean measured concentrations). In this assay the 72-hour NOEC based on the growth rate, was 200 mg/L (208 mg/L, based on mean measured concentration), the highest concentration tested. Some abnormal cells were observed in the 100 mg/L test concentration, but these had no influence on the growth of the algae.

B.9.2.6.2 Effects on growth of an additional algal species

KCA 8.2.6.2/1 (new study amendment, submitted with renewal dossier)

Author:	Hoberg, J. (amended by Kubitza, J., 2005a)
Title:	SAN 1289H Technical - toxicity to the freshwater diatom, *Navicula pelliculosa*
Date:	20.01.1997 (Amendment: 2005)
Doc ID:	96-11-6782; WAT1999-491; BASF RegDoc# 97/5171; 1997/10745 (Amendment: 2005/1003999)
Guidelines:	EPA 122-2, EPA 123-2
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item:	Dimethenamid-P (SAN 1289H; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.
Test species:	*Navicula pelliculosa*; source: Springborn culture.
Test design:	Static system; test duration 120 hours; 6 test item concentrations, each with 3 replicates per treatment including controls; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and biomass after exposure over 120 hours.
Test concentrations:	Control, 0.031, 0.063, 0.13, 0.25, 0.50 and 1.0 mg as/L (nominal), corresponding to mean measured concentrations of 0.028, 0.056, 0.10, 0.21, 0.41 and 0.89 mg as/L
Test conditions:	250-mL Erlenmeyer flasks; test volume 100 mL; AAP medium was used to prepare the exposure solutions; pH was adjusted to 7.5±0.1; pH 7.2-7.5 at test initiation and pH 7.9 - 8.5 at test termination; temperature: 25 °C; initial cell densities 1 x 10^4 cells/mL; continuous light intensity within the range 4500-4700 lux; continuous shaking.
Analytics:	Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and biomass.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Mean measured concentrations ranged from 80 to 89 % of the nominal concentrations and defined the treatment levels tested as 0.028, 0.056, 0.10,

0.21, 0.41 and 0.89 mg as/L.

Biological results: At test termination, cells exposed to the treatment levels and the control were observed to be normal. Control cultures averaged 94 x 10^4 cells/mL at test termination. Cell density in the exposure levels (0.028, 0.056, 0.10, 0.21, 0.41 and 0.89 mg as/L) averaged 94, 92, 80, 60, 40 and 24 x 10^4 cells/mL, respectively, at test termination. Statistical analysis (Williams' Test) of this data established a significant reduction in cell density at the 0.10, 0.21, 0.41 and 0.89 mg as/L treatment levels when compared to the performance of the control. Inhibitions of cell growth (cell density), NOEC, EC_{50} values and their corresponding 95 % confidence limits are summarised in Table B.9.2-43.

In the Amendment by Kubitza (2005a; Doc ID: 2005/1003999), additional endpoints related to growth rate (r) and biomass (b) after 72, 96 and 120 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). The respective endpoints were based on mean measured concentrations.

Table B.9.2-43: Effect of dimethenamid-P on the growth of the diatom *Navicula pelliculosa*; endpoints based on nominal concentrations amended by Kubitza (2005a)

Concentration [mg as/L] (nominal)	**control**	**0.031**	**0.063**	**0.13**	**0.25**	**0.50**	**1.0**
Concentration [mg as/L] (measured)	**control**	**0.028**	**0.056**	**0.10**	**0.21**	**0.41**	**0.89**
Inhibition in 72 h (biomass) [%]	--	9.3	16.3	38.8	61.4	88.1	103.5
Inhibition in 72 h (growth rate) [%]	--	3.3	6.2	16.9	31.6	63.8	138.1
Inhibition in 96 h (biomass) [%]	--	-13.8	8.5	18.1	33.0	35.1	58.5
Inhibition in 96 h (growth rate) [%]	--	-3.7	2.8	5.4	10.9	11.8	24.4
Inhibition in 120 h (biomass) [%]	--	0.0	1.4	15.1	36.6	58.1	75.6
Inhibition in 120 h (growth rate) [%]	--	0.0	0.3	3.5	10.0	19.2	30.4
Endpoints [mg as/L] (mean measured)							
E_rC_{50} (72 h)	0.287 (95 % confidence limits: 0.277 – 0.297						
E_rC_{10} (72 h)	0.082 (95 % confidence limits: 0.076 – 0.088)						
E_bC_{50} (72 h)	**0.154 (95 % confidence limits: 0.148 – 0.160)**						
E_bC_{10} (72 h)	0.031 (95 % confidence limits: 0.029 – 0.034)						
E_rC_{50} (96 h)	4.048 (95 % confidence limits: 3.039 – 5.393)						
E_rC_{10} (96 h)	0.251 (95 % confidence limits: 0.226 – 0.279)						
E_bC_{50} (96 h)	0.596 (95 % confidence limits: 0.548 – 0.649)						
E_bC_{10} (96 h)	0.070 (95 % confidence limits: 0.063 – 0.077)						
E_rC_{50} (120 h)	1.717 (95 % confidence limits: 1.480 – 1.992)						
E_rC_{10} (120 h)	0.246 (95 % confidence limits: 0.227 – 0.267)						
E_bC_{50} (120 h)	0.352 (95 % confidence limits: 0.336 – 0.369)						
E_bC_{10} (120 h)	0.088 (95 % confidence limits: 0.083 – 0.095)						
NOEC (120 h)	0.056						

Conclusions

The lowest E_bC_{50} and E_rC_{50} values for dimethenamid-P were 0.154 and 0.287 mg as/L, respectively, based on mean measured concentrations. The NOEC was 0.056 mg as/L (mean measured).

KCA 8.2.6.2/2 (study evaluated in the initial monograph, 2000)

Author: Hoberg, J.
Title: SAN 582H Technical - Toxicity to the freshwater diatom, *Navicula pelliculosa*
Date: 30.04.1992
Doc ID: 92-4-4206; WAT1999-493
BASF RegDoc# 92/12458
Guidelines: EPA 122-2 (Draft)
GLP: Yes
Validity: Not acceptable (OECD 201 validity criteria not fulfilled)

Material and Methods

Test item: Dimethenamid racemate (SAN-582-H); Batch #9022; purity: 96.9 %.

Test species: *Navicula pelliculosa*; source: Springborn culture.

Test design: Static system; test duration 120 hours; 1 test item concentrations, each with 5 replicates per treatment including controls; daily assessment of growth.

Endpoints: EC_{50}, NOEC with respect to growth rate and biomass after exposure over 120 hours.

Test concentrations: Control, 2.0 mg as/L (nominal), corresponding to mean measured concentrations of 1.2 mg as/L

Test conditions: 125 mL Erlenmeyer flasks; test volume 50 mL; AAP medium was used to prepare the exposure solutions; pH 7.4 - 7.8; temperature: 19-20 °C; initial cell densities 1 x 10^4 cells/mL; continuous light intensity within the range 1300-1600 lux; continuous shaking.

Analytics: Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and biomass, t-test for determination of NOEC

Results and Discussion

Analytical measurements: The concentration of test material in the treatment level decreased slightly between sampling intervals, averaging 60 % of nominal concentration. Based on these analyses, the treatment level was defined as 1.2 mg as/L (mean measured).

Biological results: After 120-hours of exposure, cell densities (sonicated) within the 1.2 mg as/L treatment level averaged 73 x 10^4 cells/mL. Cell densities in the control cultures averaged 72 x 10^4 cells/mL. Statistical analysis (t-est) demonstrated no significant difference ($p \leq 0.05$) between cell densities in the treatment level tested after 120 hours of exposure as compared with control cell density. Thus, the 120-hour NOEC based on cell density after 120-hours of exposure was determined to be ≥1.2 mg as/L. The 120-hour EC_{50} was estimated to be > 1.2 mg as/L.

However, it can be seen from the raw data that *N. pelliculosa* was not maintained in a state of exponential growth throughout the test period (see table below).

Table B.9.2-44: Effect of dimethenamid (SAN 582H Technical) on the growth of the diatom *Navicula pelliculosa* (Hoberg, 1992)

Cell density (×10^4 cells/mL) of *N. pelliculosa* after 1, 2, 3, 4 and 5 days of exposure to SAN 582H technical						
Control replicate	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
A	1.0	3.0	4.0	5.0	25.0	71.0
B	1.0	1.0	6.0	10.0	16.0	68.0
C	1.0	2.0	3.0	7.0	18.0	67.0
D	1.0	2.0	1.0	10.0	20.0	76.0
E	1.0	3.0	1.0	14.0	12.0	78.0
Mean (SD)		2 (1)	3 (2)	9 (3)	18 (5)	72 (5)

Conclusions

The study is not valid according to OECD 201 since exponential growth was not maintained during the test and none of the validity criteria were fulfilled:

- 16-fold increase within 72 hours (actual: 9.2-fold);
- The mean coefficient of variation for section-by-section specific growth rates coefficients of variation (actual: 131 %);
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures (actual: 18.2 %).

KCA 8.2.6.2/3 (new study amendment, submitted with renewal dossier)

Author:	Hoberg, J. (amended by Kubitza, J., 2004a)
Title:	SAN 1289 H Technical - toxicity to the freshwater blue-green alga, *Anabaena flos-aque*
Date:	20.01.1997 (Amendment: 2004)
Doc ID:	96-12-6798; WAT1999-490; BASF RegDoc# 97/5173 (Amendment: 2004/1025685)
Guidelines:	EPA 122-2, EPA 123-2
GLP:	Yes
Validity:	Not acceptable (OECD 201 validity criteria not fulfilled)

Material and Methods

Test item:	Dimethenamid-P (SAN 1289H; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.
Test species:	*Anabaena flos-aque*; source: Springborn culture.
Test design:	Static system; test duration 120 hours; 6 test item concentrations, each with 3 replicates per treatment including controls; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and biomass after exposure over 120 hours.
Test concentrations:	Control, 0.031, 0.065, 0.13, 0.25, 0.50 and 1.0 mg as/L (nominal), corresponding to mean measured concentrations of 0.028, 0.049, 0.11, 0.26, 0.41 and 0.86 mg as/L
Test conditions:	250-mL Erlenmeyer flasks; test volume 100 mL; AAP medium was used to prepare the exposure solutions; pH ranged from 7.3-7.5 at test initiation and from 8.3 - 8.9 at test termination; temperature: 25 °C; initial cell densities 0.3 x 10^4 cells/mL; continuous light intensity within the range 2200-2400 lux; continuous shaking.
Analytics:	Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and biomass.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Mean measured concentrations at test termination ranged from 75 to 110 % of the nominal concentrations and defined the treatment levels tested as 0.028, 0.049, 0.11, 0.26, 0.41 and 0.86 mg as/L.

Biological results: At test termination (120 h), cells exposed to the treatment levels and the control were observed to be normal. Control cultures averaged 94 x 10^4 cells/mL at test termination. Cell density in the exposure levels (0.028, 0.049, 0.11, 0.26, 0.41 and 0.86 mg as/L) averaged 93, 85, 78, 58, 40 and 31 x 10^4 cells/mL, respectively, at test termination. Statistical analysis (Williams' Test) of this data established a significant reduction in cell density at the 0.49, 0.11, 0.26, 0.41 and 0.86 mg as/L treatment levels when compared to the performance of the control.

However, raw data for the cell density indicated a lag phase for the the initial two days followed by a fast growth period showing that the test algae was not maintained in a state of exponential growth throughout the test period (see table below).

Table B.9.2-45: Effect of dimethenamid (BAS 656 H) on the growth of the diatom *Navicula pelliculosa* (Hoberg, 1997)

Cell density (×10^4 cells/mL) of *A. flos-aque* after 1, 2, 3, 4 and 5 days of exposure to SAN 1289H technical						
Control replicate	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
A	0.3	0.0	4.3	8.3	25	87
B	0.3	3.0	2.8	15	49	101
C	0.3	0.0	4.8	10	46	96
Mean (SD)		1.0 (1.7)	3.9 (1.0)	11 (3.6)	40 (3.6)	94 (7.0)

Inhibitions of cell growth (cell density), NOEC, EC_{50} values and their corresponding 95 % confidence limits are summarised in Table B. 9.2-46.

In the amendment by Kubitza (2004a; Doc ID: 2004/1025685), additional endpoints related to growth rate (r) and biomass (b) after 72, 96 and 120 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). The respective endpoints were based on mean measured concentrations.

Table B.9.2-46: Effect of dimethenamid-P on the growth freshwater blue-green alga, *Anabaena flos-aque* Kubitza (2004a)

Concentration [mg as/L] (nominal)	**control**	**0.031**	**0.065**	**0.13**	**0.25**	**0.50**	**1.0**
Concentration [mg as/L] (measured)	**control**	**0.028**	**0.049**	**0.11**	**0.26**	**0.41**	**0.86**
Inhibition in 72 h (biomass) [%]	--	-20.7	35.5	45.4	58.0	65.4	73.8
Inhibition in 72 h (growth rate) [%]	--	-5.9	11.6	15.9	23.3	30.4	40.3
Inhibition in 96 h (biomass) [%]	--	3.4	31.9	42.0	72.4	74.3	74.1
Inhibition in 96 h (growth rate) [%]	--	0.3	7.1	10.4	25.4	28.6	27.9
Inhibition in 120 h (biomass) [%]	--	1.4	11.0	17.3	38.5	57.9	67.5
Inhibition in 120 h (growth rate) [%]	--	0.3	2.0	3.3	8.4	15.0	19.5
Endpoints [mg as/L] (mean measured)							
E_rC_{50} (72 h)	1.34 (95 % confidence limits: 1.136 – 1.581						
E_rC_{10} (72 h)	0.073 (95 % confidence limits: 0.076 – 0.083)						
E_bC_{50} (72 h)	0.194 (95 % confidence limits: 0.182 – 0.206)						
E_bC_{10} (72 h)	0.019 (95 % confidence limits: 0.016 – 0.021)						
E_rC_{50} (96 h)	2.064 (95 % confidence limits: 1.669 – 2.551)						
E_rC_{10} (96 h)	0.099 (95 % confidence limits: 0.087 – 0.112)						
E_bC_{50} (96 h)	**0.162 (95 % confidence limits: 0.153 – 0.171)**						
E_bC_{10} (96 h)	0.019 (95 % confidence limits: 0.017 – 0.022)						
E_rC_{50} (120 h)	4.539 (95 % confidence limits: 3.339 – 6.170)						
E_rC_{10} (120 h)	0.311 (95 % confidence limits: 0.280 – 0.345)						
E_bC_{50} (120 h)	0.383 (95 % confidence limits: 0.361 – 0.407)						
E_bC_{10} (120 h)	0.060 (95 % confidence limits: 0.055 – 0.066)						
NOEC (120 h)	0.028						

Conclusions

The study is not valid according to OECD 201 (2011) for the following reasons:

- The growth of *Anabaena flos-aque* was not exponential throughout the test period;
- The mean coefficient of variation for section-by-section specific growth rates exceeded the validity criterion of 35 %.

KCA 8.2.6.2/4 (study evaluated in the initial monograph, 2000)

Author: Hoberg, J.
Title: SAN 582H Technical -Toxicity to the freshwater alga, *Anabaena flos-aquae*
Date: 12.05.1992
Doc ID: 92-5-4249; WAT98-00340
BASF RegDoc# 92/12457
Guidelines: EPA 122-2, EPA 123-2

GLP: Yes
Validity: Not acceptable (OECD 201 validity criteria not fulfilled)

Material and Methods

Test item: Dimethenamid racemate (SAN-582-H); Batch #9022; purity: 96.9 %.

Test species: *Anabaena flos-aque*; source: Springborn culture.

Test design: Static system; test duration 120 hours; 6 test item concentrations, each with 5 replicates per treatment including controls; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and biomass after exposure over 120 hours.

Test concentrations: Control, 0.063, 0.13, 0.25, 0.50, 1.0, 2.0 mg as/L (nominal), corresponding to mean measured concentrations of 0.057, 0.096, 0.22, 0.36, 0.73, 1.8 mg as/L

Test conditions: 125 mL Erlenmeyer flasks; test volume 50 mL; AAP medium was used to prepare the exposure solutions; pH ranged from 7.3-7.5 at test initiation and from 9.4-9.5 at test termination; temperature: 24-25 °C; initial cell densities 1.0 x 10^4 cells/mL; continuous light intensity within the range 1600-2300 lux; continuous shaking.

Analytics: Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and biomass.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Mean measured concentrations at test termination ranged from 77 to 90 % of the nominal concentrations and defined the treatment levels tested as 0.057, 0.096, 0.22, 0.36, 0.73 and 1.8 mg as/L. The mean measured concentrations averaged 81 % of nominal target concentrations.

Biological results: The test organism, *Anabaena flos-aquae*, tends to form chains during cell growth. This trait causes cell counts to be sporadic prior to test termination when the solutions can be vigorously shaken to provide a homogeneous distribution of cells. After 72 hours, cell densities in the control averaged 16 x 10^4 cells/mL. Cell densities in the three highest concentrations tested (1.8, 0.73 and 0.36 mg as/L) averaged 4, 6 and 4 x 10^4 cells/mL, respectively, and were determined to be significantly reduced ($p \leq 0.05$) based on Williams' Test as compared to that of the control. Cell density in the remaining concentrations tested (0.22, 0.096 and 0.057 mg as/L) averaged 14, 12 and 13 x 10^4 cells/mL and were not statistically different trom the control data (16 x 10^4 cells/mL). Thus, the 72-hour NOEC based on cell density was determined to be 0.22 mg as/L The 72-hour EC_{50}, based on cell density, was calculated to be 0.35 mg as/L with 95 % confidence limits of <0.01 - 5600 mg as/L.

However, it can be seen from the raw data that *A. flos-aque* was not maintained in a state of exponential growth throughout the test period (see table below).

Table B.9.2-47: Effect of dimethenamid (SAN 582H Technical) on the growth of the freshwater alga *Anabaena flos-aquae (*Hoberg, 1992)

Cell density (×10^4 cells/mL) of *A. flos-aque* after 1, 2, 3, 4 and 5 days of exposure to SAN 582H technical						
Control replicate	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
A	1.0	4	21	12	22	150
B	1.0	3	0	17	23	96
C	1.0	7	6	29	17	97
D	1.0	0	0	10	2	197
E	1.0	0	8	14	11	132
Mean (SD)		3.0 (3)	7 (9)	16 (7)	15 (9)	134 (42)

Inhibitions of cell growth (cell density), NOEC, EC_{50} values and their corresponding 95 % confidence limits are summarised in Table B.9.2-48.

Table B.9.2-48: Effect of SAN 582H on the growth of the blue-green algae *Anabaena flos-aque*

Concentration [mg as/L] (nominal)	**control**	**0.063**	**0.13**	**0.25**	**0.5**	**1**	**2**
Concentration [mg as/L] (measured)	**control**	**0.057**	**0.096**	**0.22**	**0.36**	**0.73**	**1.8**
Inhibition in 72 h (biomass) [%]	--	19	25	13	75	63	75
Inhibition in 72 h (growth rate) [%]	--	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
Inhibition in 120 h (biomass) [%]	--	16	20	52	49	60	63
Inhibition in 120 h (growth rate) [%]	--	25	4	48	-3	43	68
Endpoints [mg as/L] (mean measured)							
E_bC_{50} (72 h)	0.35 (95 % confidence limits: 0.00 – 5900)						
E_rC_{50} (72 h)	n.d.#						
E_bC_{50} (120 h)	0.45 (95 % confidence limits: 0.072 – 3.0)						
E_rC_{50} (120 h)	1.2 (95 % confidence limits: 0.11-65)						
NOEC (120 h)	0.22						

not determined due to insufficient concentration-response to calculate EC values

Conclusions

The study is not valid as the following validity criteria laid down in OECD 201 (2011) were not fulfilled:

- Exponential growth of the selected test alga was not maintained throughout the test period;
- The mean coefficient of variation for section-by-section specific growth rates was considerably higher than 35 % (actual: >100 %);
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was above the 10 % limit (actual: 15 %) for less frequently tested species.

KCA 8.2.6.2/5 (new study, submitted with renewal dossier)

The following algal toxicity study on the marine diatom *Skeletonema costatum* performed with the

active substance dimethenamid-P has not been evaluated previously on EU level.

Author:	Hoberg, J. (amended by Kubitza, 2005b)
Title:	SAN 1289H technical - Toxicity to the marine diatom, *Skeletonema costatum*
Date:	20.01.1997 (Amendment: 2005)
Doc ID:	1997/10743 (Amendment: 2005/1004000)
Guidelines:	EPA 122-2, EPA 123-2
GLP:	Yes
Validity:	Not acceptable (OECD 201 validity criterion not fulfilled)

Material and Methods

Test item:	Dimethenamid-P (BAS 656 H; Reg. No. 363 851), batch no. 6663-50-1, purity: 91.1 %.
Test species:	Marine diatom, *Skeletonema costatum*, strain CCMP 1332, Class Bacillariophyceae; obtained from Bigelow Laboratories, West Boothbay Harbor, Maine, maintain in stock culture at Springborn Laboratories, Inc.
Test design:	Static system (5 days); 6 test concentrations plus a control with 3 replicates for each; daily assessment of growth.
Endpoints:	EC_{25} and EC_{50} with respect to cell density, EC_{10} and EC_{50} with respect to growth rate and yield (recalculation), NOEC.
Test concentrations:	Control, 0.013, 0.031, 0.065, 0.13, 0.25, 0.50 mg as/L (nominal) equivalent to mean measured concentrations of 0, 0.013, 0.030, 0.048, 0.11, 0.22 and 0.45 mg dimethenamid-P/L
Test conditions:	250 mL Erlenmeyer flasks; test volume: 100 mL; nutrient solution (AES medium); pH 8.1 – 8.2 at test initiation and pH 8.2 – 9.0 at test termination; temperature: 20 °C ± 1 °C; initial cell densities: 1 x 10^4 cells/mL; photoperiod of 16 h light : 8 h darkness, light intensity: 3900 to 4700 lux, continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a gas chromatographic procedure.
Statistics:	Descriptive statistics, probit analysis for determination of EC_x values; Williams Test for determination of 120 h NOEC value.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for dimethenamid-P ranged from 77 % to 100 % of nominal concentrations at test initiation and from 62 % to 100 % of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest test item concentration tested. Statistically significant effects compared to the control were observed at the four highest tested concentrations after exposure over 120 hours (William`s Test, $\alpha = 0.05$).

However, it can be seen from the raw data that the test algae was not maintained in a state of

exponential growth throughout the test period (see table and figure below).

Table B.9.2-49: Effect of dimethenamid-P (SAN 1289H Technical) on the growth of the marine diatom *Skeletonema costatum* (Hoberg, 1997)

Cell density (×10^4 cells/mL) of *S. costatum* after 1, 2, 3, 4 and 5 days of exposure to SAN 1289H technical						
Control replicate	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
A	1.0	4.3	9.0	29.0	68.0	108.0
B	1.0	6.5	13.0	31.0	65.0	106.0
C	1.0	2.5	14.0	33.0	53.0	111.0
Mean (SD)		4 (2)	12 (3)	31 (2)	62 (8)	108 (3)

It was noted that the validation criterion "mean coefficient of variation for section-by-section specific growth rate in the control" required by the OECD 201 was not fulfilled as the value was >35 % (43.1 %), indicating deviation from exponential growth during the exposure period. Growth curves expressed as cells per mL for each replicate are given in the figure below. The effects on algal growth rate and development of biomass are summarised in Table B.9.2-50.

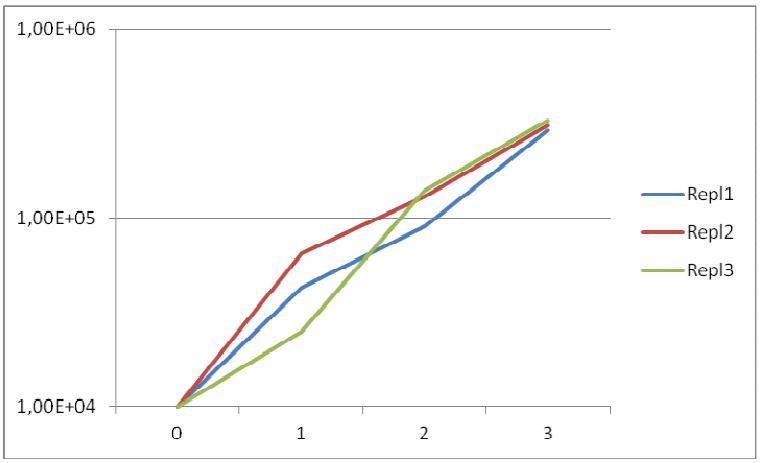

Figure B.9.2-4: Growth of the marine diatom *Skeletonema costatum*

Table B.9.2-50: Effect of dimethenamid-P on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg as/L] (nominal)	**Control**	**0.013**	**0.031**	**0.065**	**0.13**	**0.25**	**0.50**
Concentration [mg as/L] (mean measured)	**Control**	**0.013**	**0.030**	**0.048**	**0.11**	**0.22**	**0.45**
Inhibition in 120 h (cell density) [%]	--	2.2	4.6	24 *	36 *	76 *	89 *
Inhibition in 72 h (growth rate) [%] §	--	-0.9	2.6	7.9	19.1	51.6	53.1
Inhibition 72 h (biomass) [%] §	--	-3.3	8.9	24.4	48.9	84.1	86.2
Endpoints [mg as/L] (mean measured)							
EC_{50} (120 h)	0.120 (95 % confidence limits: 0.054 - 0.260)						
NOEC (120 h)	0.030						
E_rC_{50} (72 h) #	0.309 (95 % confidence limits: 0.290 - 0.330)						
E_rC_{10} (72 h) #	0.060 (95 % confidence limits: 0.055 - 0.064)						
E_bC_{50} (72 h) #	**0.109 (95 % confidence limits: 0.105 - 0.114)**						
E_bC_{10} (72 h) #	0.030 (95 % confidence limits: 0.028 - 0.032)						
E_rC_{50} (96 h) #	0.362 (95 % confidence limits: 0.339 - 0.386)						
E_rC_{10} (96 h) #	0.083 (95 % confidence limits: 0.077 - 0.088)						
E_bC_{50} (96 h) #	0.125 (95 % confidence limits: 0.120 - 0.130)						
E_bC_{10} (96 h) #	0.041 (95 % confidence limits: 0.038 - 0.043)						
E_rC_{50} (120 h) #	0.478 (95 % confidence limits: 0.438 - 0.522)						
E_rC_{10} (120 h) #	0.088 (95 % confidence limits: 0.082 - 0.095)						
E_bC_{50} (120 h) #	0.124 (95 % confidence limits: 0.119 - 0.129)						
E_bC_{10} (120 h) #	0.034 (95 % confidence limits: 0.032 - 0.037)						

recalculated data (see study amendment)
* Statistically significant differences compared to the control (William`s Test, $\alpha = 0.05$).
§ Statistical test was not performed for days 3 and 4, neither in the original report nor in the amendment

Conclusions

The study is not valid as the mean coefficient of variation for section-by-section specific growth rate in the control was above the limit value of 35 % demanded by OECD 201 (2011).

B.9.2.7 Effects on aquatic macrophytes

KCA 8.2.7/1 (new study amendment, submitted with renewal dossier)

Author: Hoberg, J. (amended by Kubitza, 2004c)
Title: SAN 1289 H Technical - toxicity to duckweed, *Lemna gibba*
Date: 20.01.1997 (Amendment: 2004)
Doc ID: 96-11-6787; WAT1999-492
BASF RegDoc# 97/10742 (Amendment: 2004/1025686)
Guidelines: EPA 122-2, EPA 123-2
GLP: Yes
Validity: Acceptable

Material and Methods

Test item:	Dimethenamid-P (SAN 1289H; BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1 %.
Test species:	*Lemna gibba* G3, supplier: University of California, Los Angeles, California, six days since previous transfer
Test design:	14-day duration with solution renewal on days 3, 6, 9 and 12; 5 test item concentrations, each with 3 replicates per treatment including controls; daily assessment of growth.
Endpoints:	EC_{50} and NOEC with respect to growth rate and biomass after exposure over 14 days.
Test concentrations:	Control, 0.0010, 0.0030, 0.0089, 0.027 and 0.081 mg as/L (nominal), corresponding to geometric mean measured concentrations of 0.000424, 0.00146, 0.00372, 0.0148 and 0.0463 mg as/L.
Test conditions:	Replicate sterile 270-mL crystallising dishes, three per treatment level and the control; test volume 100 mL; Hoagland's medium was used to prepare the exposure solutions; pH was adjusted to 7.5±0.1; pH ranged from 4.9 to 5.0 in new solutions and from 4.9 to 6.6 in old solutions; temperature: 24-25 °C; initial frond number: 15; continuous light intensity within the range 4500-5400 lux.
Analytics:	Analytical verification of the test item was conducted using gas chromatography with Nitrogen-Phosphorus Detection (GC-NPD).
Statistics:	Descriptive statistics; probit/logit analysis for determination of EC_x values for growth rate and biomass.

Results and Discussion

Analytical measurements: Measured concentrations of dimethenamid-P ranged from 82 % to 120 % of nominal concentrations at test initiation. Measured concentrations at test solution renewal (3-day old solution) decreased significantly and ranged from 15 % to 36 % of nominal concentrations. However, no analytical verification of test item concentrations was conducted for the remaining renewal periods (days 6, 9, and 12). Therefore, contrary to the study author's (initial measured) and applicant's opinion (nominal), the biological results presented below are based on geometric mean measured test concentrations. For the sake of transparency, the measured concentrations (value in mg as/L and corresponding recovery in % of nominal) for each treatment group are given as follows: 0.0012 (120 %), 0.0032 (110 %), 0.0073 (82 %), 0.026 (95 %), 0.074 (92 %) in freshly prepared solutions, respectively, and 0.00015 (15 %), 0.00067 (22 %), 0.0019 (21 %), 0.0084 (31 %), 0.029 (36 %) in 3-day old solutions, respectively (see table below, copied from the original study report).

Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L)[a]			
	Day 0[b]	Day 0 Percent Nominal	Day 3[c]	Day 3 Percent Nominal
Control	<0.0003	NA[d]	<0.0003	NA
0.0010	0.0012	120	0.00015[e]	15
0.0030	0.0032	110	0.00067	22
0.0089	0.0073	82	0.0019	21
0.027	0.026	95	0.0084	31
0.081	0.074	92	0.029	36
QC[f]#1 0.00100	0.00112	112	0.000923	92.3
QC#2 0.0200	0.0200	100	0.0204	102
QC#3 0.100	0.0956	95.6	0.0936	93.6

[a] Calculated values are based on actual analytical results and not on rounded values presented in this table.
[b] Freshly prepared solutions which are representative of day 0, 3, 6 and 12 freshly prepared solutions.
[c] Three-day-old solutions which are representative of day 6, 9 and 12 aged solutions.
[d] NA = Not applicable
[e] Extrapolated value
[f] QC = Quality Control sample

Figure B.9.2-5: Measured concentrations of dimethenamid-P

Biological results: The duckweed population in the control vessels showed sufficient growth (e.g. after six days of exposure, at least seven-fold increase in number of fronds was observed). After 14 days of exposure, the number of fronds was statistically significantly reduced compared to the control at the two highest test item concentrations of 0.0148 and 0.0463 mg as/L. Statistically significant effects on frond dry weight (biomass) were observed at test item concentrations of ≥ 0.00372 mg as/L. No morphological effects were observed in the control and at the lowest tested concentration of 0.000424 mg as/L throughout the test duration. At test termination, fronds exposed to 0.00146 and 0.00372 mg as/L were smaller and had less root formation compared to the control fronds. In addition, fronds exposed to the 0.00372 mg as/L were observed to be slightly chlorotic and curled. Fronds exposed to the two highest test item concentrations of 0.0148 and 0.0463 mg as/L were curled, slightly chlorotic, had very little root formation and were smaller compared to the fronds in the control. The effects on plant growth (based on frond densities and frond dry weight) are summarised in Table B.9.2-51.

In the amendment by Kubitza (2004c; Doc ID: 2004/1025686), additional endpoints related to EC_{10} and EC_{50} for 3, 6, 9, 12 and 14 days of exposure were recalculated on the basis of nominal concentrations. However, as stated above, measured concentrations showed a sharp decline after 3 days, endpoints (EC_{10}, EC_{50}, NOEC) were recalculated by the RMS based on geometric mean concentrations. Statistical analyses were performed with the software programme "ToxRat Professional XT", version 3.0.

Table B.9.2-51: Effect (14 d) of dimethenamid-P on the growth of duckweed *Lemna gibba*

Concentration [mg as/L] (nominal)	**control**	**0.0010**	**0.0030**	**0.0089**	**0.027**	**0.081**
Concentration [mg as/L] (geometric mean measured)	**--**	**0.000424**	**0.00146**	**0.00372**	**0.0148**	**0.0463**
Inhibition in 3 d (yield frond number) [%]	--	0.0	2.5	1.3	44.3*	60.8*
Inhibition in 0-3 d (growth rate) [%]	--	0.0	1.4	0.5	33.9*	48.4*
Inhibition in 6 d (yield frond number) [%]	--	1.1	2.3	2.0	65.5*	85.2*
Inhibition in 0-6 d (growth rate) [%]	--	0.5	1.0	0.8	40.6*	64.8*
Inhibition in 9 d (yield frond number) [%] #	--	-9.5	-8.5	-12.5	80.5*	93.3*
Inhibition in 0-9 d (growth rate) [%] #	--	-2.9	-2.7	-3.8	48.1*	72.6*
Inhibition in 12 d (yield frond number) [%] #	--	-2.8	-4.6	1.9	85.8*	94.9*
Inhibition in 0-12 d (growth rate) [%] #	--	-0.8	-1.2	0.5	50.9*	72.4*
Inhibition in 14 d (yield frond number) [%]	--	5.0	-8.0	8.3	91.4*	97.6*
Inhibition in 14 d (growth rate) [%] #	--	1.2	-2.0	2.2	56.8*	79.2*
Inhibition in 14 d (yield dry weight) [%]	--	11.1	18.1*	29.2*	92.1*	95.8*
Inhibition in 0-14 d (yield dry weight) [%]	--	2.9	5.2	9.0	63.6*	74.4*
Endpoints [mg as/L] (geometric mean measured)						
E_yC_{50} (3 d)	0.02471 (95 % confidence limits: 0.00489– 0.11850					
E_yC_{10} (3 d)	0.00346 (95 % confidence limits: 0.00089– 0.01347					
E_rC_{50} (3 d)	0.04168 (95 % confidence limits: 0.00691– 0.22287					
E_rC_{10} (3 d)	0.00422 (95 % confidence limits: 0.00108– 0.01653					
E_yC_{50} (6 d)	0.01174 (95 % confidence limits: 0.0054 – 0.02544)					
E_yC_{10} (6 d)	0.00403 (95 % confidence limits: 0.00201 – 0.00806)					
E_rC_{50} (6 d)	0.02429 (95 % confidence limits: 0.01542 – 0.03770)					
E_rC_{10} (6 d)	0.00430 (95 % confidence limits: 0.00294 – 0.00628)					
E_yC_{50} (9 d)	0.01294 (95 % confidence limits: --) 1)					
E_yC_{10} (9 d)	0.01071 (95 % confidence limits: --) 1)					
E_rC_{50} (9 d)	0.01829 (95 % confidence limits: 0.01549 – 0.02148)					
E_rC_{10} (9 d)	0.00419 (95 % confidence limits: 0.00364 – 0.00482)					
E_yC_{50} (12 d)	0.0086 (95 % confidence limits: 0.00686 – 0.01110)					
E_yC_{10} (12 d)	0.00462 (95 % confidence limits: 0.00368 – 0.00581)					
E_rC_{50} (12 d)	0.01750 (95 % confidence limits: 0.01497 – 0.02035)					
E_rC_{10} (12 d)	0.00366 (95 % confidence limits: 0.00321 – 0.00417)					
E_yC_{50} (14 d)	0.00736 (95 % confidence limits: 0.00485 – 0.01136)					

Concentration [mg as/L] (nominal)	**control**	**0.0010**	**0.0030**	**0.0089**	**0.027**	**0.081**
Concentration [mg as/L] (geometric mean measured)	**--**	**0.000424**	**0.00146**	**0.00372**	**0.0148**	**0.0463**
E_yC_{10} (14 d)	0.00384 (95 % confidence limits: 0.00258 – 0.00570)					
E_rC_{50} (14d)	0.01443 (95 % confidence limits: 0.00878 – 0.02360)					
E_rC_{10} (14 d)	0.00367 (95 % confidence limits: 0.00236 – 0.00570)					
E_yC_{50} (14 d) based on dry weight	**0.00599 (95 % confidence limits: 0.00323 – 0.01095)**					
E_yC_{10} (14 d) based on dry weight	0.00235 (95 % confidence limits: 0.00143 – 0.00390)					
E_rC_{50} (14 d) based on dry weight	0.01314 (95 % confidence limits: 0.00568 – 0.03027)					
E_rC_{10} (14 d) based on dry weight	0.00242 (95 % confidence limits: 0.00121 – 0.00491)					
NOEC (14 d) based on dry weight	0.000424 (based on biomass dry weight and phytotoxicity)					

[#] Negative values indicate stimulated growth compared to the control.
* Statistically significantly different compare to the control (Williams' Test; $\alpha = 0.05$; one-sided smaller).
[1)] 95 % confidence limits not determined due to mathematical reasons

Conclusions

In the 14-day semi-static toxicity test with the aquatic plant *Lemna gibba*, the lowest E_yC_{50} of dimethenamid-P for the parameters frond density and frond dry weight was determined to be 0.00736 and 0.00599 mg as/L (geometric mean measured), respectively.

The lowest NOEC was 0.000424 mg as/L (geometric mean measured) based on frond dry weight and phytotoxicity. Geometric mean of the recalculated six and nine days endpoints for yield frond number and growth rate was determined to be 0.0123 and 0.0211 mg as/L (geometric mean measured), respectively.

KCA 8.2.7/2 (new study, submitted with renewal dossier)

Author: Backfisch, K., Kubitza, J.
Title: Effect of dimethenamid-P (BAS 656 H, Reg.No. 363851) on the growth of *Lemna gibba* in presence and absence of sediment
Date: 30.11.2012
Doc ID: 2012/1215555
Guidelines: OECD 221, EPA 850.4400 (draft 1996), ASTM E 1415-91
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 %.

Test species: Duckweed (*Lemna gibba* G3), inocula 7 to 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system (7 days); two experiments: one with sediment and one without sediment, each with 6 treatment groups (5 test item concentrations, control) using 3 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 3, 5 and 7.

Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Standard test without sediment: control, 0.0050, 0.010, 0.020, 0.040 and 0.080 mg as/L (nominal); test with sediment: 0.010, 0.020, 0.040, 0.080 and 0.160 mg as/L (nominal)
Test conditions:	400 mL glass beakers, test volume: 160 mL, 20x-AAP nutrient medium, in the test with sediment the glass beakers contained 100 g sediment (according to OECD 219; pH 7.41) corresponding to a layer of 1.0 - 1.5 cm; pH 7.48 - 7.51 at test initiation and pH 8.72 - 8.82 termination in the standard test without sediment; pH 7.48 - 7.51 at test initiation and pH 7.69 - 7.88 at test termination in the test with sediment; water temperature 24.8 °C - 24.9 °C, continuous light, light intensity: 7950 lux - 8970 lux.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of the EC_x values based on frond no. and dry weight.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. In the standard test without sediment, the analysed contents of dimethenamid-P ranged from 101.5 % to 105.7 % of nominal at test initiation and from 67.6 % to 73.3 % of nominal at test termination. In the test with sediment, the measured concentrations of dimethenamid-P were between 101.5 % and 105.7 % of nominal in samples taken at test initiation and between 52.2 % and 58.5 % of nominal at test termination. As the measured concentrations decreased <80 % of nominal in both experimental setups, the following biological results are based on geometric mean concentrations.

Biological results:
Standard test without sediment: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 127.5 fronds per vessel, corresponding to a 12 x multiplication or a doubling time of 1.98 d. The dry weight increased from 1.01 mg to an average of 13.91 mg per vessel in the control at test termination. Morphological symptoms like smaller fronds and shorter roots were observed at the four highest test item concentrations. Effects on growth rate and yield in the standard test without sediment are summarised in Table B.9.2-52a.

Table B.9.2-52a: Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in the standard test without sediment

Concentration [mg as/L] (nominal)		**0.0050**	**0.010**	**0.020**	**0.040**	**0.080**
Concentration [mg as/L] (geometric mean measured)		**0.00433**	**0.0088**	**0.01655**	**0.03401**	**0.06957**
Inhibition after 7 d [%] (growth rate based on frond no.)		0.7	11.8	35.3	43.8	48.0
Inhibition after 7 d [%] (yield based on frond no.)		1.9	27.6	63.4	72.0	75.7
Inhibition after 7 d [%] # (growth rate based on dry weight)		-6.7	5.1	28.6	37.3	66.6
Inhibition after 7 d [%] # (yield based on dry weight))		-20.4	13.6	56.8	67.3	89.0
		Endpoints [mg as/L] (geometric mean)				
E_rC_{50} (7 d) based on frond no.		0.0568 (95 % confidence limits: 0.0266 - > 0.0800)				
E_rC_{10} (7 d) based on frond no.		0.0050 (95 % confidence limits: 0.0 - 0.0138)				
E_yC_{50} (7 d) based on frond no.		**0.0168 (95 % confidence limits: 0.0026 - > 0.0800)**				
E_yC_{10} (7 d) based on frond no.		0.0036 (95 % confidence limits: 0.0 - 0.0089)				
E_rC_{50} (7 d) based on dry weight		0.0434 (95 % confidence limits: 0.0304 - > 0.0800)				
E_rC_{10} (7 d) based on dry weight		0.0093 (95 % confidence limits: 0.0021 - 0.0158)				
E_yC_{50} (7 d) based on dry weight		0.0190 (95 % confidence limits: 0.0098 - 0.0389)				
E_yC_{10} (7 d) based on dry weight		0.0058 (95 % confidence limits: 0.0002 - 0.0107)				

Negative values indicate stimulated growth compared to the control.

Test with sediment: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 242 fronds per vessel, corresponding to a 22 x multiplication or a doubling time of 1.57 d. The dry weight increased from 1.01 mg to an average of 29.38 mg per vessel in the control at test termination. Morphological symptoms like smaller fronds and shorter roots were observed at the four highest test item concentrations. Effects on growth rate and yield in the test with sediment are summarised in Table B.9.2-52b.

Table B.9.2-52b: Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in the test with sediment

Concentration [mg as/L] (nominal)	**0.010**	**0.020**	**0.040**	**0.080**	**0.160**
Concentration [mg as/L] (geometric mean measured)	**0.00742**	**0.01508**	**0.03107**	**0.06117**	**0.12421**
Inhibition after 7 d [%] # (growth rate based on frond no.)	-0.4	7.6	34.6	47.9	57.4
Inhibition after 7 d [%] # (yield based on frond no.)	-1.3	22.1	68.8	81.0	87.0
Inhibition after 7 d [%] # (growth rate based on dry weight)	-5.9	0.0	27.0	31.7	38.5
Inhibition after 7 d [%] # (yield based on dry weight)	-22.6	0.1	61.9	67.9	75.1
	Endpoints [mg as/L] (geometric mean)				
E_rC_{50} (7 d) based on frond no.	0.0763 (95 % confidence limits: 0.0452 - > 0.160)				
E_rC_{10} (7 d) based on frond no.	0.0115 (95 % confidence limits: 0.0004 - 0.0242)				
E_yC_{50} (7 d) based on frond no.	**0.0255 (95 % confidence limits: 0.0129 - 0.0484)**				
E_yC_{10} (7 d) based on frond no.	0.0094 (95 % confidence limits: 0.0003 - 0.0163)				
E_rC_{50} (7 d) based on dry weight	> 0.1242 (extrapolated: n.d.)				
E_rC_{10} (7 d) based on dry weight	0.0166 (95 % confidence limits: n.d.)				
E_yC_{50} (7 d) based on dry weight	0.0380 (95 % confidence limits: n.d.)				
E_yC_{10} (7 d) based on dry weight	0.0109 (95 % confidence limits: n.d.)				

# Negative values indicate stimulated growth compared to the control.
n.d. = not determined

Conclusions

In the 7-day aquatic plant test on *Lemna gibba* without sediment, the E_rC_{50} of dimethenamid-P based on frond number was determined to be the 0.0568 mg as/L and the E_yC_{50} was 0.0168 mg as/L (geometric mean measured). The E_rC_{50} of dimethenamid-P based on dry weight was determined to be the 0.0434 mg as/L and the E_yC_{50} was 0.019 mg as/L (geometric mean measured).
In the test with sediment, the E_rC_{50} of dimethenamid-P based on frond no. was determined to be the 0.0763 mg as/L and the E_yC_{50} was 0.0255 mg as/L (geometric mean measured). The E_rC_{50} of dimethenamid-P based on dry weight was determined to be the > 0.124 mg as/L and the E_yC_{50} was 0.038 mg as/L (geometric mean measured).

KCA 8.2.7/3 (new study, submitted with renewal dossier)

Author: Kubitza, J. and Grund, S.
Title: Effect of BAS 656P H (dimethenamid-P, Reg.No. 363851) on the growth of *Lemna gibba* in different peak exposure scenarios
Date: 16.08.2013
Doc ID: 2013/1291744
Guidelines: OECD 221, EPA 850.4400 (draft 1996), ASTM E 1415-91
GLP: No
Validity: Acceptable

Material and Methods

Test item:	Dimethenamid-P (BAS 656P H; Reg. no.: 363 851), batch no. COD-001509, purity: 95.9 % ± 1 %.
Test species:	Duckweed (*Lemna gibba* G3), inocula approximately 10 days old; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.
Test design:	Static system, exposure over 1 or 2 x 24 h; 8 different exposure scenarios, 3 replicates for the test item treatment and the control in each scenario. Scenario "A – G": two consecutive exposure peaks over 24 h; the consecutive peaks are separated by non-exposure periods varying between 1 and 7 days, after the second peak the plants were rinsed and transferred to fresh medium, the second peak was followed by a cultivation period over 6 days (growth phase). Scenario "H": single exposure peak over 24 h followed by a 7 day cultivation period (growth phase). In each scenario, two plants with four fronds and one plant with three fronds were added impartially to each vessel under axenic conditions giving a total number of 11 fronds at test initiation. Assessment of growth at the end of each exposure peak, at the end of the non-exposure period and at the end of the cultivation period; in scenario "H", plant growth was additionally assessed two days after the single peak treatment.
Endpoints:	Effects on plant growth based on growth rate and yield; no endpoints values were calculated.
Test concentrations:	0 (control) and 0.250 mg dimethenamid-P/L (nominal) in all exposure scenarios.
Test conditions:	400 mL glass beakers, test volume: 160 mL, dilution water: 20 x-AAP medium; pH 7.50 - 7.54 at test initiation in all bulk solutions; temperature 20 °C - 22 °C; continuous light at approx. 10 klux.
Analytics:	None.
Statistics:	Descriptive statistics; ANOVA followed by Dunnett`s test (two-sided $p < 0.05$).

Results and Discussion

Analytical measurements: No analytical verification of the test item concentrations was carried out. The following biological results are based on nominal test concentrations.

Biological results: In scenarios "A - G", the duckweed population in the control vessels increased from 11 fronds per vessel at test initiation to an average of 426, 656, 591, 729, 849, 931 and 1314 fronds per vessel in the control after 9, 10, 11, 12, 13, 14 and 15 days, respectively. In scenario "H", the frond number increased to an average of 680 fronds per vessel in the control after 9 days. Thus, good and continuous exponential growth was achieved in all exposure scenarios.

In the scenarios with non-exposure periods of ≤ 2 days between two consecutive peaks (scenario "A"

and “B”), plant growth was statistically significantly decreased based on both parameters growth rate and yield when compared to the single peak treatment (scenario “H”; Dunnett`s test, $p < 0.05$) by about 6 % based on growth rate and about 15 % based on yield. The relative percent differences between the inhibition values in scenario “A” and “B” compared to those in scenario “H” were +62 % and +63 % based on growth rate and +33 % and +43 % based on yield. Exposure of *Lemna gibba* to two consecutive peaks separated by > 2 days resulted in similar plant growth (scenarios “D and F”, as well as scenarios “G and H”) or statistically significantly increased plant growth (scenarios “C” and “F”) when compared to the single peak treatment (Dunnett`s test, $p < 0.05$).

The data show that the impact of consecutive peaks on the growth of *Lemna gibba* is influenced by the duration of the non-exposure period between the peaks. Non-exposure periods > 2 days between two consecutive 24 hour peaks of 250 mg BAS 656P H/L (scenario “C” to “G”) caused no additional growth reduction as compared to the effects measured after a single peak treatment at the same concentration of BAS 656P H. In these scenarios, the second peak did not cause an increase of the magnitude of the effect.

Furthermore, there is no effect-addition between the number of exposure peaks and the magnitude of the measured effects (*i.e.* two consecutive peaks of the same test item concentration do not induce 2-fold of the effect caused by one single peak even if they are separated by only 1 day). Effects on growth rate and yield are summarised in Table B.9.2-53.

Table B.9.2-53: Effects of dimethenamid-P on the growth of *Lemna gibba* based on frond numbers in different exposure scenarios

Exposure scenario				**Inhibition rel. to the control [%]** (rel. difference compared to single peak scenario “H”)	
Serial code	Peak no. & duration	Peak concentration [mg as/L]	NEP between peaks [d]	growth rate	yield
“A”	2 x 24 h	0.250	1	16.3 * (+62)	46.1 * (+33)
“B”	2 x 24 h	0.250	2	16.4 * (+63)	49.6 * (+43)
“C”	2 x 24 h	0.250	3	8.0 ** (-21)	27.7 ** (-20)
“D”	2 x 24 h	0.250	4	10.2 (+2)	35.5 (+2)
“E”	2 x 24 h	0.250	5	9.6 (-4)	34.8 (+0.2)
“F”	2 x 24 h	0.250	6	7.3 ** (-28)	27.9 ** (-20)
“G”	2 x 24 h	0.250	7	8.3 (-17)	33.2 (-4)
“H”	1 x 24 h	0.250	--	10.0	34.7

NEP = Non-Exposure Period
Values in brackets give the relative percent differences of the inhibition values compared those of scenario “H”.
* Statistically significant decrease in plant growth compared to the single peak exposure scenario “H” (Dunnett`s test $p < 0.05$).
** Statistically significant increase in plant growth compared to the single peak exposure scenario “H” (Dunnett`s test $p < 0.05$).

Conclusions

The results of this study show that the impact of consecutive peaks on the growth of *Lemna gibba* is influenced by the duration of the non-exposure period between the peaks. For *Lemna gibba* two consecutive 24 hour peaks of 0.250 mg dimethenamid-P/L can be considered toxicologically independent from each other if the interval between the single peaks is longer than 2 days. In this case, the second peak did not contribute to the magnitude of the response anymore.

KCA 8.2.7/4 (new study, submitted with renewal dossier)

Author:	Janson, G.-M.
Title:	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the aquatic plant *Ceratophyllum demersum* after different exposure durations
Date:	06.09.2013
Doc ID:	2013/1286175
Guidelines:	OECD 221, OECD 219, ASTM E 1913-04
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656P H; Reg. no.: 363 851), batch no. COD-001509, purity: 95.9 % ± 1 %.

Test species: *Ceratophyllum demersum* (Ceratophyllaceae), a dicotyledonous aquatic plant species, cultivated in-house (non-GLP) after collected from natural running water.

Test design: Static system including sediment, three test item concentrations were tested over two different exposure durations, *i.e.* 24 and 48 hours, each followed by a 7 day cultivation period; 4 treatment groups (3 test item concentrations, control) for both exposure scenarios with 3 plants per replicate; the tests were run with 3 replicates for the test item treatments and 6 replicates for the control. After the respective exposure times the plants were transferred to fresh sediment and medium and cultivated for further seven days; assessment of total length of the plants (main shoot above the sediment plus side shoots) at test initiation, after the respective exposure phase, once during the cultivation period and at the end of the test; the length of the side shoots were recorded once during the study and at test termination; assessment of fresh weight at test initiation and test end; visual observations once during the growth phase and at test end; determination of dry weight at test termination. The starting dry weight was determined by calculating a mean factor based on the ratios of the final dry weights and lengths of the control replicates, which is then multiplied by the initial length data for each plant in all treatments.

Endpoints: EC_{50} and NOEC with respect to growth rate and yield after exposure over two different exposure durations, each followed by a 7 day cultivation period.

Test concentrations: Control, 0.3, 1.0 and 3.0 mg as/L for both exposure scenarios.

Test conditions: 2.0 L glass beakers and flower pots (Ø 9 cm), standard artificial sediment (OECD 219) and 1.8 L Smart & Barko medium (pH 7.63 at test initiation); air temperature 20.0 ± 2 °C; water temperature: 21.1 °C - 21.9 °C in the 24 h exposure scenario and 20.1 °C - 21.7 °C in the 48 h exposure scenario; oxygen saturation: 91.6 % - 183.0 % in the 24 h exposure scenario and 81.5 % - 161.2 % in the 48 h exposure scenario; pH 7.29 - 9.83 in the 24 h exposure scenario and pH 7.34 - 9.57 in the 48 h exposure scenario; light : dark - rhythm 16 : 8 h, light intensity: 10 - 12 klux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics; Probit analysis using linear max. likelihood regression for calculation of the EC_x values; ANOVA followed by Dunnett's Multiple Sequential t-test Procedure, Student-t test for Homogeneous Variances and Welch-t test for Inhomogeneous Variances ($\alpha = 0.05$) for determination of the NOEC values.

Results and Discussion

Analytical measurements: Analytical verification of the test item concentrations was conducted in the bulk solutions prepared for each concentration at test initiation, and in mixed samples (pooled replicates of each treatment) at the end of the different exposure periods. The mean measured concentrations of dimethenamid-P in the bulk solutions ranged from 93 % to 95 % of the nominal concentrations at test initiation. At the end of the different 24 h exposure phase, measured values were between 93 % and 96 % of nominal. At the end of the 48 h exposure time the measured values were between 89 % and 94 % of nominal. Since the analytical measured values confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No statistically significant effects on plants were observed after exposure over 24 hours at concentrations of up to and including 3.0 mg as/L, except for the total length (based on growth rate and yield) in the highest tested concentration (Student-t test, $\alpha = 0.05$). After exposure over 48 hours, total length was statistically significantly impacted at the two highest test item concentrations of 1.0 and 3.0 mg as/L (Student-t test for growth rate data and Welch-t test for yield data, $\alpha = 0.05$). Additionally, plant fresh weight based on growth rate was statistically significantly reduced after exposure over 48 hours at 3.0 mg as/L (Dunnett`s Multiple t-test Procedure, $\alpha = 0.05$). Plant dry weight was not significantly impacted at any test item concentrations in both exposure scenarios. Generally, the overall impact was rather limited and none of the scenarios produced an impact strong enough to calculate an EC_{50}. At test end slight growth of algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study. The results are summarised in Table B.9.2-54.

Table B.9.2-54: Effect of dimethenamid-P on the aquatic plant *Ceratophyllum demersum* in different exposure scenarios followed by a 7 day cultivation period

Exposure scenario	**24 h exposure period + 7 d cultivation period**			**48 h exposure period + 7 d cultivation period**		
Concentration [mg as/L] (nominal)	**0.3**	**1.0**	**3.0**	**0.3**	**1.0**	**3.0**
Inhibition after 7 d cultivation [%] (growth rate based on total length)	4.2	15.9	19.6 *	17.9	32.4 *	35.3 *
Inhibition after 7 d cultivation [%] (yield based on total length)	5.3	25.3	31.9 *	22.4	42.0 *	46.7 *
Inhibition after 7 d cultivation [%] (growth rate based on fresh weight)	5.4	9.7	15.7	14.4	20.9	29.2 *
Inhibition after 7 d cultivation [%] (yield based on fresh weight)	2.0	11.2	16.3	16.1	25.3	31.2
Inhibition after 7 d cultivation [%] # (growth rate based on dry weight)	-14.6	12.1	14.4	10.9	8.9	12.0
Inhibition after 7 d cultivation [%] # (yield based on dry weight)	-23.6	13.9	22.4	5.5	10.3	16.0
	Endpoints [mg as/L] (nominal)					
E_rC_{50} based on total length, fresh weight and dry weight	> 3.0			> 3.0		
E_yC_{50} based on total length, fresh weight and dry weight	> 3.0			> 3.0		
NOE_rC / NOE_yC based on total length	1.0			0.3		
NOE_rC based on fresh weight	3.0			1.0		
NOE_yC based on fresh weight	3.0			3.0		
NOE_rC / NOE_yC based on dry weight	3.0			3.0		

Negative values indicate stimulated growth
* Statistically significant differences compared to the control (Dunnett's Multiple Sequential t-test Procedure, Student-t test for Homogeneous Variances or Welch-t test for Inhomogeneous Variances, $\alpha = 0.05$, one-sided smaller).

Conclusions

The results of the study demonstrated that exposure times typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to long-term constant exposure simulated in the standard study on *Ceratophyllum demersum* (exposure over 9 days). No significant effects were observed on plants exposed to dimethenamid-P for 24 h at up to and including the concentration of 1.0 mg as/L (nominal). Significant effects on plants exposed for 48 h were observed at the test item concentrations of 1.0 and 3.0 mg as/L. However, the impacts were rather low and not sufficient to produce an EC_{50} endpoint; thus, all EC_{50} values for all measured parameters and all treatment groups are > 3.0 mg as/L (nominal).

KCA 8.2.7/5 (new study, submitted with renewal dossier)

Author: Janson, G.-M.
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the aquatic plant *Glyceria maxima*
Date: 06.09.2013
Doc ID: 2013/1286172
Guidelines: OECD 221, OECD 219, ASTM E 1913-04
GLP: Yes
Validity: Acceptable

Material and Methods

Test item:	Dimethenamid-P (BAS 656P H; Reg. no.: 363 851), batch no. COD-001509, purity: 95.9 % ± 1 %.
Test species:	*Glyceria maxima* (Poaceae), a monocotyledonous aquatic plant species, cultivated in-house (non-GLP) after purchase from the plant nursery "Petrowsky" Eschede, Germany.
Test design:	Static system (including sediment); test duration 14 days; 6 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates; one grass blade with 2 - 4 leaves per replicate; one plant per replicate was potted to fresh sediment and medium and cultivated for 14 days; assessment of leaf number and length at test initiation, once during the and at the end of the test; assessment of fresh weight at test initiation and test end; visual observations once during the growth phase and at test end; determination of dry weight at test termination. The starting dry weight was determined by calculating a mean factor based on the ratios of the final dry weights and lengths of the control replicates, which is then multiplied by the initial length data for each plant in all treatments.
Endpoints:	EC_{50} with respect to growth rate and yield related to wet weight, dry weight, total length as well as number of leaves after 14 days of exposure.
Test concentrations:	Control, 0.0030, 0.010, 0.030, 0.10, 0.30 and 1.0 mg as/L (nominal).
Test conditions:	2.0 L glass beakers and flower pots (Ø 9 cm), standard artificial sediment (OECD 219 with slight modifications, pH 6.76) and 1000 mL Smart & Barko medium (pH 7.71 at test initiation); oxygen saturation: 88.5 % - 94.0 % at the test initiation and 102.6 % - 123.6 % at test termination; pH 7.60 - 7.63 at test initiation and 8.02 - 8.81 at test termination; conductivity: 286 - 287 µS/cm; water temperature: 20.3 °C - 20.7 °C; light : dark - rhythm 16 : 8 h, light intensity: 10 klux ± 2 klux.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection. At test initiation the analytical samples were taken from the respective bulk solutions and at the end from mixed samples (pooled replicates of each treatment).
Statistics:	Descriptive statistics; probit analysis using linear maximum likelihood regression for EC_{50} calculations.

Results and Discussion

Analytical measurements: Analytical verification of active substance concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured concentrations of dimethenamid-P ranged from 99 % to 102 % of nominal concentrations at test initiation and from 73 % to 83 % nominal at test termination. As the analytically measured values (at test initiation) confirmed the correct application of the test item in both exposure scenarios, the following biological results are based on nominal test concentrations.

Biological results: At test end, slight growth of algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study. The results are summarised in Table B.9.2-55.

Table B.9.2-55: Effect of dimethenamid-P on the growth of the aquatic plant *Glyceria maxima*

Concentration [mg as/L] (nominal)	**0.0030**	**0.010**	**0.030**	**0.10**	**0.30**	**1.0**
Inhibition in 14 d [%] # (growth rate based on total length)	5.9	0.4	-4.6	33.4	65.8	90.2
Inhibition in 14 d [%] (yield based on total length)	8.6	4.6	7.7	51.0	79.0	95.1
Inhibition in 14 d [%] # (growth rate based on dry weight)	-5.6	4.9	-14.8	6.5	28.0	28.0
Inhibition in 14 d [%] # (yield based on dry weight)	-17.3	12.1	-7.3	15.6	45.6	45.1
Inhibition in 14 d [%] (growth rate based on wet weight)	4.3	11.8	4.2	27.8	42.2	68.4
Inhibition in 14 d [%] # (yield based on wet weight)	-10.7	11.8	2.4	33.5	58.5	81.3
Inhibition in 14 d [%] # (yield based on number of leaves)	6.1	-6.1	2.0	26.5	38.8	87.8
Endpoints [mg as/L] (nominal)						
$E_rC_{50\ total\ length}$ (14 d)	0.184 (95 % confidence limits: 0.136 - 0.250)					
$E_yC_{50\ total\ length}$ (14 d)	**0.109** (95 % confidence limits: 0.074 - 0.160)					
$E_rC_{50\ dry\ weight}$ (14 d)	> 1.0					
$E_yC_{50\ dry\ weight}$ (14 d)	0.934 (95 % confidence limits: 0.315 - > 1.)					
$E_rC_{50\ wet\ weight}$ (14 d)	0.402 (95 % confidence limits: 0.242 - 0.817)					
$E_yC_{50\ wet\ weight}$ (14 d)	0.221 (95 % confidence limits: 0.136 - 0.375)					
$E_yC_{50\ no.\ of\ leaves}$ (14 d)	0.318 (95 % confidence limits: 0.179 - 0.608)					

Negative values indicate stimulated growth compared to the control.

Conclusions

In a 14-day aquatic-plant test with *Glyceria maxima*, the E_rC_{50} of dimethenamid-P was determined to be 0.184 mg as/L based on total length, > 1.0 mg as/L based on dry weight and 0.402 mg as/L based on wet weight (nominal). The E_yC_{50} was 0.109 mg as/L based on total length, 0.934 mg as/L based on dry weight and 0.221 mg as/L based on wet weight (nominal). The E_yC_{50} based on the number of leaves was 0.318 mg as/L, based on nominal concentrations.

KCA 8.2.7/6 (new study, submitted with renewal dossier)

Author: Hoffmann, F., Grund, S. (amended by Hoffmann, F., 2012a)
Title: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of *Lemna gibba* after different exposure scenarios
Date: 10.07.2012 (Amendment: 17.07.2012)
Doc ID: 2012/1084264 (Amendment: 2012/1202274)
Guidelines: OECD 221, EPA 850.4400, ASTM E 1415-91
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656P H; Reg. no.: 363 851), batch no. COD-001509, purity: 95.9 % ± 1 %.

Test species: Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system, two different exposure scenarios each followed by a 7 day cultivation period (growth phase):

Scenario A: Three different concentration were tested over three different exposure durations, *i.e.* 12, 24, and 36 hours

Scenario B: Plants were exposed in two different exposure peak designs, each simulating two exposure peaks of the test item with decreasing consecutive concentrations over defined time intervals which are separated by a "no-exposure" period of 13 h; at the end of the respective exposure period after 34 h (see following table):

Consecutive exposure time [h]	**"0.250 mg/L max. peak exposure" [mg/L]**	**"0.500 mg/L max. peak exposure" [mg/L]**
0	0.250	0.500
5	0.175	0.350
9	0.050	0.100
12	0	0
25	0.090	0.180
31	0.050	0.100
34	start of 7 d growth phase	

In both scenarios, two plants with four fronds and one plant with three fronds were added impartially to each vessel under axenic conditions giving a total number of 11 fronds at initiation of the exposure phase. The tests were run with three replicates for the test item treatments and with six replicates for the control. After the respective exposure times the plants were transferred to fresh medium and afterwards cultivated for further seven days (growth phase). Assessment of growth and other effects was conducted at the end of the exposure periods, once during and at the end of the seven day growth phase. The yield based on the dry weight was determined at test beginning from a sample of the inoculum culture and at test termination with the plant material from each test concentration and control.

Endpoints: EC_{50} and NOEC with respect to growth rate and yield after exposure over different exposure durations and at different exposure designs, followed by a 7 day cultivation period.

Test concentrations: Scenario A: Control, 0.100, 0.300, 0.500 mg as/L (nominal);
Scenario B: "0.250 mg/L maximum peak exposure" design with consecutive test concentrations of 0.250 - 0.175 - 0.050 - 0 - 0.090 - 0.050 mg/L; "0.500 mg/L maximum peak exposure" design with consecutive test concentrations of 0.500 - 0.350 - 0.100 - 0 - 0.180 - 0.100 mg/L (nominal).

Test conditions: 400 mL glass beakers, test volume: 160 mL, 20x-AAP nutrient medium, continuous light in both scenarios;
Scenario A: Temperature: 23.9 – 24.2 °C, pH 7.51 - 7.53 at test initiation and pH 7.63- 8.42 at test end, light intensity: approx. 8100 lux
Scenario B: Temperature: 24.0 – 24.2 °C, pH 7.50 - 7.55 at test initiation and

pH 7.63- 7.71 at test end, light intensity: approx. 8400 lux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection. Samples from replicates of each test concentration were pooled.

Statistics: Descriptive statistics; Dunnett`s Multiple t-test for scenario A and Student`s t-test for scenario B for determination of the NOEC values; EC_{50} values were determined by Probit analysis using linear maximum likelihood regression for scenario A data and estimated based on the raw data in scenario B.

Results and Discussion

Analytical measurements:
Analytical verification of the test item concentrations was carried out in the bulk solutions of each test concentration at test initiation and in mixed samples (pooled replicates) of each treatment at the end of the respective exposure period in scenario A. The mean measured values determined in the bulk solutions at test initiation were between 111.3 % and 115.4 % of nominal. At the end of the different exposure times (12 h, 24 h, 36 h) the means of the analytically determined values were between 82.7 % and 105.0 %. In scenario B, analytical verification of the test item concentrations in bulk solutions was performed at the start of exposure to each test concentration for both test designs. Measured values were between 92.7 % and 113.3 % for the "0.250 mg/L maximum peak exposure" design and between 93.5 % and 117.4 % of nominal for the "0.500 mg/L maximum peak exposure" design. As the analytically measured values confirmed the correct application of the test item in both exposure scenarios, the following biological results are based on nominal test concentrations.

Biological results:
Scenario A: In the 12, 24 and 36 hour exposure treatment group, the duckweed population in the control vessels increased from 11 fronds per vessel at test initiation to an average of 128, 164 and 155 fronds per vessel in the control after 7.5 days, 8 days and 8.5 days, respectively. Thus, good and continuous exponential growth was achieved in all exposure scenarios. A 12 hour exposure period caused no negative impact on plant morphology after the growth phase at up to and including a test concentration of 0.300 mg dimethenamid-P/L. However, at the highest tested concentration of 0.500 mg as/L, single fronds appeared smaller than those in the controls at the end of the growth phase. Statistically significant effects on the growth of *Lemna gibba* compared to the control were observed at the two highest tested concentrations after exposure over 24 h and 36 h based on all tested parameters as well as in the 0.100 mg as/L treatment group after 36 h of exposure for the test parameters growth rate and yield based on dry weight (Dunnett`s Multiple t-test, $\alpha = 0.05$). Furthermore, morphological effects like smaller fronds and single necrotic fronds were recorded after exposure over 24 hours at the two highest treatment levels. After exposure over 36 hours, morphological changes like smaller and concaved fronds were observed for all treatment levels. The effects on plant growth in the exposure scenario A are summarised in Table B.9.2-56a.

Table B.9.2-56a: Exposure Scenario A - Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in different exposure scenarios followed by a 7 day cultivation period

Exposure scenario	**12 h exposure period**			**24 h exposure period**			**36 h exposure period**		
Concentration [mg as/L] (nominal)	**0.100**	**0.300**	**0.500**	**0.100**	**0.300**	**0.500**	**0.100**	**0.300**	**0.500**
Inhibition after 7 d cultivation [%] + (growth rate based on frond no.)	0.0	1.4	0.5	-0.6	8.2*	23.7*	1.7	22.1*	43.2*
Inhibition after 7 d cultivation [%] + (yield based on frond no.)	-4.3	3.1	-2.3	-1.5	21.4*	50.7*	4.8	47.5*	73.3*
Inhibition after 7 d cultivation [%] + (growth rate based on dry weight)	1.2	3.8	5.9	1.9	26.4*	44.7*	7.5*	23.5*	56.3*
Inhibition after 7 d cultivation [%] + (yield based on dry weight)	3.2	10.0	14.5	5.3	54.8*	74.8*	19.5*	49.8*	83.6*
Endpoints [mg as/L] (nominal)									
E_rC_{50} based on frond no.	> 0.500 #			> 0.500			> 0.500		
$NOEC_r$ based on frond no.	≥ 0.500			0.100			0.100		
E_yC_{50} based on frond no.	> 0.500 #			0.495			**0.317**		
$NOEC_y$ based on frond no.	≥ 0.500			0.100			0.100		
E_rC_{50} based on dry weight	> 0.500 #			> 0.500			0.458		
$NOEC_r$ based on dry weight	≥ 0.500			0.100			< 0.100		
E_yC_{50} based on dry weight	> 0.500 #			0.288			**0.253**		
$NOEC_y$ based on dry weight	≥ 0.500			0.100			< 0.100		

+ Negative values indicate stimulated growth.
# EC_{50} values for the 12 h exposure period could not be determined by statistical analysis due to the lack of meaningful concentration-response-relationship; therefore, EC_{50} values for this exposure period were expressed as "> 0.500 mg/L".
* Statistically significant differences compared to the control (Dunnett`s Multiple t-test, α = 0.05, one-sided smaller).

Scenario B: Both peak exposures designs were conducted with the same controls, due to the same test conditions and incubator. The duckweed population in the control vessels increased from 11 fronds per replicate at test initiation to an average of 255 fronds per vessel at the end of the growth phase, corresponding to a multiplication of 23.2. Thus, continuous exponential growth was achieved in all exposure scenarios during the growth phase. There was no statistically significant difference between the growth of *Lemna gibba* in the control and the test item treatments in the "0.250 mg/L max. peak exposure" design, referring to the test parameters growth rate and yield (both based on frond number and dry weight; Student`s t-test, α = 0.05) and no morphological effects were determined at the end of the growth phase. For the "0.500 mg/L max. peak exposure" design, statistically significant differences between the control and the test item treatments could be determined for all test parameters. However, the effects were negligible with inhibition for the different parameters between 2 % and 16 %. Single fronds appeared smaller at day 4 and at termination of the growth phase. The effects on plant growth in the exposure scenario B are summarised in Table B.9.2-56b.

Table B.9.2-56b: Exposure Scenario B - Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in different exposure scenarios followed by a 7 day cultivation period

Exposure design	"0.250 mg/L max. peak exposure"	"0.500 mg/L max. peak exposure"
Inhibition after 7 d cultivation [%] + (growth rate based on frond no.)	1.0	3.0*
Inhibition after 7 d cultivation [%] + (yield based on frond no.)	3.2	9.4*
Inhibition after 7 d cultivation [%] + (growth rate based on dry weight)	-0.8	5.0*
Inhibition after 7 d cultivation [%] + (yield based on dry weight)	-2.7	15.9*
	Endpoints [mg as/L] (nominal) #	
E_rC_{50} based on frond no. and dry weight	> 0.250	> 0.500
$NOEC_r$ based on frond no. and dry weight	≥ 0.250	< 0.500
E_yC_{50} based on frond no. and dry weight	> 0.250	> 0.500
$NOEC_y$ based on frond no. and dry weight	≥ 0.250	< 0.500

\+ Negative values indicate stimulated growth.
\# Endpoints were visually estimated based on raw data (E_xC_{50}) and based on statistical calculation ($NOEC_x$), respectively
* Statistically significant differences compared to the control (Student`s t-test, α = 0.05, one-sided smaller).

Table B.9.2-56c: Raw data (Scenario A, 24 h and 36 h exposure)[1)], Yield[2)] at each observation date during 7 day cultivation period. Please note that there is an increase of effects over time

	Yield during 7 days cultivation period – 24 h exposure (mean frond numbers)			Yield during 7 days cultivation period – 36 h exposure (mean frond numbers)		
	1	**5**	**8**	**1.5**	**5.5**	**8.5**
control	19.00	53.83	152.67	17.50	54.33	143.50
100	20.00	53.33	155.00	15.00	43.00	136.67
300	15.67	46.67	120.00	10.00	31.67	75.33
500	14.00	46.33	75.33	8.67	29.33	38.33
E_yC_{50} µg/L	n.d.	n.d.	495	n.d.	564	317

[1)] results from 12 h exposure scenario not shown as there were no significant effects;
[2)] 11 fronds per vessel at test initiation (0 d)

Conclusions

The results of the study demonstrate that short-term exposure times typical for moving water bodies after run off events (hours to days) cause less effects than long-term constant exposure to dimethenamid-P simulated in the standard *Lemna* studies. A 12 h exposure period followed by a growth phase over 7 days caused no significant impact on the plants at up to and including concentrations of 0.500 mg as/L. Exposure periods of 24 h caused significant effects at the two highest concentrations and the EC_{50} value was > 0.500 mg/L for the parameter "growth rate" and 0.495 mg/L and 0.288 mg/L for the parameter "yield" based on frond number and dry weight, respectively. Plants exposed for 36 h showed statistically significant differences to the control at all treatments levels. The determined EC_{50} values for growth rate were > 0.500 mg/L and 0.458 mg/L based on frond number and dry weight, respectively. The corresponding EC_{50} value for yield was 0.317 mg/L based on frond number and 0.253 mg/L based on dry weight.
In the exposure scenarios simulating double peak exposure with maximum concentrations of 0.500 and 0.250 mg/L for the first peaks, there was no significant effect on the growth of *Lemna gibba* in the "0.250 mg/L max. peak exposure" design for all test parameters. The EC_{50} values were

> 0.500 mg/L and > 0.250 mg/L for all test parameters in the respective exposure designs.
However, an increase of effects over time (carry-over of effects) during the growth phase was noted (see Table B.9.2-56c), indicating that there might be even higher effect levels if the observation period would have lasted longer. It appears that a concern for macrophytes in terms of delayed effects (especially for slower growing aquatic plants) cannot be exluded as similar studies are lacking for other macrophytes.

KCA 8.2.7/7 (new study, submitted with renewal dossier)

Author:	Kubitza, J., Dohmen, G.-P. (amended twice by Kubitza, J., 2013a, 2014a)
Title:	Effect of dimethenamid-P - Tested as formulated product - BAS 656 08 H - On emergent aquatic plants
Date:	28.02.2003 (Amendment No.1: 28.11.2013 / Amendment No.2: 27.02.2014)
Doc ID:	2002/1012788 (Amendment No.1: 2013/1361973 / Amendment No.2: 2014/1082325)
Guidelines:	HARAP (Campbell et al. 1999) Guidance Document on Higher-tier Aquatic Risk Assessment of Pesticides, CLASSIC (Workshop on Community Level Aquatic System Studies May-June 1999)
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item: BAS 656 08 H, batch no. 2001-1; content of as: dimethenamid-P (BAS 656 H, Reg. no. 363 851): 711.4 g/L (nominal: 720.0 g/L); density: 1.127 g/cm^3.

Test species: Emergent aquatic plants; monocotyledonous: *Acorus calamus* (Araceae), *lris pseudacorus* (Iridaceae), *Sparganium erectum* (Sparganiaceae); dicotyledonous: *Mentha aquatic* (Lamiaceae), *Ludwigia palustris* (Onagraceae), *Veronica beccabunga* (Scrophulariaceae). Monocotyledonous plants purchased from the garden centre “Germann”, dicotyledonous plants purchased from "Harster", both resident in Speyer, Germany; *Sparganium erectum* cultivated in-house.

Test design: Static system (including sediment); test duration 13 days, 7 test item concentrations, each with 3 replicates plus a control with 6 replicates; the number of plants per replicate (one to several) varied depending on the test species; plants were treated using a laboratory spray track system simulating a realistic spraying event; visual observations one day after treatment, one week after treatment and at the end of the test; determination of wet weight, leave length and root weight for monocotyledonous plants and determination of wet weight and shoot length as well as visual assessment of root formation for dicotyledonous plants at test end. The test with *S. erectum* had been repeated because of high variability of results, which were probably due to the very differing growth stages of the initial plants at the start of the first test (only the results of the repeated test are reported here).

Endpoints: EC_{50} with respect to growth rate related to wet weight, length data and, for monocotyledonous plants, root formation after 13 days of exposure.

Test concentrations: The amounts of BAS 656 08 H (and respective values for dimethenamid-P) applied *via* spray application with 200 L/ha as well as the recalculated nominal and initial measured water concentrations are presented in the following table.

Table B.9.2-57: Amounts of BAS 656 08 H (and respective values for dimethenamid-P) applied via spray application

nominal application rates		**nominal water concentrations**				**initial measured water concentrations**	
all tested species		**monocotyledonous** (*A. calamus, I. pseudacorus, S. erectum)*		**dicotyledonous** *(M. aquatica, L. palustris, V. beccabunga)*		***S. erectum***	
BAS 656 08 H [L/ha]	dimethen-amid-p [kg/ha]	BAS 656 08 H [mg/L]	dimethenamid-P [mg/L]	BAS 656 08 H [mg/L]	dimethen-amid-P [mg/L]	BAS 656 08 H [mg/L]	dimethen-amid-P [mg/L]
0.010	0.007	0.015	0.010	0.017	0.011	0	0
0.023	0.016	0.034	0.022	0.038	0.024	0.009	0.006
0.052	0.037	0.078	0.049	0.086	0.054	0.023	0.014
0.118	0.084	0.177	0.112	0.197	0.124	0.085	0.054
0.270	0.192	0.404	0.255	0.449	0.283	0.257	0.162
0.614	0.437	0.921	0.581	1.023	0.646	0.506	0.319
1.400	0.996	2.098	1.324	2.332	1.472	0.905	0.572

Test conditions: 0.8 L and 1 L glass beakers, standard artificial sediment (OECD 219) and 3x AAP medium (pH: 7.2); oxygen content: 6.9 mg/L - 8.3 mg/L; pH: 7.93 - 10.13; conductivity: 265 - 275 µS/cm; water temperature: 19.6 °C - 25.2 °C; air temperature: 21 ± 1 °C; light : dark - rhythm 16 : 8 h, light intensity: approx. 8.5 ± 1.5 klux.

Analytics: Analytical verification of test item concentrations was conducted using a SPME-method with GC/MS detection.

Statistics: Descriptive statistics; determination of EC_{50} values by probit analysis, William's, Dunnett`s and Bonferroni test ($p \leq 0.05$) for determination of NOEC values. In addition to the visual assessments, which were included as qualitative parameters for the determination of the NOEC, the following calculations / transformations have been performed with the different plant species to assess statistically significant differences in plant growth. Depending on plant species and initial biomass determinations feasible for the respective species, the final growth parameters were compared to different initial determinations as follows:

- *Acorus calamus, Iris pseudacorus, Sparganium erectum*:

  Wet weight: ln(final wet weight) - ln(initial wet weight)
  Length: ln(final length) - ln(initial length)
  Root weight: final root wet weight - initial root wet weight

- *Mentha aquatica, Ludwigia palustris, Veronica beccabunga*:

  Wet weight: ln(final wet weight) - ln(initial wet weight)
  Length: ln(final length) - ln(initial length)

Results and Discussion

Analytical measurements: The nominal test item concentrations in samples taken from additional plant-free vessels were confirmed by analytical analysis showing recovery of 106.6 % ± 11 %. Test item recovery in the repeated part of the study was 108.6 % ± 16 %, except for the lowest test concentration at which 33.9 % was found. In addition, analytical verification of test item concentrations was conducted in treated vessels for each plant species in each concentration at the

beginning and at the end of the test. The measured concentrations in samples taken from the test vessels with *A. calamus*, *I. pseudacorus*, *M. aquatic*a and *L. palustris* ranged from 84.2 % to 134.0 % of nominal concentrations at test initiation and from 30.6 % to 120.0 % of nominal at test termination. The following biological results for these species are given in nominal as well as geometric mean water concentrations of the test item. Regarding analytical measurements for *V. beccabunga,* 5 out of 7 samples showed very high recoveries (470 % - 10719 %) at test initiation (see table below), however the other samples showed relatively normal recovery (111 % and 156 %) and concentrations at test termination were in the range of 50 % - 76 % results for *V. beccabunga*. Therefore, the geomean of nominal (day 0) and measured concentrations (day 13) was calculated for each test level as the initial measured concentrations with recoveries raging from 111 % - 10719 % were not deemed plausible, especially in view of the clear dose-response relationship on the basis of nominal concentrations.

Table B.9.2-58: Determination of BAS 656 08 H in *Veronica beccabunga* water samples at test initation / termination

Nominal[1)] Appl. Rate [L/ha]	Nominal conc. [mg/L]	Measured conc. [mg/L] at day 0 (% of nominal)	Measured conc. [mg/L] at day 13 (% of nominal)	Geomean of measured conc. [mg/L] days 0-13 (% of nominal)	Geomean[2)] of nominal (0 d) + measured conc. [mg/L] days 0-13 (% of nominal)
control	-	n.d.	n.d.	-	-
0.01	0.017	0.0889 (523)	0.0116 (68)	0.032 (189)	0.014 (82)
0.023	0.038	3.82 (10063)	0.0263 (69)	0.317 (834)	0.032 (83)
0.052	0.086	9.218 (10719)	0.0633 (74)	0.764 (888)	0.074 (86)
0.118	0.197	1.027 (521)	0.150 (76)	0.393 (199)	0.172 (87)
0.27	0.449	0.7015 (156)	0.248 (55)	0.417 (93)	0.334 (74)
0.614	1.023	4.809 (470)	0.548 (54)	1.624 (159)	0.749 (73)
1.4	2.332	2.58 (111)	1.16 (50)	1.731 (74)	1.646 (71)

[1)] All nominal and measured concentrations refer to the formulation BAS 656 08 H

[2)] Explanation: The observed discrepancy between the initial measured concentrations in *Veronica beccabunga* water samples (which would result in highly varying spacing factors) and the monotonic dose-response relationship with respect to the nominal values led to the decision to calculate geometric mean measured concentrations using nominal at test start and measured values at test termination.

The measured concentrations in samples from vessels with *S. erectum* ranged from 26.0 % to 63.6 % of nominal at test initiation and from 15.2 % to 51.6 % of nominal at test termination. The low recovery could be explained by interception of the leaves as verification samples of the additional plant free vessels demonstrated sufficient recovery (*i.e.* 83.5 % - 121.7 %). In view of the strong decline of the test item concentration at test termination, the biological results for this plant species are thus based on recalculated, geometric mean measured water concentrations.

Given the generally low recovery values observed in the study, and for the sake of consistency, the biological results for the remaining plant species were also based on geometric mean of the measured concentrations.

Biological results: No morphological effects on the monocotyledonous plants were observed in the controls and at any of the concentrations tested. At test termination some plants of the dicotyledonous species *Mentha aquatica* were yellowish and cambered at test item concentrations of 0.197 mg BAS 656 08 H/L (0.124 mg as/L) and higher, furthermore the plants appeared smaller with increasing test item concentrations. The root formation of *Ludwigia palustris* and *Veronica*

beccabunga was affected at concentrations of 0.086 mg BAS 656 08 H/L (0.054 mg as/L) and higher at test termination. For *Veronica beccabunga* the effect was not uniform and some replicates at the two highest test item concentrations showed strong root formation.

The most sensitive species in this test was the dicotyledonous *Ludwigia palustris* which showed statistically significant effects compared to the control at the 6 highest test item concentrations (William's test, $p \leq 0.05$) based on both wet weight and length data after exposure over 13 days. Effects on plant growth are summarised in Table B.9.2-59a to Table B.9.2-59f separately for each tested plant species.

Table B.9.2-59a: Effect of dimethenamid-P on the growth of the aquatic plant *Acorus calamus*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.015**	**0.034**	**0.078**	**0.177**	**0.404**	**0.921**	**2.098**
Concentration [mg BAS 656 08 H/L] (geomean)	**0.013**	**0.041**	**0.094**	**0.164**	**0.339**	**0.840**	**2.080**
Concentration [mg dimethenamid-P/L] (nominal)	**0.010**	**0.022**	**0.049**	**0.112**	**0.255**	**0.581**	**1.324**
Concentration [mg dimethenamid-P/L] (geomean)	**0.008**	**0.026**	**0.059**	**0.104**	**0.214**	**0.530**	**1.314**
Inhibition in 13 d [%] [#] (growth rate based on wet weight)	-13.6	-27.5	-33.0	-12.9	15.9	11.5	13.0
Inhibition in 13 d [%] [#] (growth rate based on leave length)	-18.7	-6.1	4.5	-15.5	-19.7	-23.3	0.6
Inhibition in 13 d [%] [#] (growth rate based on root weight)	-4.8	-13.6	-11.4	-3.6	0.1	1.1	4.0

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight, leave length and root formation (13 d)	> 2.098	> 1.324
NOEC based on wet weight, leave length and root weight (13 d)	≥ 2.098	≥ 1.324
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight, leave length and root formation (13 d)	> 2.080	> 1.314
NOEC based on wet weight, leave length and root weight (13 d)	≥ 2.080	≥ 1.314

[#] Negative values indicate stimulated growth compared to the control.

Table B.9.2-59b: Effect of dimethenamid-P on the growth of the aquatic plant *Iris pseudacorus*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.015**	**0.034**	**0.078**	**0.177**	**0.404**	**0.921**	**2.098**
Concentration [mg BAS 656 08 H/L] (geomean)	**0.011**	**0.029**	**0.079**	**0.118**	**0.249**	**0.597**	**1.195**
Concentration [mg dimethenamid-P/L] (nominal)	**0.010**	**0.022**	**0.049**	**0.112**	**0.255**	**0.581**	**1.324**
Concentration [mg dimethenamid-P/L] (geomean)	**0.007**	**0.018**	**0.050**	**0.074**	**0.157**	**0.377**	**0.754**
Inhibition in 13 d [%] # (growth rate based on wet weight)	8.4	-13.7	41.7	37.9	64.8 *	44.3 *	82.9 *
Inhibition in 13 d [%] # (growth rate based on leave length)	9.3	9.7	16.0	26.3	23.4	47.6 *	36.6 *
Inhibition in 13 d [%] # (growth rate based on root weight)	7.8	-15.5	26.3 *	18.6 *	24.4 *	25.1 *	38.1 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight (13 d)	0.363 (95 % confidence limits: 0.337 - 0.392)	0.229 (95 % confidence limits: 0.213 - 0.247)
E_yC_{50} based on leave length and root formation (13 d)	> 2.098	> 1.324
NOEC based on wet weight (13 d)	0.177	0.112
NOEC based on leave length (13 d)	0.404	0.255
NOEC based on root formation (13 d)	0.034	0.022
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight (13 d)	0.244 (95 % confidence limits: 0.063 - 2.61)	**0.154** (95 % confidence limits: 0.040- 1.65)
E_yC_{50} based on leave length and root formation (13 d)	> 1.195	> 0.754
NOEC based on wet weight (13 d)	0.029**	0.018**
NOEC based on leave length (13 d)	0.249	0.157
NOEC based on root formation (13 d)	0.029	0.018

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).
* NOEC by visual interpretation of wet weight data (effect values ranged from 38 % to 42 % at the two higher test levels)

Table B.9.2-59c: Effect of dimethenamid-P on the growth of the aquatic plant *Sparganium erectum*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.015**	**0.034**	**0.078**	**0.177**	**0.404**	**0.921**	**2.098**
Concentration [mg BAS 656 08 H/L] (geomean)	**n.d.**	**0.007**	**0.016**	**0.064**	**0.231**	**0.432**	**0.714**
Concentration [mg dimethenamid-P/L] (nominal)	**0.010**	**0.022**	**0.049**	**0.112**	**0.255**	**0.581**	**1.324**
Concentration [mg dimethenamid-P/L] (geomean)	**n.d.**	**0.004**	**0.010**	**0.041**	**0.146**	**0.273**	**0.451**
Concentration [mg BAS 656 08 H/L] (initial measured)	**0**	**0.009**	**0.023**	**0.085**	**0.257**	**0.506**	**0.905**
Concentration [mg dimethenamid-P/L] (initial measured)	**0**	**0.006**	**0.014**	**0.054**	**0.162**	**0.319**	**0.572**
Inhibition in 13 d [%] # (growth rate based on wet weight)	-5.7	-8.2	15.2	-14.1	32.7 *	42.6 *	53.7 *
Inhibition in 13 d [%] # (growth rate based on leave length)	-3.7	19.2	26.0	-20.6	33.7	63.7 *	13.9 *
Inhibition in 13 d [%] # (growth rate based on root weight)	-14.4	-12.7	-8.7	-17.7	26.4 *	25.7 *	26.7 *

Endpoints	**related to BAS 656 08 H [mg/L] (initial measured)**	**related to dimethenamid-P [mg/L] (initial measured)**
E_yC_{50} based on wet weight (13 d)	0.720	0.455
E_yC_{50} based on leave length and root weight (13 d)	> 0.905	> 0.572
NOEC based on wet weight and root weight (13 d)	0.085	0.054
NOEC based on leave length (13 d)	0.257	0.162
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight (13 d)	0.591 (95 % confidence limits: 0.399 - 0.875)	**0.373** (95 % confidence limits: 0.252 - 0.552)
E_yC_{50} based on leave length and root weight (13 d)	> 0.714	> 0.451
NOEC based on wet weight and root weight (13 d)	0.064	0.041
NOEC based on leave length (13 d)	0.231	0.146

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).
n.d. not determined

Table B.9.2-59d: Effect of dimethenamid-P on the growth of the aquatic plant *Mentha aquatica*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.017**	**0.038**	**0.086**	**0.197**	**0.449**	**1.023**	**2.332**
Concentration [mg BAS 656 08 H/L] (geomean)	**0.017**	**0.035**	**0.067**	**0.143**	**0.305**	**0.678**	**1.724**
Concentration [mg dimethenamid-P/L] (nominal)	**0.011**	**0.024**	**0.054**	**0.124**	**0.283**	**0.646**	**1.472**
Concentration [mg dimethenamid-P/L] (geomean)	**0.011**	**0.022**	**0.042**	**0.090**	**0.193**	**0.428**	**1.088**
Inhibition in 13 d [%] # (growth rate based on wet weight)	-27.3	-4.5	-23.0	18.3	41.6	-12.9	30.6
Inhibition in 13 d [%] # (growth rate based on shoot length)	-20.1	17.2	34.3	40.5	85.5 *	44.8 *	56.6 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight (13 d)	> 2.332	> 1.472
E_yC_{50} based on shoot length (13 d)	0.441 (95 % confidence limits: 0.400 - 0.487)	0.278 (95 % confidence limits: 0.252 - 0.307)
E_yC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight (13 d)	≥ 2.332	≥ 1.472
NOEC based on shoot length (13 d)	0.197	0.124
NOEC based on root weight (13 d)	0.086 +	0.054 +
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight (13 d)	> 1.724	> 1.088
E_yC_{50} based on shoot length (13 d)	0.326 (95 % confidence limits: 0.154 - 0.689)	**0.206** (95 % confidence limits: 0.097 - 0.435)
E_yC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight (13 d)	≥ 1.724	≥ 1.088
NOEC based on shoot length (13 d)	0.143	0.090
NOEC based on root weight (13 d)	0.067 +	0.042 +

n.d. = not determined
# Negative values indicate stimulated growth compared to the control.
+ based on visual observation
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table B.9.2-59e: Effect of dimethenamid-P on the growth of the aquatic plant *Ludwigia palustris*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.017**	**0.038**	**0.086**	**0.197**	**0.449**	**1.023**	**2.332**
Concentration [mg BAS 656 08 H/L] (geomean)	**0.011**	**0.027**	**0.061**	**0.152**	**0.283**	**0.696**	**1.299**
Concentration [mg dimethenamid-P/L] (nominal)	**0.011**	**0.024**	**0.054**	**0.124**	**0.283**	**0.646**	**1.472**
Concentration [mg dimethenamid-P/L] (geomean)	**0.007**	**0.017**	**0.039**	**0.096**	**0.179**	**0.439**	**0.819**
Inhibition in 13 d [%] (growth rate based on wet weight)	12.1	39.3 *	59.8 *	59.8 *	71.8 *	85.2 *	76.6 *
Inhibition in 13 d [%] (growth rate based on shoot length)	10.4	36.5 *	61.3 *	68.3 *	82.3 *	98.3 *	96.6 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight (13 d)	0.098 (95 % confidence limits: 0.089 - 0.107)	0.062 (95 % confidence limits: 0.056 - 0.068)
E_yC_{50} based on shoot length (13 d)	0.075 (95 % confidence limits: 0.072 - 0.080)	0.047 (95 % confidence limits: 0.045 - 0.050)
E_yC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight and shoot length (13 d)	0.017	0.011
NOEC based on root weight (13 d)	0.017 +	0.011 +
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight (13 d)	0.068 (95 % confidence limits: 0.027 - 0.139)	0.043 (95 % confidence limits: 0.017 - 0.088)
E_yC_{50} based on shoot length (13 d)	0.053 (95 % confidence limits: 0.037 - 0.073)	**0.033** (95 % confidence limits: 0.023 - 0.046)
E_yC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight and shoot length (13 d)	0.011	0.007
NOEC based on root weight (13 d)	0.011 +	0.007 +

n.d. = not determined
+ based on visual observation
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table B.9.2-59f: Effect of dimethenamid-P on the growth of the aquatic plant *Veronica beccabunga*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.017**	**0.038**	**0.086**	**0.197**	**0.449**	**1.023**	**2.332**
Concentration [mg BAS 656 08 H/L] (geomean)[1)]	**0.014**	**0.032**	**0.074**	**0.172**	**0.334**	**0.749**	**1.646**
Concentration [mg dimethenamid-P/L] (nominal	**0.011**	**0.024**	**0.054**	**0.124**	**0.283**	**0.646**	**1.472**
Concentration [mg dimethenamid-P/L] (geomean) [1)]	**0.009**	**0.020**	**0.047**	**0.109**	**0.211**	**0.473**	**1.039**
Inhibition in 13 d [%] (growth rate based on wet weight)	1.9	10.7	35.6 *	42.8 *	44.5 *	50.4 *	63.4 *
Inhibition in 13 d [%] (growth rate based on shoot length)	10.0	35.2 *	43.3 *	46.6 *	55.2 *	67.0 *	90.0 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight (13 d)	0.683 (95 % confidence limits: 0.615 - 0.756)	0.431 (95 % confidence limits: 0.388 - 0.477)
E_yC_{50} based on shoot length (13 d)	0.205 (95 % confidence limits: 0.188 - 0.222)	0.129 (95 % confidence limits: 0.118 - 0.140)
E_yC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight (13 d)	0.038	0.024
NOEC based on shoot length (13 d)	0.017	0.011
NOEC based on root weight (13 d)	0.038 +	0.024 +
Endpoints	**related to BAS 656 08 H [mg/L] (geomean)**	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight (13 d)	0.512 (95 % confidence limits: 0.263 - 1.519)	0.323 (95 % confidence limits: 0.166 - 0.958)
E_yC_{50} based on shoot length (13 d)	0.165 (95 % confidence limits: 0.086 - 0.316)	**0.104** (95 % confidence limits: 0.054 - 0.199)
E_yC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight (13 d)	0.032	0.020
NOEC based on shoot length (13 d)	0.014	0.009
NOEC based on root weight (13 d)	0.032 +	0.020 +

1) nominal values were used instead of inital measured (0 d) for reasons of plausibility
n.d. = not determined
+ based on visual observation
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Conclusions

In a 13-day static toxicity test with six emergent aquatic plant species, *Ludwigia palustris* was the most sensitive species generating an E_yC_{50} of 0.068 mg BAS 656 08 H/L (0.043 mg dimethenamid-P/L) based on wet weight and an E_yC_{50} of 0.053 mg BAS 656 08 H/L (0.033 mg as/L) based on length data (geometric mean of the measured concentrations).

KCA 8.2.7/8 (new study, submitted with renewal dossier)

Author: Kubitza, J., Dohmen, G.P.
Title: Effect of dimethenamid-P - Tested as formulated product BAS 656 08 H - on submersed aquatic plants

Date:	28.02.2003
Doc ID:	2002/1012789
Guidelines:	HARAP (Campbell et al. 1999) Guidance Document on Higher-tier Aquatic Risk Assessment of Pesticides, CLASSIC (Workshop on Community Level Aquatic System Studies May-June 1999)
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item: BAS 656 08 H, batch no. 2001-1; content of as: dimethenamid-P (BAS 656 H, Reg. no. 363 851): 711.4 g/L (nominal: 720.0 g/L); density: 1.127 g/cm^3.

Test species: Submersed aquatic plants;
Monocotyledonous: *Elodea densa* (Hydrocharitaceae) (source: "Aquaristic Reier", Frankfurt, Germany), *Potamogeton crispus* (Potamogetonaceae) (source: field collected from river "Eisbach" near Bachern, Germany), *Vallisneria spiralis* (Hydrocharitaceae) (source: first test, "Aquaristic Reier", Frankfurt, Germany; repeated test: in-house cultivation);
Dicotyledonous: *Ceratophyllum demersum* (Ceratophyllaceae) (source: field collected from a pond in "Fischach" near Augsburg, Germany), *Myriophyllum spicatum* (Haloragaceae) (in-house cultivation), *Crassula recurva* (Crassulaceae) (source: garden market "Glaß", Augsburg, Germany).

Test design: Static system (including sediment), test duration 9 - 12 days (plant dependent), 6 test item concentrations, each with 3 replicates plus a control with 6 replicates; the number of plants per replicate (one to several) varied depending on the test species; the plants (except *C. demersum*) were potted in sediment; *C. demersum* was kept free floating within the vessels; nevertheless pots with the same sediment were placed on the bottom of the vessels; visual observations one day after treatment, one week after treatment and at the end of the test; determination of wet weight and plant length at test end, visual assessment of root formation. At test end the potential for recovery was tested in the control and the three highest test item concentrations for all tested species (except for *V. spiralis*) over 9, respectively 10 days.

Endpoints: EC_{50} with respect to growth rate related to wet weight and length data after exposure over 9 to 12 days (plant dependent).

Test concentrations: Control, 0.0015, 0.0030, 0.0063, 0.0146, 0.0634 and 0.526 mg BAS 656 08 H/L (nominal), 0, 0.00096, 0.00189, 0.0040, 0.0092, 0.040 and 0.332 mg dimethenamid-P/L (nominal; based on a measured content of 711.4 g as/L and a formulation density of 1.127 g/cm^3).

Test conditions: 2 L glass beakers, standard artificial sediment (OECD 219 amended with 1 g plant fertiliser per pot) and 3 x AAP medium (amended with 2.24 mg/L $FeCl_3$, pH 6.5); oxygen content: 7.5 mg/L - 9.4 mg/L: 80.0 % - 248.0 %; pH: 7.27 - 10.95; conductivity: 268 - 283 µS/cm; carbonate hardness: 0.33 - 0.35 mmol/L; water temperature: 19.8 °C - 24.0 °C; air temperature: 21 ± 1 °C; light : dark - rhythm 16 : 8 h, light intensity: approx.. 8.5 klux.

Analytics: Analytical verification of test item concentrations was conducted using a SPME-method with GC/MS detection.

Statistics: Descriptive statistics; determination of EC_{50} values by probit analysis, William's, Dunnett`s and Bonferroni test ($p \leq 0.05$) for determination of NOEC values. The following transformations have been performed with the raw data to determine EC_{50} and to assess statistically significant differences in plant growth:

- *Elodea densa, Potamogeton crispus, Ceratophyllum demersum, Myriophyllum spicatum, Crassula recurva*:

  Wet weight: ln(final wet weight) - ln(initial wet weight)
  Length: ln(final length) - ln(initial length)

- *Vallisneria spiralis*:

  Wet weight: final wet weight
  Length: ln(final length)

Note: At the end of the test with *Vallisneria spiralis* the old leaves of the plants were decayed. Therefore weight and length of the old leaves (the introduced leaves from test initiation) were not included in the final calculation. Only the new leaves were weighed and metered at test end.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted for each tested plant species in all test item concentrations at the beginning and at the end of the test, except for the two lowest test concentrations which were below the limit of quantification. The measured concentrations of the test item for all plant species ranged from 80.2 % to 112.0 % of nominal concentrations at test initiation and from 46.7 % to 99.8 % of nominal at test termination. As the analytically measured values at test end were in most cases <80 % of nominal, the following biological results are based on geometric mean measured concentrations.

Biological results: At the highest test item concentration plant tips of *E. densa* appeared slightly dark colored at test termination the plants in one replicate had no side branches. Root formations and side branch development of *E. densa* was generally normal except for the highest test concentration. The leaves of *P. crispus* were necrotic in the highest test item concentration one week after treatment and in the three highest test item concentrations at test termination. Furthermore, no root formation was observed, neither in the control nor in the test item treatments. Regarding *V. spiralis*, no particular test substance related visual assessments were made throughout the test. At test termination shoot tips of *C. demersum* were slightly dark colored at 0.0146 mg BAS 656 08 H/L (0.0092 mg as/L) and shoot-tips at the highest test item concentration were necrotic. At test termination leaves of *M. spicatum* tended to drop off when handled and the root formation was poor; however both effects were independent of the treatment level. No root formation was observed in two replicates at 0.0146 mg BAS 656 08 H/L (0.0092 mg as/L). Some plants of *C. recurva* showed necrotic leaf parts at the two highest test item concentrations one week after treatment and plants appeared dark colored at the highest test item concentration at test termination.
The most sensitive species in this test was the dicotyledonous *C. demersum* which showed statistically significant effects compared to the control at the 4 highest test item concentrations (William's test, $p \leq 0.05$) based on plant length development after exposure over 9 days. The recovery test showed that all plant species were able to recover rapidly from a previous treatment with the test item. Effects on plant growth are summarised in Table B.9.2-60a to Table B.9.2-60f separately for each plant species.

Table B.9.2-60a: Effect of dimethenamid-P on the growth of the aquatic plant *Elodea densa*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg BAS 656 08 H/L] (geomean)	**<LOQ**	**<LOQ**	**0.00453**	**0.0116**	**0.0501**	**0.378**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Concentration [mg dimethenamid-P/L] (geomean)	**<LOQ**	**<LOQ**	**0.0029**	**0.0073**	**0.0316**	**0.239**
Inhibition in 12 d [%] # (wet weight)	-36.5	-9.4	-10.4	-10.3	15.4	32.2 *
Inhibition in 12 d [%] # (length)	-18.4	-55.5	-78.9	-51.4	-0.9	66.3 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight (12 d)	> 0.526	> 0.332
E_yC_{50} based on length (12 d)	0.438 (95 % confidence limits: n.d.)	0.276 (95 % confidence limits: n.d.)
NOEC based on wet weight and length (12 d)	0.0634	0.040
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC50 based on wet weight (12 d)	> 0.378	> 0.239
E_yC50 based on length (12 d)	0.330 (95 % confidence limits: n.d.)	**0.208** (95 % confidence limits: n.d.)
NOEC based on wet weight and length (12 d)	0.0501	0.0316

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).
n.d. not determined due to mathematical reasons or inappropriate data).

Table B.9.2-60b: Effect of dimethenamid-P on the growth of the aquatic plant *Potamogeton crispus*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg BAS 656 08 H/L] (geomean)	**<LOQ**	**<LOQ**	**0.00454**	**0.00984**	**0.0467**	**0.339**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Concentration [mg dimethenamid-P/L] (geomean)	**<LOQ**	**<LOQ**	**0.00287**	**0.0062**	**0.0295**	**0.214**
Inhibition in 9 d [%] # (wet weight)	-1.8	-16.6	6.9	-8.5	2.8	3.1
Inhibition in 9 d [%] # (length)	-9.8	-26.9	2.7	-4.2	8.6	56.4 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight (9 d)	> 0.526	> 0.332
E_yC_{50} based on length (9 d)	0.444 (95 % confidence limits: 0.396 - 0.498)	0.280 (95 % confidence limits: 0.250 - 0.314)
NOEC based on wet weight (9 d)	≥ 0.526	≥ 0.332
NOEC based on length (9 d)	0.0634	0.040
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight (9 d)	> 0.33872	> 0.214
E_yC_{50} based on length (9 d)	0.275 (95 % confidence limits: 0.212 – 0.364)	**0.174** (95 % confidence limits: 0.133 – 0.230)
NOEC based on wet weight (9 d)	≥ 0.339	≥ 0.332
NOEC based on length (9 d)	0.0467	0.0295

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table B.9.2-60c: Effect of dimethenamid-P on the growth of the aquatic plant *Vallisneria spiralis*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg BAS 656 08 H/L] (geomean)	**<LOQ**	**<LOQ**	**0.00477**	**0.01051**	**0.0490**	**0.414**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Concentration [mg dimethenamid-P/L] (geomean)	**<LOQ**	**<LOQ**	**0.0030**	**0.0066**	**0.0310**	**0.261**
Inhibition in 12 d [%] [#] (wet weight)	-3.3	-3.8	1.3	-3.8	3.8	-1.8
Inhibition in 12 d [%] [#] (length)	12.0	10.9	5.7	0.2	8.0	7.3
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_yC_{50} based on wet weight and length (12 d)	> 0.526			> 0.332		
NOEC based on wet weight and length (12 d)	≥ 0.526			≥ 0.332		
	related to BAS 656 08 H [mg/L] (geomean)			**related to dimethenamid-P [mg/L] (geomean)**		
E_yC50 based on wet weight and length (12 d)	> 0.414			**> 0.261**		
NOEC based on wet weight and length (12 d)	≥ 0.414			≥ 0.261		

[#] Negative values indicate stimulated growth compared to the control.

Table B.9.2-60d: Effect of dimethenamid-P on the growth of the aquatic plant *Ceratophyllum demersum*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg BAS 656 08 H/L] (geomean)	**<LOQ**	**<LOQ**	**0.00603**	**0.01235**	**0.05406**	**0.4361**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Concentration [mg dimethenamid-P/L] (geomean)	**<LOQ**	**<LOQ**	**0.00381**	**0.00779**	**0.0341**	**0.2753**
Inhibition in 9 d [%] # (wet weight)	-3.8	-7.7	10.6	33.2 *	64.8 *	72.8 *
Inhibition in 9 d [%] # (length)	13.7	3.3	27.7 *	31.4 *	74.8 *	94.0 *
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_yC_{50} based on wet weight (9 d)	0.067 (95 % confidence limits: 0.062 - 0.074)			0.042 (95 % confidence limits: 0.039 - 0.047)		
E_yC_{50} based on length (9 d)	0.025 (95 % confidence limits: 0.023 - 0.026)			0.016 (95 % confidence limits: 0.015 - 0.016)		
NOEC based on wet weight (9 d)	0.0063			0.0040		
NOEC based on length (9 d)	0.0030			0.0019		
	related to BAS 656 08 H [mg/L] (geomean)			**related to dimethenamid-P [mg/L] (geomean)**		
E_yC_{50} based on wet weight (9 d)	0.0438 (95 % confidence limits: n.d.)			0.0276 (95 % confidence limits: 0.039 - 0.047)		
E_yC_{50} based on length (9 d)	0.021 (95 % confidence limits: 0.011 - 0.039)			**0.0133** (95 % confidence limits: 0.007 - 0.025)		
NOEC based on wet weight (9 d)	0.00603			0.0040		
NOEC based on length (9 d)	<0.00603			<0.00381		

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table B.9.2-60e: Effect of dimethenamid-P on the growth of the aquatic plant *Myriophyllum spicatum*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg BAS 656 08 H/L] (geomean)	**<LOQ**	**<LOQ**	**0.00612**	**0.01378**	**0.06449**	**0.4855**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Concentration [mg dimethenamid-P/L] (geomean)	**<LOQ**	**<LOQ**	**0.0039**	**0.0087**	**0.0407**	**0.3065**
Inhibition in 9 d [%] # (wet weight)	-18.6	-16.0	-15.3	-16.0	-38.6	-17.3
Inhibition in 9 d [%] # (length)	-20.7	-49.8	-55.2	16.3	43.3 *	66.8 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_yC_{50} based on wet weight (9 d)	> 0.526	> 0.332
E_yC_{50} based on length (9 d)	0.152 (95 % confidence limits: 0.138 - 0.168)	0.096 (95 % confidence limits: 0.087 - 0.106)
NOEC based on wet weight (9 d)	≥ 0.526	≥ 0.332
NOEC based on length(9 d)	0.0146	0.0092
	related to BAS 656 08 H [mg/L] (geomean)	**related to dimethenamid-P [mg/L] (geomean)**
E_yC_{50} based on wet weight (9 d)	> 0.4855	> 0.3065
E_yC_{50} based on length (9 d)	0.140 (95 % confidence limits: 0.021 - 1082)	**0.088** (95 % confidence limits: 0.013 - 683)
NOEC based on wet weight (9 d)	≥ 0.4855	≥ 0.3065
NOEC based on length(9 d)	0.0092	0.0087

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table B.9.2-60f: Effect of dimethenamid-P on the growth of the aquatic plant *Crassula recurva*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg BAS 656 08 H/L] (geomean)	**<LOQ**	**<LOQ**	**0.00575**	**0.0119**	**0.0618**	**0.539**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Concentration [mg dimethenamid-P/L] (geomean)	**<LOQ**	**<LOQ**	**0.0036**	**0.0075**	**0.039**	**0.340**
Inhibition in 12 d [%] # (wet weight)	-27.0	7.2	24.8	9.4	18.6	7.7
Inhibition in 12 d [%] # (length)	9.0	-5.1	-23.3	-14.5	33.8	79.2 *
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_yC_{50} based on wet weight (12 d)	> 0.526			> 0.332		
E_yC_{50} based on length (12 d)	0.156 (95 % confidence limits: 0.141 - 0.173)			0.0984 (95 % confidence limits: 0.089 - 0.109)		
NOEC based on wet weight (12 d)	≥ 0.526			≥ 0.332		
NOEC based on length(12 d)	0.0634			0.040		
	related to BAS 656 08 H [mg/L] (geomean)			**related to dimethenamid-P [mg/L] (geomean)**		
E_yC_{50} based on wet weight (12 d)	> 0.539			> 0.340		
E_yC_{50} based on length (12 d)	0.137 (95 % confidence limits: 0.0585 - 0.400)			**0.0865** (95 % confidence limits: 0.037 - 0.25)		
NOEC based on wet weight (12 d)	≥ 0.539			≥ 0.340		
NOEC based on length(12 d)	0.0618			0.039		

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Conclusions

In a static toxicity test with six submersed aquatic plant species, *Ceratophyllum demersum* was the most sensitive species generating an E_yC_{50} of 0.0438 mg BAS 656 08 H/L (0.0276 mg dimethenamid-P/L) based on wet weight and an E_yC_{50} of 0.021 mg BAS 656 08 H/L (0.0133 mg as/L) based on length data (geometric mean of the measured concentrations).

KCA 8.2.7/9 (new study, submitted with renewal dossier)

Author: Hoffmann, F.
Title: Effect of Reg.No. 360712 (M31, metabolite of dimethenamid-P) on the growth of *Lemna gibba*
Date: 18.08.2008
Doc ID: 2008/1035918
Guidelines: OECD 221, EPA 850.4400, ASTM E 1415-91
GLP: Yes
Validity: Acceptable

Material and Methods

Test item:	M656H031 (M31, Reg. No. 360 712); metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. RS-582TAS-050495, purity: 99.4%.
Test species:	Duckweed (*Lemna gibba* G3), inocula 7 - 10 day old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.
Test design:	Static system (7 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 3, 5 and 7.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control, 10, 18, 31, 56 and 100 mg M656H031/L (nominal).
Test conditions:	400 mL glass beakers, test volume 160 mL, 20x-AAP nutrient medium, pH 7.50 - 7.52 at test initiation and pH 8.62 - 8.87 at test termination; water temperature: 24.2 °C - 24.5 °C, continuous light, average light intensity: about 8200 lux.
Analytics:	Analytical verification of the test item was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656H031 ranged from 98.8 % to 110.1 % of nominal at test initiation and from 86.5 % to 105.4 % of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 222 fronds per vessel, corresponding to a 20.2 x multiplication. The dry weight increased from 2.1 mg to an average of 28.9 mg per vessel in the control at test termination. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Effects on growth rate and yield are summarised in Table B.9.2-61.

Table B.9.2-61: Effect of M656H031 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna gibba*

Concentration [mg/L] (nominal)	**10**	**18**	**32**	**56**	**100**
Inhibition after 7 d [%] * (growth rate based on frond no.)	0.3	0.3	0.3	-0.1	-1.2
Inhibition after 7 d [%] * (growth rate based on dry weight)	0.8	-1.7	0.1	-0.2	-3.4
Inhibition after 7 d [%] * (yield based on frond no.)	1.0	0.9	0.9	-0.4	-3.7
Inhibition after 7 d [%] * (yield based on dry weight)	2.4	-4.7	0.2	-0.9	-10.2
Endpoints [mg M656H031/L] (nominal)					
E_rC_{50} (7 d) based on frond no. and dry weight	> 100				
E_rC_{10} (7 d) based on frond no. and dry weight	> 100				
E_yC_{50} (7 d) based on frond no. and dry weight	> 100				
E_yC_{10} (7 d) based on frond no. and dry weight	> 100				

* Negative values indicate stimulated growth compared to the control.

Conclusions

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656H031 (metabolite of dimethenamid-P) based on frond no. and dry weight were determined to be both > 100 mg/L (nominal).

KCA 8.2.7/10 (new study, submitted with renewal dossier)

Author: Swierkot, A.
Title: Reg.No. 5749263 (metabolite of BAS 656 H, dimethenamid-P M656H055, M55) - *Lemna gibba* CPCC 310 growth inhibition test
Date: 27.08.2013
Doc ID: 2013/1063800
Guidelines: OECD 221
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M656H055 (M55; Reg. No. 5 749 263) metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. L80-154, purity: 69.8 ± 1 %.

Test species: Duckweed (*Lemna gibba* G3), inocula 7 day old cultures; cultures maintained in-house; stock obtained from "University of Waterloo, Canadian Phycological Culture Centre", Ontario, Canada.

Test design: Static system (7 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 3, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield based on frond number and dry weight after exposure over 7 days.

Test concentrations: Control, 13.6, 24.5, 44.1, 79.4 and 143 mg M656H055/L (nominal).

Test conditions: 600 mL glass beakers, test volume 400 mL, 20x-AAP nutrient medium, pH 7.54 - 7.83 at test initiation and pH 9.53 - 9.71 at test termination; water temperature: 23.8 °C - 24.3 °C, continuous light, light intensity: 8955 - 9248 lux.

Analytics: Analytical verification of the test item was conducted using a liquid chromatography-method with UV-VIS detection.

Statistics: Descriptive statistics, probit analysis for calculation of EC_x values; Williams Multiple Sequential t-test Procedure ($\alpha = 0.05$) for determination of the NOEC.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656H055 ranged from 100.3 % to 102.9 % of nominal at test initiation and from 110.5 % to 115.0 % of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 9 fronds per vessel to an average of 108 fronds per vessel, corresponding to a 12 x multiplication. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. No statistically significant effects on algal growth compared to the control were observed at any test item concentration tested (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$). Effects on growth rate and yield are summarised in Table B.9.2-62.

Table B.9.2-62: Effect of M656H055 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna gibba*

Concentration [mg/L] (nominal)	**13.6**	**24.5**	**44.1**	**79.4**	**143**
Inhibition after 7 d [%] (growth rate based on frond no.)	0.0	0.0	0.0	0.0	2.23
Inhibition after 7 d [%] (yield based on frond no.)	0.0	0.0	0.0	0.0	5.88
Inhibition after 7 d [%] (growth rate based on dry weight)	0.0	0.0	0.0	0.0	0.0
Inhibition after 7 d [%] (yield based on dry weight)	0.0	0.0	0.0	0.0	0.0
Endpoints [mg M656H055/L] (nominal)					
E_rC_{50} / E_yC_{50} (7 d) based on frond no. and dry weight	> 143				
E_rC_{10} / E_yC_{10} (7 d) based on frond no. and dry weight	> 143				
NOE_rC / NOE_yC (7 d) based on frond no. and dry weight	≥ 143				

Conclusions

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656H055 (metabolite of dimethenamid-P) based on both frond no. and dry weight were determined to be both > 143 mg/L (nominal).

KCA 8.2.7/11 (new study, submitted with renewal dossier)

Author: Swierkot, A.
Title: Reg.No. 403121 (metabolite of BAS 656 H, dimethenamid-P, M39) - *Lemna gibba* CPCC 310 growth inhibition test
Date: 09.10.2013
Doc ID: 2013/1191249
Guidelines: OECD 221
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Reg. No. 403 121, metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. L74-88, purity: 99.1 ± 1 %.

Test species: Duckweed (*Lemna gibba* G3), inocula 7 days old cultures; cultures maintained in-house; stock obtained from "University of Waterloo, Canadian Phycological Culture Centre, Department of Biology", Ontario, Canada.

Test design: Semi-static system (7 days); renewal of test solutions on days 2, 4 and 6; 7 treatment groups (6 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 4, 6 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control, 0.41, 1.23, 3.7, 11.11, 33.33 and 100 mg Reg. No. 403 121/L (nominal).

Test conditions: Glass vessels, test volume 150 mL, 20x-AAP nutrient medium, pH 7.24 - 7.67 at test initiation and pH 8.35 - 8.99 at test termination; water temperature: 24.2 °C - 24.6 °C, continuous light, average light intensity 8350 lux - 9225 lux.

Analytics: Analytical verification of the test item was conducted using a liquid chromatography-method with DAD detection.

Statistics: Descriptive statistics, probit analysis for calculation of EC_x values; Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment and Williams Multiple Sequential t-test Procedure (both $\alpha = 0.05$) for determination of the NOEC.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in the fresh and old solutions of each test concentration at the beginning of the test, on day 2, 4 and 6 and at the end of the test, except for the 0.41 mg/L treatment since this concentration was below the limit of detection (LoD = 0.50 mg/L). Mean measured values for Reg. No. 403 121 ranged from 85.4 % to 102.4 % of nominal concentrations in fresh solutions and from 36.5 % to 102.0 % of nominal in old solutions. The determined mean concentrations in old solutions were in the range of < LoD and 77.2 % of initial concentrations, what shows that the test item was not stable under test condition. However, as initially measured concentrations confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 9 fronds per vessel to an average of 198 fronds per vessel, corresponding to a 22 x multiplication. After 7 days of exposure, no morphological effects on algae were observed in the control group and at test item concentrations of up to and including 33.33 mg/L. At the highest test item concentration of 100 mg/L, smaller fronds, shorter roots and plants with single fronds were observed. Statistically significant effects on algal growth rate and yield compared to the control were determined in the two highest test item concentrations based on frond numbers and in the highest concentration based on dry weight (Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment and Williams Multiple Sequential t-test Procedure, both $\alpha = 0.05$). Effects on growth rate and yield are summarised in Table B.9.2-63.

Table B.9.2-63: Effect of Reg. No. 403 121 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna* gibba

Concentration [mg/L] (nominal)	**0.41**	**1.23**	**3.70**	**11.11**	**33.33**	**100**
Inhibition after 7 d [%] (growth rate based on frond no.)	0.00	0.01	0.00	1.45	10.44 *	40.68 *
Inhibition after 7 d [%] (yield based on frond no.)	0.00	0.00	0.00	4.67	28.99 *	74.98 *
Inhibition after 7 d [%] (growth rate based on dry weight)	0.00	0.62	0.00	0.00	5.83	34.67 *
Inhibition after 7 d [%] (yield based on dry weight)	0.00	2.97	0.00	0.00	16.69	65.27 *
Endpoints [mg Reg. No. 403 121/L] (nominal)						
E_rC_{50} (7 d) based on frond no.	> 100					
E_rC_{10} (7 d) based on frond no.	10.88 (95 % confidence limits: 1.68 - 36.15)					
E_yC_{50} (7 d) based on frond no.	54.57 (95 % confidence limits: 53.39 - 55.78)					
E_yC_{10} (7 d) based on frond no.	17.07 (95 % confidence limits: 6.28 - 17.85)					
NOE_rC / NOE_yC (7 d) based on frond no.	11.11					
E_rC_{50} (7 d) based on dry weight	> 100					
E_rC_{10} (7 d) based on dry weight	14.22 (95 % confidence limits: 1.22 - > 100)					
E_yC_{50} (7 d) based on dry weight	72.87 (95 % confidence limits: 67.37 - 78.93)					
E_yC_{10} (7 d) based on dry weight	26.05 (95 % confidence limits: 21.42 - 30.25)					
NOE_rC / NOE_yC (7 d) based on dry weight	33.33					

* Statistically significant differences compared to the control (Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment and Williams Multiple Sequential t-test Procedure; both $\alpha = 0.05$).

Conclusions

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} of Reg. No. 403 121 (metabolite of dimethenamid-P) was determined to be > 100 mg/L based on both frond no. and dry weight (nominal). The E_yC_{50} was 54.57 mg/L based on frond no. and 72.87 mg/L based on dry weight (nominal).

KCA 8.2.7/12 (new study, submitted with renewal dossier)

Author: Swierkot, A.
Title: Reg.No. 5917262 (metabolite of BAS 656 H, dimethenamid-P, M43) - *Lemna gibba* CPCC 310 growth inhibitation test
Date: 26.11.2013
Doc ID: 2013/1191248

Guidelines: OECD 221
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M656PH043 (M43, Reg. No. 5 917 262), metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. L82-113, purity: 94.6 ± 1 %.

Test species: Duckweed (*Lemna gibba* G3), inocula 7 day old cultures; cultures maintained in-house; stock obtained from "University of Waterloo, Canadian Phycological Culture Centre", Ontario, Canada.

Test design: Static system (7 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 3, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield based on frond number and dry weight after exposure over 7 days.

Test concentrations: Control, 2.56, 6.4, 16, 40 and 100 mg M656PH043/L (nominal).

Test conditions: 600 mL glass beakers, test volume 400 mL, 20x-AAP nutrient medium, pH 7.58 - 7.76 at test initiation and pH 9.56 - 9.98 at test termination; water temperature: 24.0 °C - 24.4 °C, continuous light, light intensity: 7674 - 7910 lux.

Analytics: Analytical verification of the test item was conducted using a liquid chromatography-method with DAD detection.

Statistics: Descriptive statistics, probit analysis for calculation of EC_x values.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656PH043 ranged from 98.1 % to 105.8 % of nominal at test initiation and from 104.6 % to 108.1 % of nominal at test termination. As analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 9 fronds per vessel to an average of 136 fronds per vessel, corresponding to a 15 x multiplication. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Effects on growth rate and yield are summarised in Table B.9.2-64.

Table B.9.2-64: Effect of M656PH043 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna gibba*

Concentration [mg/L] (nominal)	**2.56**	**6.4**	**16**	**40**	**100**
Inhibition after 7 d [%] (growth rate based on frond no.)	1.00	2.60	6.98	10.43	12.30
Inhibition after 7 d [%] (yield based on frond no.)	2.87	7.31	18.54	25.85	30.29
Inhibition after 7 d [%] (growth rate based on dry weight)	0.00	0.00	1.49	3.18	6.28
Inhibition after 7 d [%] (yield based on dry weight)	0.00	0.00	4.53	8.73	17.03
Endpoints [mg M656PH043/L] (nominal)					
E_rC_{50} (7 d) based on frond no. and dry weight	> 100				
E_rC_{10} (7 d) based on frond no.	50.5 (95 % confidence limits: 24.3 - > 100)				
E_rC_{10} (7 d) based on dry weight	> 100				
E_yC_{50} (7 d) based on frond no. and dry weight	> 100				
E_yC_{10} (7 d) based on frond no.	7.0 (95 % confidence limits: 0.5 - 15.6)				
E_yC_{10} (7 d) based on dry weight	48.3 (95 % confidence limits: 35.6 - 60.0)				

Conclusions

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656PH043 (metabolite of dimethenamid-P) based on both frond no. and dry weight were determined to be both > 100 mg/L (nominal).

B.9.2.8 Further testing on aquatic organisms

No further studies required; thus, this point is not addressed *via* new toxicity studies.

B.9.3 Effects on arthropods

B.9.3.1 Effects on bees

In order to re-evaluate dimethenamid-P (BAS 656 H) a total of four laboratory studies with technical dimethenamid-P were submitted. Since the Annex I inclusion of dimethenamid-P, new toxicity studies on the active substance on bees have been performed. Table B.9.3-1 presents a summary of these studies. Further details regarding the studies are provided in section B.9.3.1.1.

Table B.9.3-1 Toxicity to bees of dimethenamid-P

Test substance	Test species	Endpoint	Value	Reference
Dimethenamid-P (BAS 656 H)	honeybee	24 h acute oral LD_{50}	> 1000 µg as/bee	Donat H.J, 1986 1986/11170
		24 h acute contact LD_{50}	94 µg as/bee	
	honeybee	48 h acute oral LD_{50}	118.8 µg as/bee*	Zenker K., 2011** Study no. 2010/1126065
		48 h acute contact LD_{50}	93.8 µg as/bee*	
	honeybee larvae	96 h oral LD_{50} 96 h oral LC_{50}	69.6 µg as/larva 2.054 g as/kg food	Kleebaum K., 2014** Study no. 2013/1132510
	bumblebee	48 h acute oral LD_{50}	> 158 µg as/bumblebee	Roehlig U., 2014** Study no. 2013/1275562
		48 h acute contact LD_{50}	> 200 µg as/bumblebee	

* corrected for the purity of the test item
** new study submitted for the re-evaluation of dimethenamid-P (BAS 656 H)

B.9.3.1.1 Acute toxicity (KCA 8.3.1.1.)

Acute oral (KCA 8.3.1.1.1) and contact (KCA 8.3.1.1.2) toxicity

Report: B 9.3.1.1/1
Zenker K., 2011
Acute toxicity of BAS 656-H (Reg.No. 363 851, dimethenamid-P) to the honeybee *Apis mellifera* L. under laboratory conditions
2010/1126065
Guidelines: OECD 213 (1998)
GLP: yes
Validity Acceptable

Executive Summary

In an oral dose response toxicity test, young adult worker bees (*Apis mellifera* L.) were exposed to dimethenamid-P. The toxicity of the test item was determined at nominal doses of 12.9, 25.8, 51.7, 103.3 and 206.6 µg/bee, resulting in an uptake of 12.9, 25.8, 51.1, 101.8 and 193.2 µg of dimethenamid-P/bee. Additionally, honeybees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.063 to 0.500 µg dimethoate/bee (analysed) or with a solution of water and sucrose or of water, sucrose and acetone as controls. The test was conducted with 3 replicates each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of oral exposure, a mortality of 3.3 % was observed in both controls. In the test item treated groups, mortalities between 0.0 % and 90.0 % were observed. At the highest tested doses of 193.2 µg and 101.8 µg dimethenamid-P/bee statistically significant differences compared to the control were observed. Some of the surviving bees treated with 193.2 µg or 101.8 µg consumed product/bee showed an abnormal behaviour after 48 hours, like uncoordinated movements or lying on the back.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. 6261B01BH, purity: 96.8 % (± 1 %).

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera carnica* P.), young adult worker bees, age: 3 - 5 weeks, deriving from a healthy and queen-right colony; source: Bienenfarm Kern GmbH, Leipzig, Germany; collected in the morning of use.

Test design: Dose response test for oral toxicity; duration 48 h, 3 replicates, each replicate consisting of 10 bees per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioural abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: 50 % (w/v) aqueous sucrose solution, 50 % (w/v) aqueous sucrose solution + 1 % v/v acetone; test item (nominal): 12.9, 25.8, 51.7, 103.3 and 206.6 µg as/bee, resulting in an uptake of dimethenamid-P of 12.9, 25.8, 51.1, 101.8 and 193.2 µg as/bee.

Test conditions: Temperature: 24.8 – 25.2 °C; relative humidity: 59 % - 61 %, photoperiod: 24 h darkness. Food: 50 % w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction ($\alpha = 0.05$), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, a mortality of 3.3 % was observed in both controls. In the test item treated groups, mortalities between 0.0 % and 90.0 % were observed. At the highest tested doses of 193.2 µg and 101.8 µg consumed dimethenamid-P/bee statistically significant differences compared to the control (Fisher's Exact Binominal test, $\alpha = 0.05$) were observed. Some of the surviving bees treated with 193.2 µg and 101.8 µg consumed product/bee showed an abnormal behaviour after 48 hours, like uncoordinated movements or lying on the back.
The results are summarised in Table B.9.3-2.

Table B.9.3-2: Toxicity of dimethenamid-P to honeybees (*Apis mellifera carnica* P.) in an oral toxicity test

Treatment		Uptake	Mortality [%]		Corrected mortality [%] [1]	
Test item [µg dimethenamid-P/bee] (nominal)	**Test item [µg dimethenamid-P/bee]** (purity: 96.8 %)	**Test item [µg dimethenamid-P/bee]**	**24 h**	**48 h**	**24 h**	**48 h**
Control (sucrose solution)	Control (sucrose solution)	--	3.3	3.3	--	--
Control (sucrose sol. + acetone)	Control (sucrose sol. + acetone)	--	0.0	3.3	--	--
12.9	12.5	12.9	3.3	3.3	--	0.0
25.8	25.0	25.8	0.0	0.0	--	-3.4
51.7	50.0	51.1	0.0	0.0	--	-3.4
103.3	100.0	101.8	33.3 *	33.3 *	--	31.0
206.6	200.0	193.2	86.7 *	90.0 *	--	89.7
Endpoint [µg/bee]						
LD_{50} (95 % CL) [2] (48 h)		**Test item dimethenamid-P**				
		122.7 (106.9 – 140.7) 118.8 (103.5 – 136.2), corrected for the purity of 96.8 %				

[1] according to Abbott (1925)
[2] Median lethal dose after 48 hours of exposure calculated by Probit analysis (with 95 % Confidence Limits)
* Statistically significant differences compared to the control (Fisher's Exact Binominal Test, $\alpha = 0.05$).

The LD_{50} value (24 h) for the toxic reference item in the oral toxicity test was LD_{50} = 0.196 µg as/bee (95 % confidence limits: 0.158 - 0.244 µg as/bee).

III. CONCLUSION

In an acute oral toxicity study with dimethenamid-P on honeybees the LD_{50} value (48 h) was determined to be 122.7 µg dimethenamid-P/bee (118.8 µg as/bee, if corrected for the purity of the test item).

The study is considered valid and acceptable for the risk assessment.

Report: B 9.3.1.1/2
Zenker K., 2011
Acute toxicity of BAS 656-H (Reg.No. 363 851, dimethenamid-P) to the honeybee Apis mellifera L. under laboratory conditions
2010/1126065
Guidelines: OECD 214 (1998)
GLP: yes
Validity: Acceptable

Executive Summary
In a contact dose response toxicity test, young adult worker bees (*Apis mellifera* L.) were exposed to dimethenamid-P. The toxicity of the test item was determined at doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg as/bee (based on analysed purity). Additionally, honeybees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.063 to 0.500 µg dimethoate/bee (analysed) and with deionised water, Tween solution and Acetone as control. The test was conducted with 3 replicates each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of contact exposure, a mortality of 0.0 % was observed in all controls. In the test item treated groups, mortalities between 0.0 % and 100.0 % were observed. At the highest tested doses of 103.3 µg and 206.6 µg dimethenamid-P/bee statistically significant differences compared to the control were observed. 92 % of the surviving bees treated with 103.3 µg dimethenamid-P/bee showed some abnormal behaviour after 48 hours, like uncoordinated movements or lying on the back.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. 6261B01BH, purity: 96.8 % (± 1 %).

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera carnica* P.), young adult worker bees, age: 3 -5 weeks, deriving from a healthy and queen-right colony; source: Bienenfarm Kern GmbH, Leipzig, Germany; collected in the morning of use.

Test design: Dose response test for contact toxicity; duration 48 h, 3 replicates, each replicate consisting of 10 bees per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioural abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: deionised water, Tween control (1.0 % (v/v) Tween solution), acetone control (undiluted); test item: 12.9, 25.8, 51.7, 103.3 and 206.6 µg dimethenamid-P/bee (based on a purity of 96.8 %: 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee).

Test conditions: Temperature: 24.8 – 25.2 °C; relative humidity: 59 % - 61 %, photoperiod: 24 h darkness. Food: 50 % w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction ($\alpha = 0.05$), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, a mortality of 0.0 % was observed in all controls. In the test item treated groups, mortalities between 0.0 % and 100.0 % were observed. At the highest tested doses of 103.3 µg and 206.6 µg dimethenamid-P/bee statistically significant differences compared to the control (Fisher's Exact Binominal test, $\alpha = 0.05$) were observed. 92 % of the surviving bees treated with 103.3 µg dimethenamid-P/bee showed some abnormal behaviour after 48 hours, like

uncoordinated movements or lying on the back.
The results are summarised in Table B.9.3-3.

Table B.9.3-3: Toxicity of dimethenamid-P to honeybees (*Apis mellifera carnica* P.) in a contact toxicity test

Treatment [µg dimethenamid-P/bee] (nominal)	Treatment [µg dimethenamid-P/bee] (purity: 96.8 %)	Mortality [%]		Corrected mortality [%]	
		24 h	48 h	24 h	48 h
--	Control (water)	0.0	0.0	--	--
--	Tween control	0.0	0.0	--	--
--	Acetone control	0.0	0.0	--	--
12.9	12.5	0.0	0.0	--	--
25.8	25.0	0.0	3.3	--	--
51.7	50.0	0.0	3.3	--	--
103.3	100.0	46.7 *	56.7 *	--	--
206.6	200.0	100.0 *	100.0 *	--	--
Endpoint [µg/bee]					
LD_{50} (95 % CL) [1] (48 h)		**Test item dimethenamid-P**			
		96.9 (76.2 – 123.2) 93.8 (73.8 – 119.3), corrected for the purity of 96.8 %			

[1] Median lethal dose after 48 hours of exposure calculated by Probit analysis (with 95 % Confidence Limits)
* Statistically significant differences compared to the control (Fisher's Exact Binominal Test $\alpha = 0.05$).

The LD_{50} value (24 h) for the toxic reference item in the contact toxicity test was LD_{50} = 0.147 µg as/bee (95 % confidence limits: 0.113 - 0.190 µg as/bee).

III. CONCLUSION

In an acute contact toxicity study with dimethenamid-P on honeybees the LD_{50} value (48 h) was determined to be 96.9 µg dimethenamid-P/bee (93.8 µg as/bee, if corrected for the purity of the test item).

The study is considered valid and acceptable for the risk assessment.

Report: B 9.3.1.1/3
Roehlig U., 2014a
Acute toxicity of BAS 656 H (dimethenamid-P) to the bumblebee *Bombus terrestris* L. under laboratory conditions
2013/1275562
Guidelines: OECD 213 (1998), EFSA Guidance Document on bees (2013), Van der Steen (2009), Hanewald et al. (2013)
GLP: yes
Validity: Acceptable

Executive Summary

In an oral dose response toxicity test, young adult worker bumblebees (*Bombus terrestris*) were

exposed to dimethenamid-P. The toxicity of the test item was determined at doses of 12.5, 25.0, 50.0, 100, and 200 µg active substance/bumblebee (based on analysed purity) and resulting in an actual uptake of 11.1, 19.7, 44.6, 87.8 and 158 µg active substance/bumblebee. Additionally, bumblebees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.25 to 2.0 µg dimethoate/bumblebee (analysed) or with a solution of water and sucrose or of water, sucrose and acetone as controls. The test was conducted with 30 replicates each containing one bumblebee. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of oral exposure, a mortality of 3.3 % was observed in both controls. In the test item treated groups, mortalities between 0.0 % and 10.0 % were observed. No statistically significant differences compared to the control were observed in all treatment groups. No behavioural abnormalities of surviving bumblebees occurred throughout the oral toxicity test.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. COD-001509, purity: 95.9 %.

B. STUDY DESIGN

Test species: Bumblebee (*Bombus terrestris*) young adult worker bumblebees deriving from a healthy and queen-right micro-hive; source: Biobest Belgium N.V., Westerlo, Belgium and delivered by Katz Biotech AG, Baruth, Germany; individuals were collected in the morning prior to use.

Test design: Dose response test for oral toxicity; duration 48 h, 30 replicates, each replicate consisting of 1 bumblebee per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value and behavioural abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: 50 % (w/v) aqueous sucrose solution, 50 % (w/v) aqueous sucrose solution + 1 % v/v acetone; test item: 12.5, 25.0, 50.0, 100, and 200 µg as/bumblebee (based on analysed purity) and resulting in an actual uptake of 11.1, 19.7, 44.6, 87.8 and 158 µg as/bumblebee.

Test conditions: Temperature: 24.6 – 25.6 °C; relative humidity: 58 % - 62 %, photoperiod: 24 h darkness. Food: 50 % w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction (one-sided greater $\alpha = 0.05$), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, a mortality of 3.3 % was observed in both controls. In the test item treated groups, mortalities between 0.0 % and 10.0 % were observed. No statistically significant differences compared to the control (Fisher's Exact Binominal test, $\alpha = 0.05$) were observed in all treatment groups. No behavioural abnormalities of surviving bumblebees occurred throughout the oral toxicity test. The results are summarised in Table B.9.3-4.

Table B.9.3-4: Toxicity of dimethenamid-P to bumblebees (*Bombus terrestris* L.) in an oral toxicity test

Treatment	Uptake of test item	Mortality [%]		Corrected mortality [%] [1)]	
[µg as/bumblebee]	[µg as/bumblebee]	24 h	48 h	24 h	48 h
Control (sucrose solution)	--	0.0	3.3	--	--
Control (sucrose sol. + acetone)	--	0.0	3.3	--	--
12.5	11.1	0.0	0.0	0.0	0.0
25.0	19.7	3.3	3.3	0.0	0.0
50.0	44.6	3.3	6.7	0.0	3.4
100	87.8	6.7	10.0	0.0	6.9
200	158	3.3	10.0	0.0	6.9
Endpoint [µg as/bumblebee]					
LD_{50} (48 h)	> 158				

[1)] Calculated from mean mortality data in the 1 % acetone control group, according to the formula of Abbott (1925), corrected by Schneider-Orelli (1947); negative values are given as 0.0.

The LD_{50} value (24 h) for the toxic reference item in the oral toxicity test was LD_{50} = 0.504 µg as/bumblebee (95 % confidence limits: 0.269 - 0.943 µg as/bumblebee).

III. CONCLUSION

In an acute oral toxicity study with dimethenamid-P on bumblebees the LD_{50} value (48 h) was determined to be > 158 µg as/bumblebee.

The study is considered valid and acceptable for the risk assessment.

Report: B 9.3.1.1/4
Roehlig U., 2014
Acute toxicity of BAS 656 H (dimethenamid-P) to the bumblebee Bombus terrestris L. under laboratory conditions
2013/1275562
Guidelines: OECD 213 (1998), OECD 214 (1998), EFSA Guidance Document on bees (2013), Van der Steen (2009), Hanewald et al. (2013)
GLP: yes
Validity: Acceptable

Executive Summary
In a contact dose response toxicity test, young adult worker bumblebees (*Bombus terrestris*) were exposed to dimethenamid-P. The toxicity of the test item was determined at doses of 12.5, 25.0, 50.0, 100, and 200 µg active substance/bumblebee (based on analysed purity). Additionally, bumblebees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.25 to 1.999 µg dimethoate/bee (analysed) or with deionised water, Tween solution and acetone as control. The test was conducted with 3 replicates each of the test cages contained 10 bumblebees. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of contact exposure, no mortality occurred in the control groups treated with deionised water or tween solution; 3.3 % mortality occurred in the acetone control group. In the test item treated groups, mortalities between 0.0 % and 3.3 % were observed. No statistically significant differences

compared to the control were observed in all treatment groups. No behavioural abnormalities of surviving bumblebees occurred throughout the contact toxicity test.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. COD-001509, purity: 95.9 %.

B. STUDY DESIGN

Test species: Bumblebee (*Bombus terrestris*) young adult worker bumblebees deriving from a healthy and queen-right micro-hive; source: Biobest Belgium N.V., Westerlo, Belgium and delivered by Katz Biotech AG, Baruth, Germany; collected in the morning prior to use.

Test design: Dose response test for contact toxicity; duration 48 h, 3 replicates, each replicate consisting of 10 bumblebees per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioural abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: deionised water, Tween control (deionised water plus 1.0 % (v/v) Tween solution), acetone control (pure acetone); test item: 12.5, 25.0, 50.0, 100.0, and 200 µg as/bumblebee (based on analysed purity).

Test conditions: Temperature: 24.6 – 25.6 °C; relative humidity: 58 % - 62 %, photoperiod: 24 h darkness. Food: 50 % w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction (one-sided greater $\alpha = 0.05$), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, no mortality occurred in the control groups treated with deionised water or tween solution; 3.3 % mortality occurred in the acetone control group. In the test item treated groups, mortalities between 0.0 % and 3.3 % were observed. No statistically significant differences compared to the control (Fisher's Exact Binominal test, $\alpha = 0.05$) were observed in all treatment groups. No behavioural abnormalities of surviving bumblebees occurred throughout the contact toxicity test. The results are summarised in Table B.9.3-5.

Table B.9.3-5: Toxicity of dimethenamid-P to bumblebees (*Bombus terrestris* L.) in a contact toxicity test

Treatment [µg as/bumblebee]	Mortality [%]		Corrected mortality[1] [%]	
	24 h	48 h	24 h	48 h
Control (water)	0.0	0.0	--	--
Tween control	0.0	0.0	--	--
Acetone control	0.0	3.3	--	--
12.5	0.0	0.0	0.0	0.0
25.0	0.0	0.0	0.0	0.0
50.0	0.0	3.3	0.0	0.0
100	0.0	0.0	0.0	0.0
200	0.0	3.3	0.0	0.0
Endpoint [µg as/bumblebee]				
LD_{50} (48 h)	> 200			

[1] Calculated from mean mortality data in the 1 % acetone control group, according to the formula of Abbott (1925), corrected by Schneider-Orelli (1947); negative values are given as 0.0.

The LD_{50} value (24 h) for the toxic reference item in the contact toxicity test was LD_{50} = 1.122 µg as/bumblebee (95 % confidence limits: 0.907 - 1.387 µg as/bumblebee).

III. CONCLUSION

In an acute contact toxicity study with dimethenamid-P on bumblebees the LD_{50} value (48 h) was determined to be > 200 µg as/bumblebee.

The study is considered valid and acceptable for the risk assessment.

B.9.3.1.2 Chronic toxicity (KCA 8.3.1.2)

No new studies are available.

B.9.3.1.3 Effects on honeybee brood (KCA 8.3.1.3)

Report: B 9.3.1.3/1
Kleebaum K., 2014
Acute toxicity of BAS 656 H (dimethenamid-P) to honeybee larvae (*Apis mellifera* L.) under laboratory conditions (*in vitro*)
2013/1132510

Guidelines: OECD 237 (2013) Honey bee (*Apis mellifera*) larval toxicity test single exposure

GLP: yes

Validity: **A**cceptable

Executive Summary

In an acute toxicity test, honeybee larvae (*Apis mellifera carnica* P.) were exposed to BAS 656 H (dimethenamid-P). The toxicity of the test item was determined at doses of 12.4, 24.8, 49.6, 99.2 and 198.5 µg as/larva. The corresponding concentrations of the test item in the diet were 0.366, 0.732,

1.464, 2.927 and 5.855 g as/kg. Additionally, honeybee larvae were treated with dimethoate as reference item or with an untreated control and a solvent control.

After 72 hours of oral exposure, a mortality of 5.6 % was observed in both controls. In the test item group, mortalities ranged between 2.8 and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg as/larva with mortalities of each 100 %. After 96 hours of oral exposure, a mortality of 8.3 % was observed in both controls. In the test item group, mortalities ranged between 8.3 % and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg as/larva with mortalities of each 100 %.

After 72 hours of exposure 3.0 %, 13.0 % and 30.3 % of the surviving larvae showed deviations to the normal food consuming behaviour. These deviations occurred proportionally to the test item doses (being 12.4, 24.6 and 49.6 µg as/larva, respectively). 96 hours after treatment with 12.4, 24.6 and 49.6 µg as/larva, 3.3 %, 0.0 % and 12.1 % of the surviving larvae showed deviations to the normal food consuming behaviour and correspondingly to develop into an average sized larva. Still, a dose-relation could be detected. Nevertheless, if compared to the previous day it becomes obvious, that development of some larvae was only slightly delayed and not irreversibly disturbed.

In an acute larval toxicity test with BAS 656 H (dimethenamid-P), the LD_{50} (72 h) was determined to be 65.8 µg as/larva, which is equivalent to an LC_{50} (72 h) of 1.941 g as/kg food. The LD_{50} (96 h) was determined to be 69.6 µg as/larva, which is equivalent to a LC_{50} (96 h) of 2.054 g as/kg food.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 656 H (dimethenamid-P) (Reg. No. 363851), batch no.: COD-001509; Content of active substance: analysed purity of 95.9 % (*w/w*) (tolerance ±1.0 %).

Test species: *Apis mellifera carnica* P. (honeybee), first instar larvae; derived from three healthy and queen-right colonies; source: Bienenfarm Kern GmbH, Leipzig, Germany.

B. STUDY DESIGN

Test design: One day old honeybee larvae (D1) of *Apis mellifera carnica* P. were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. Afterwards, in a 72 hour acute test, the 4 day old (D4) larvae were exposed to a single application of BAS 656 H (dimethenamid-P) diluted in the larvae food (aqueous sugar solution mixed with royal jelly). In total, 3 treatment groups were set up: 5 doses of the test item, two untreated control groups (with and without solvent) and 4 doses of the reference item with 3 replicates per dose and 12 larvae per replicate. After the day of application, additional feeding of the larvae took place 24 hours (D5) and 48 hours later (D6). Assessments of larval mortality were done after 24, 48, 72 and 96 hours (respectively D5, D6, D7 and D8). Additionally, other observations as small body size or large quantities of remaining food on D7 and D8 were noted. In an analytical phase of the study the concentration of the active substance in the test item stock solution A was determined.

Endpoint: Mortality, body size, food uptake.

Reference item: Dimethoate (99.8 % *w/w* analysed).

Test concentrations:	control (untreated diet: aqueous sugar solution with gelee royal, 1:1) and solvent control (aqueous sugar solution with gelee royal, 1:1 including 1 % (*v/v*) acetone); BAS 656 H: 12.4, 24.8, 49.6, 99.2 and 198.5 µg as/larva, corresponding to 0.366, 0.732, 1.464, 2.927 and 5.855 g as/kg food (nominal); reference item: 1.1, 2.2, 4.4 and 8.8 µg dimethoate/larva.
Test conditions:	Temperature: 34.0 °C – 34.5 °C, relative humidity: 92 % - 96 % with two short periods of lower humidity, photoperiod: darkness (except during assessments), food: aqueous sugar solution mixed with gelee royal (1:1).
Statistics:	Descriptive statistics; Fisher's Exact Binomial test with Bonferroni Correction for mortality data and no effect levels (one-sided greater, $\alpha = 0.05$); Probit and Weibull analysis for calculation of LC_{50} and LD_{50} values.

II. RESULTS AND DISCUSSION

After 72 hours of oral exposure, a mortality of 5.6 % was observed in both controls. In the test item group, mortalities ranged between 2.8 % and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg as/larva with mortalities of each 100 % (Fisher's Exact Binomial test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).
After 96 hours of oral exposure, a mortality of 8.3 % was observed in both controls. In the test item group, mortalities ranged between 8.3 % and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg as/larva with mortalities of each 100 % (Fisher's Exact Binomial test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).
After 72 hours of exposure 3.0 %, 13.0 % and 30.3 % of the surviving larvae showed deviations to the normal food consuming behaviour. These deviations occurred proportionally to the test item doses (being 12.4, 24.6 and 49.6 µg as/larva, respectively).
96 hours after treatment with 12.4, 24.6 and 49.6 µg as/larva, 3.3 %, 0.0 % and 12.1 % of the surviving larvae showed deviations to the normal food consuming behaviour and correspondingly to develop into an average sized larva. Still a dose-relation could be detected. Nevertheless, if compared to the previous day it becomes obvious, that development of some larvae was only slightly delayed and not irreversibly disturbed.

The LD_{50} (96 h) was determined to be 69.6 µg as/larva, which is equivalent to a LC_{50} (96 h) of 2.054 g as/kg food.

The results are summarised in Table B.9.3-6.

Table B.9.3-6: Toxicity of BAS 656 H (dimethenamid-P) to *Apis mellifera carnica* P. in an acute larval toxicity test

Treatment		Mortality after 72 hours			Mortality after 96 hours		
Dosage [µg as/larva]	**Concentra-tion [g as/kg food]**	**mean mortality [%]**	**corrected mortality**	**mean other observa-tions [%] 1)**	**mean mortality [%]**	**corrected mortality**	**mean other observa-tions [%] 1)**
Control	--	5.6	--	0.0	8.3	--	0.0
Solvent control	--	5.6	--	0.0	8.3	--	0.0
12.4	0.366	2.8	0.0	3.0	8.3	0.0	3.3
24.8	0.732	8.3	2.9	13.0	13.9	6.1	0.0
49.6	1.464	8.3	2.9	30.3	8.3	0.0	12.1
99.2	2.927	100.0 *	100.0	--	100.0 *	100.0	--
198.5	5.855	100.0 *	100.0	--	100.0 *	100.0	--

Endpoints		72 hours	96 hours
Test item dose [µg as/larva]	**LD_{50} (95 % CL)**	65.8 (45.9 – 94.3)	69.6 (48.8 – 99.4)
	NOED	49.6	49.6
Test item concentrations [g as/kg food]	**LC_{50} (95 % CL)**	1.941 (1.353 – 2.783)	2.054 (1.438 – 2.933)
	NOEC	1.464	1.464

* Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher`s Exact Binominal Test with Bonferroni Correction; $\alpha = 0.05$; one sided greater).

1) Other observations (large quantities of remaining food, smaller body size of larva).

The LD_{50} value (72 h) for the reference item in the acute larval toxicity test could not be determined. Mortality at 8.8 µg/larvae was above 50 % across all replicates (D7/72 h), being 55.6 % (corrected for control mortality 52.9 %).

III. CONCLUSION

In an acute larval toxicity test with BAS 656 H (dimethenamid-P), the LD_{50} (72 h) was determined to be 65.8 µg as/larva, which is equivalent to an LC_{50} (72 h) of 1.941 g as/kg food. The LD_{50} (96 h) was determined to be 69.6 µg as/larva, which is equivalent to a LC_{50} (96 h) of 2.054 g as/kg food.

The study is considered valid and acceptable for the risk assessment.

B.9.3.1.4 Sublethal effects (KCA 8.3.1.4)

No new studies are available.

B.9.3.2 Effects on non-target arthropods other than bees

No new studies with the active substance were required or submitted for the renewal assessment.
Laboratory tests using formulation BAS 656 07 H and BAS 656 07 H (both: EC; dimethenamid-P 64 %) were submitted. Please refer to the evaluation of the representative formulation BAS 656 12 H (dimethenamid-P_RAR_20_Volume_3CP_BAS 656 12 H_B-9).

B.9.4 Effects on non-target soil meso- and macrofauna

One new study on the acute toxicity of the dimethenamid-P metabolite M31 to earthworms was submitted with the renewal dossier and is summarised below.

The dossier for the initial EU evaluation contained studies on the acute toxicity of the active substance as well as the metabolites M23 and M27 which were all assessed in the initial monograph. To increase the transparency and comprehensibility of the overall assessment, summaries of the studies assessed with the initial evaluation of dimethenamid-P have been added by the RMS. No new evaluation of the previously submitted studies was performed.

KCA 8.4/1 Van Dijk, 1988 (study evaluated in the initial monograph, 2000)

Author:	Van Dijk, A.
Title:	Acute toxicity (LC_{50}) study of SAN 582 H to earthworms
Date:	22.06.1988
Doc ID:	RCC 204614; ARW96-00062; BASF RegDoc.# 88/11372
Guidelines:	OECD 207
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test substance:	technical dimethenamid (SAN 582 H)
Purity:	91.4 %
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	62.5/125/250/500/1000 mg/kg

Results and Discussion

LC_{50}:	294.4 (260.6 - 339.9) mg/kg
NOEC:	125 mg/kg

Conclusion

The study is acceptable. In a 14-day toxicity study to earthworms (*Eisenia fetida)* with of dimethenamid (SAN 582, purity 91.4 %), the LC_{50} was 294.4 mg/kg dry soil. The NOEC related to mortality was determined to be 125 mg/kg soil. The test substance was incorporated in artificial soil (10 % peat). As there are no studies containing only the active substance available, the results will be used further in the risk assessment.

KCA 8.4/2 Krieg, 1998a (study evaluated in the initial monograph, 2000)

Author:	Krieg, W.
Title:	Effect of M23 (dimethenamid-metabolite) on the mortality of the earthworm *Eisenia foetida*
Date:	19.03.1998
Doc ID:	47842; ARW1999-48; BASF RegDoc.# 98/10299
Guidelines:	OECD 207
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test substance:	metabolite M23
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	0/79/158/316/632/1264 mg/kg

Results and Discussion

LC_{50}:	>1264 mg/kg
NOEC:	1264 mg/kg

Conclusion

The study is acceptable. In a 14-day earthworm reproduction study with M23, a metabolite of dimethenamid-P, the LC_{50} was > 1264 mg/kg dry soil. The NOEC related to mortality was determined to be 1264 mg/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (10 % peat).

KCA 8.4/3 Krieg, 1998b (study evaluated in the initial monograph, 2000)

Author:	Krieg W.
Title:	Effect of M27 (dimethenamid-metabolite) on the mortality of the earthworm *Eisenia foetida*
Date:	20.03.1998
Doc ID:	47843; ARW1999-49; BASF RegDoc# 98/10300
Guidelines:	OECD 207
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test substance:	metabolite M27
Guideline:	OECD 207
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	0/79/158/316/632/1264 mg/kg

Results and Discussion

LC_{50}:	>1264 mg/kg
NOEC:	1264 mg/kg

Conclusion

The study is acceptable. In a 14-day earthworm reproduction study with M27, a metabolite of dimethenamid-P, the LC_{50} was > 1264 mg/kg dry soil. The NOEC related to mortality was determined to be 632 mg/kg dry soil. The test substance was incorporated in artificial soil (10 % peat).

KCA 8.4/4 Krome, 2008 (new study, submitted with renewal dossier)

Author:	Krome, K.
Title:	Acute toxicity (14 days) of Reg. No. 360712 (Metabolite of BAS 656 H, M31) to the earthworm *Eisenia fetida* in artificial soil

Date: 26.09.2008
Doc ID: RRA 12620; 080818BO; BASF RegDoc# 2008/1052695
Guidelines: OECD 207, DIN ISO 11268-1 (April 1997)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, purity of as: [[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbomoyl]-methane-sulfinyl]-acetic acid, Reg. No. 360 712): 98.7 ± 1 %.

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum and weight of 300 – 600 mg), age: between 2 and 12 months; source: in-house.

Test design: 14-d exposure in treated artificial soil (10 % peat) according to OECD 207; different concentrations of the test item were mixed homogeneously into the soil, which was then used to fill glass vessels after which the earthworms were introduced on top of the soil; 6 treatment groups (5 test item concentrations, water control); 4 replicates/treatment group with 10 worms each. Assessment of worm mortality was carried out after 7 and 14 d, measurement of behavioural effects and weight change as sub-lethal parameter after 14 d.

Endpoints: LC_{50} (50 % mortality of earthworms after exposure over 14 days), behavioural effects, weight change.

Test concentrations: Control (Water); 62.5, 125, 250, 500 and 1000 mg M31/kg dry soil (nominal).

Reference item: 2-chloroacetamide; LC_{50} = 55.8 mg/kg dry soil. The effects of the reference item were investigated in a separate study.

Test conditions: Artificial soil according to OECD 207 (10 % peat); pH 5.58 – 5.87 at test initiation, pH 5.40 – 5.63 at test termination; water content: approx. 54 % of water holding capacity (WHC); temperature: 18 °C – 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: 544 ± 22 lux.

Statistics: Descriptive statistics, Normality and Equal Variance test followed by one-way ANOVA ($\alpha = 0.05$).

Results and Discussion

The LC_{50} was determined to be > 1000 mg M31/kg dry soil.

After 14 days of exposure, no mortality was observed in any test item group and the control. The biomass development was not statistically significant different compared to the control at all test item concentrations (ANOVA, $\alpha = 0.05$). The results are summarised in the following table.

Table B.9.4-1: Effect of M31, a metabolite of dimethenamid-P, on earthworm (*Eisenia fetida*) mortality and biomass (14 d)

M31 [mg/kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality [%]	0	0	0	0	0	0
Weight change [%]	-3	-3	-5	-2	-6	-4
	Endpoint [mg/kg dry soil]					
LC_{50}	> 1000					
NOEC	≥ 1000					

Conclusion

The study is acceptable. In a 14-day toxicity study to earthworms (*Eisenia fetida)* with M31, a metabolite of dimethenamid-P, the LC_{50} was > 1000 mg/kg dry soil. The NOEC related to mortality and biomass was determined to be 1000 mg/kg soil, the highest concentration tested. The test substance was incorporated in artificial soil (10 % peat).

B.9.4.1 Earthworm – sub-lethal effects

Four new studies on the reproductive toxicity of the active substance dimethenamid-P as well as its metabolites M23 (Reg.No. 360715), M27 (Reg.No. 360714) and M31 (Reg.No. 360712) to earthworms were submitted with the renewal dossier and are summarised below.

KCA 8.4.1/1 Friedrich, 2012 (new study, submitted with renewal dossier)

Author:	Friedrich S.
Title:	Sublethal toxicity of BAS 656 H (dimethenamid-P) to the earthworm *Eisenia fetida* in artificial soil with 5 % peat
Date:	06.11.2012
Doc ID:	12 10 48 093 S; BASF RegDoc# 2012/1129456
Guidelines:	OECD 222 (2004)
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item: BAS 656 H (dimethenamid-P), batch no. COD-001509, Reg. No. 363 851, purity: 95.9 %.

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight of 300 mg – 448 mg), approximately 3 months old; source: "W. Neudorff GmbH KG" followed by in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5 % peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects (feeding activity) and biomass development after 28 days; assessment of reproduction rate after additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Nutdazim 50 Flow (carbendazim, SC 500). The effects of the reference item were investigated in a separate study.

Test rates: Control, 15.0, 19.5, 25.4, 33.0, 42.8 mg as/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with reduced content of peat: 5 %); pH 6.12 – 6.19 at test initiation and pH 5.82 – 5.88 at test termination; water content: 58.3 % – 58.5 % of maximum water holding capacity (WHC) at test initiation and 56.9 % – 58.3 % of WHC at test termination; temperature: 18.0 °C – 21.1 °C; photoperiod: 16 h light : 8 h dark, light intensity: 610 lux.

Statistics: Descriptive statistics. Fisher's Exact Binominal test for mortality ($\alpha = 0.05$, one-sided greater), Williams t-test for weight change and reproduction ($\alpha = 0.05$, one-sided smaller), Probit analysis.

Results and Discussion

After 28 days of exposure the mortality between 0 % and 5.0 % in the test item groups and 1.3 % in the control. No statistically significant mortality compared to the control was observed at any test item concentration (Fisher's Exact Binominal test with Bonferroni correction, α = 0.05). Body weight was not statistically significantly different from the control up to highest concentration of 42.8 mg as/kg dry soil (Williams t-test, α = 0.05, one-sided smaller). The reproduction rate was significantly different from the control in the two highest concentration of 33.0 and 42.8 mg as/kg dry soil (Williams t-test, α = 0.05, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups.

Table B.9.4-2: Effect of BAS 656 H on earthworms (*Eisenia fetida*) in a 56-day reproduction study

BAS 656 H [mg/kg dry soil]	**Control**	**15.0**	**19.5**	**25.4**	**33.0**	**42.8**
Mortality (day 28) [%]	1.3	2.5	0.	5.0	5.0	5.0
Weight change (day 28) [%]	46.7	46.4	49.6	46.6	43.5	39.1
No. of juveniles (day 56)	84.1	77.5	82.3	70.3	43.3 *	23.0 *
Reproduction in [%] of control (day 56)	100	92.1	97.8	83.5	51.4	27.3
	Endpoints [mg as/kg dry soil]					
NOEC (day 28 mortality and weight)	42.8					
NOEC (day 56 reproduction)	25.4					
EC_{50} (95 % confidence limits)	34.3 (31.3 – 37 6)					

* Statistically significantly different from control (Williams t-test; α = 0.05, one-sided smaller.

In a separate study the reference item inhibited the reproduction rate by 72.7 % and 98.8 % compared to a control at 5 and 10 mg product/kg dry soil.

Conclusion

The study is acceptable. In a 56-day earthworm reproduction study with BAS 656 H (dimethenamid-P), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be 25.4 mg as/kg dry soil. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.1/2 Luehrs, 2007a (new study, submitted with renewal dossier)

Author: Luehrs, U.
Title: Effects of Reg.No. 360715 on reproduction and growth of earthworms *Eisenia fetida* in artificial soil with 5 % peat
Date: 08.11.2007
Doc ID: 37431022; BASF RegDoc# 2007/1037731
Guidelines: OECD 222, ISO 11268-2 (1998)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M23 (metabolite of dimethenamid-P), batch no. L59-52, Reg. No. 360 715, purity: 97.1 %.

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum and weight of 301 mg – 575 mg), 9 - 10 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5 % peat); different concentrations of the test item were incorporated into the soil; 6

treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects (feeding activity) and biomass development after 28 days; assessment of reproduction rate after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Brabant Carbendazim Flowable (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test rates: Control, 0.52, 1.04, 2.08, 4.16 and 8.32 mg M23/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with reduced content of peat: 5 %); pH 6.0 – 6.1 at test initiation and termination; water content: 48.5 – 53.4 % of maximum water holding capacity (WHC) at test initiation and 52.8 - 58.8 % WHC at test termination; temperature: 19 - 21 °C; photoperiod: 16 h light : 8 h dark, light intensity: 490 lux - 710 lux.

Statistics: Descriptive statistics. Two-sided Bonferroni-Welch-t-test for weight change and one-sided Bonferroni-Welch-t-test for reproduction data ($\alpha = 0.05$).

Results and Discussion

After 28 days of exposure no mortality was observed in any treatment group. Body weight changes and reproduction of the earthworms exposed to M23, a metabolite of dimethenamid-P, were not statistically significantly different compared to the control up to the highest test item concentration of 8.32 mg/kg dry soil (Bonferroni-Welch-t-test, $\alpha = 0.05$). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. The results are summarised in the following table.

Table B.9.4-3: Effect of M23, a metabolite of dimethenamid-P, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

M23 [mg/kg dry soil]	**Control**	**0.52**	**1.04**	**2.08**	**4.16**	**8.32**
Mortality (day 28) [%]	0	0	0	0	0	0
Weight change (day 28) [%]	29.7	29.7	27.2	29.6	29.4	26.8
No. of juveniles (day 56)	243	241	241	249	247	212
Reproduction in [%] of control (day 56)	--	99.3	99.3	102.6	101.6	87.1
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg dry soil]					
NOEC (day 28 mortality and weight)	≥ 8.32					
NOEC (day 56 reproduction)	≥ 8.32					

Conclusion

The study is acceptable. In a 56-day earthworm reproduction study with M23, a metabolite of dimethenamid-P, the NOEC for mortality, biomass, reproduction, and feeding activity was 8.32 mg/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.1/3 Luehrs, 2007b (new study, submitted with renewal dossier)

Author: Luehrs U.
Title: Effects of Reg.No. 360714 on reproduction and growth of earthworms *Eisenia fetida* in artificial soil with 5 % peat

Date: 08.11.2007
Doc ID: 37421022; BASF RegDoc# 2007/1037732
Guidelines: OECD 222, ISO 11268-2 (1998), EEC 96/12, EEC 91/414
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M27 (metabolite of dimethenamid-P), batch no. 01311-28, Reg. No. 360 714, purity: 97.1 %.

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum, weight: 304 mg - 600 mg), approx.10 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5 % peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects (feeding activity) and biomass development after 28 days of exposure; assessment of reproduction rate after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Brabant Carbendazim Flowable (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test rates: Control, 0.66, 1.32, 2.64, 5.28 and 10.56 mg M27/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with reduced content of peat (5 %); pH 6.3 – 6.4 at test initiation, 5.9 – 6.1 at test termination; water content: 51.8 % – 55.0 % of maximum water holding capacity (WHC) at test initiation and 50.8 %- 63.8 % WHC at test termination; temperature: 19 - 21 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 750 lux.

Statistics: Descriptive statistics. Fisher's exact test for mortality data, Dunnett's test for weight change data (two-sided) and Bonferroni-Welch-t-test (one-sided) for reproduction data ($\alpha = 0.05$).

Results and Discussion

After 28 days of exposure no mortality was observed in any treatment group except for a mortality of 2.5 % at 10.56 mg M27/kg dry soil, which was not statistically significant different (Fisher's exact test, $\alpha = 0.05$). Body weight changes of earthworms exposed to M27 were not statistically significantly different compared to the control up to the highest test item concentration of 10.56 mg/kg dry soil (Dunnett's test, $\alpha = 0.05$). No statistically significant differences on reproduction were observed in any of the treatment groups (Bonferroni-Welch-test, $\alpha = 0.05$). No behavioural abnormalities were observed in any of the treatment groups and the feeding activity in all treated groups was comparable to the one in the control. The results are summarised in the following table.

Table B.9.4-4: Effect of M27, a metabolite of dimethenamid-P, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

M27 [mg/kg dry soil]	**Control**	**0.66**	**1.32**	**2.64**	**5.28**	**10.56**
Mortality (day 28) [%]	0	0	0	0	0	2.5
Weight change (day 28) [%]	8.3	11.7	5.5	11.8	9.5	20.8
No. of juveniles (day 56)	254	229	256	277	194	254
Reproduction in [%] of control (day 56)	--	90.1	101	109	76.4	100
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg dry soil]					
$NOEC_{mortality, weight}$ (day 28)	≥ 10.56					
$NOEC_{reproduction}$ (day 56)	≥ 10.56					

Conclusion

The study is acceptable. In a 56-day reproduction study with M27, a metabolite of dimethenamid-P, on earthworms, the NOEC for mortality, biomass, reproduction, and feeding activity was 10.56 mg/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.1/4 Luehrs, 2009 (new study, submitted with renewal dossier)

Author: Luehrs U.
Title: Effects of Reg. No. 360712 (metabolite of BAS 656 H, M31) on the reproduction and growth of earthworms *Eisenia fetida* in artificial soil with 5 % peat
Date: 08.01.2009
Doc ID: 46551022; BASF RegDoc# 2008/1070910
Guidelines: OECD 222, ISO 11268-2 (1998)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7 %.

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum, weight: 305 mg - 596 mg), approx.10 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5 % peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects (feeding activity) and biomass development after 28 days of exposure; assessment of reproduction rate after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Brabant Carbendazim Flowable (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test rates: Control, 6.25, 12.5, 25, 50 and 100 mg M31/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with reduced content of peat (5 %); pH

6.4 – 6.5 at test initiation, 6.1 – 6.5 at test termination; water content: 50.5 % – 54.5 % of maximum water holding capacity (WHC) at test initiation and 53.4 % – 60.0 % WHC at test termination; temperature: 18 - 21 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 640 lux.

Statistics: Descriptive statistics. Dunnett's test for weight change (two-sided) and reproduction data (one-sided smaller), both $\alpha = 0.05$.

Results and Discussion

After 28 days of exposure no mortality was observed in any treatment group. Body weight changes of earthworms exposed to M31 were not statistically significantly different compared to the control up to the highest test item concentration of 100 mg/kg dry soil (Dunnett's test, $\alpha = 0.05$). No statistically significant differences on reproduction were observed in any of the treatment groups (Dunnett's test, $\alpha = 0.05$). No behavioural abnormalities were observed in any of the treatment groups and the feeding activity in all the treated groups was comparable to the control. The results are summarised in the following table.

Table B.9.4-5: Effect of M31, a metabolite of dimethenamid-P, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

M31 [mg/kg dry soil]	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality (day 28) [%]	0	0	0	0	0	0
Weight change (day 28) [%]	42.1	52.6	49.3	47.4	44.8	39.1
No. of juveniles (day 56)	167	177	226	162	230	185
Reproduction in [%] of control (day 56)	--	106.2	135.6	97.1	138.0	110.9
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg dry soil]					
NOEC (day 28 mortality and weight)	≥ 100					
NOEC (day 56 reproduction)	≥ 100					

Conclusion

The study is acceptable. In a 56-day reproduction study with M31, a metabolite of dimethenamid-P, on earthworms, the NOEC for mortality, biomass, reproduction, and feeding activity was 100 mg/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

B.9.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A risk assessment for soil macro-organisms, i.e. collembolans and/or soil mites was not conducted in the initial monograph. Evidence from beneficial arthropod toxicity tests suggest toxicity of dimethenamid-P residues to non-target invertebrate fauna. According to data requirements studies on the possible effects of dimethenamid-P and its metabolites M23, M27 and M31 on the reproduction of collembolans were submitted and are documented below.

B.9.4.2.1 Species level testing

KCA 8.4.2.1/1 Friedrich, 2011a (new study, submitted with renewal dossier)

Author: Friedrich, S.
Title: Effects of BAS 656 H (Reg.No. 363 851, dimethenamid-P) on the reproduction of the collembolans *Folsomia candida*
Date: 29.03.2011
Doc ID: 11 10 48 015 S; BASF RegDoc# 2011/1000481
Guidelines: OECD 232 (2009), ISO 11267 (1999)

GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Dimethenamid-P (BAS 656 H, batch no. 6261B01BH, Reg. No. 363 851, purity: 96.8 ± 1.0 %.

Test species: Collembola (*Folsomia candida*), age: 9-12 days; source: in-house culture.

Test design: In a 28-day test, adults of *Folsomia candida* were exposed to six soil concentrations of dimethenamid-P. The test substrate was artificial soil according to OECD 232 (5 % peat). In total, 7 treatment groups were set up (6 concentrations of the test item and an untreated control group) with 4 replicates for the test item treatments and 8 replicates for the control, each with 10 collembolans. The artificial soil was treated and filled into glass vessels before collembolans were introduced on the top of the soil. Assessment of adult collembolans mortality, behavioural effects and reproduction (number of juveniles) was done after 28 days.

Endpoints: Mortality and reproduction rate.

Reference item: Boric acid (100 % analysed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 3.13, 6.25, 12.5, 25, 50 and 100 mg as/kg dry soil.

Test conditions: Artificial soil according to OECD 232 with a content of 5 % peat; pH 5.89 - 6.00 at test initiation and 5.79 – 6.08 at test termination; water content at test initiation 57.8 % – 58.0 % of the maximum water holding capacity (WHC) and 56.8 % – 57.5 % of the max. WHC at test termination; temperature: 18.0 - 21.4 °C; photoperiod: 16 h light : 8 h dark, light intensity: 680 lux.

Statistics: Descriptive statistics. Fisher's exact test with Bonferroni Correction for mortality data (one-sided greater), Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for reproduction data (one-sided smaller), both $\alpha = 0.05$. Probit analysis was performed for determination of LC_{50} and EC_{50}.

Results and Discussion

In the test item treatments mortality rates of 5 % to 47.5 % were observed, compared to 5 % in the control. Statistically significant differences compared to the control were observed at concentrations of 25, 50 and 100 mg test item/kg dry soil (Fisher's exact test with Bonferroni Correction, $\alpha = 0.05$).
In the control, a mean of 637.9 juveniles was counted. In the treatment groups, a mean number of juveniles between 138.5 and 664.8 was counted corresponding to a reproduction rate between 21.7 % and 104.2 %. Statistically significant differences on reproduction compared to the control were recorded at concentrations tested of 50 and 100 mg test item/kg dry soil (Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for reproduction data, $\alpha = 0.05$). The results are summarised in the following table.

Table B.9.4-6: Effects of BAS 656 H on Collembola (*Folsomia candida*) in 28-day reproduction study

Dimethenamid-P [mg/kg dry soil]	**Control**	**3.13**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality [%] (day 28)	5.0	5.0	10.0	7.5	27.5*, 1)	30.0*, 1)	47.5*, 1)
No. of juveniles (day 28)	637.9	606.3	611.8	664.8	443.5	237.3*, 2)	138.5*, 2)
Reproduction in [%] of control (day 28)	100.0	95.0	95.9	104.2	69.5	37.2	21.7
	Endpoints [mg BAS 656 H/kg dry soil]						
NOEC (mortality)	12.5						
LC_{50} (mortality)	118.3 (95 % confidence limit: 77.1 – 251.1)						
NOEC (reproduction)	25.0						
EC_{50} (reproduction)	41.6 (95 % confidence limit: 31.3 – 56.2)						

* Statistically significantly different compared to the control.
1) Fisher's exact test with Bonferroni Correction for mortality data, one-sided greater ($\alpha = 0.05$).
2) Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for reproduction data, one-sided smaller ($\alpha = 0.05$).

Conclusion

The study is acceptable.
Accepted endpoints: EC_{50} = 41.6 mg as/kg dw and the LC_{50} = 118.3 mg as/kg dw. The NOEC = 12.5 mg as/kg dw (mortality) and NOEC = 25 mg as/kg dw (reproduction). The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.2.1/2 Friedrich, 2011b (new study, submitted with renewal dossier)

Author: Friedrich S.
Title: Effects of Reg.No. 360 712 (metabolite of BAS 656 H, M31) on the reproduction of the collembolans *Folsomia candida*
Date: 13.01.2011
Doc ID: 10 10 48 110 S; BASF RegDoc# 2011/1000222
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7 ± 1.0 %.

Test species: Collembola (*Folsomia candida*), age: 9-12 days; source: in-house culture.

Test design: In a 28-day test, adults of *Folsomia candida* were exposed to five soil concentrations of M31. The test substrate was artificial soil according to OECD 232 (5 % peat). In total, 7 treatment groups were set up (5 concentrations of the test item, an untreated control and a solvent control) with 4 replicates for the test item treatments and 8 replicates for the control groups, each with 10 collembolans. The artificial soil was treated and filled into glass vessels, before the collembolans were introduced on the top of the soil. Assessment of adult collembolans mortality, behavioural effects and reproduction (number of juveniles) was done after 28 days.

Endpoints: Mortality and reproduction rate.

Reference item: Boric acid (100 % analysed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 12.5, 25, 50, 100 and 200 mg M31/kg dry soil.

Test conditions: Artificial soil according to OECD 232 with a content of 5 % peat; pH 5.73 – 5.88 at test initiation and 5.64 – 6.03 at test termination; water content at test initiation 58.2 % - 58.5 % of the maximum water holding capacity (WHC) and 57.7 %- 58.2 % of WHC at test termination; temperature 18.0 - 20.1 °C; photoperiod: 16 h light : 8 h dark, light intensity: 710 lux.

Statistics: Descriptive statistics. Fisher's Exact Binomial test with Bonferroni Correction for mortality data, Student-t-test, Dunnett-t-test for reproduction data (α = 0.05).

Results and Discussion

In the test item treatments mortality rates of 0.0 % to 7.5 % were observed, compared to 3.8 % in the control. No statistically significant differences compared to the control were observed at any test item concentration (Fisher's exact binomial test with Bonferroni Correction $\alpha = 0.05$).
In the control groups, a mean of 830.5 (untreated control) and 824.5 (solvent control) juveniles was counted. In the treatment groups, a mean number of juveniles between 805.5 and 880.5 was counted corresponding to a reproduction relative to the control between 97.7 % and 106.8 %. No statistically significant differences on reproduction compared to the control were observed at any concentration tested (Student-t-test, Dunnett-t-test for reproduction data, $\alpha = 0.05$). The results are summarised in the following table.

Table B.9.4-7: Effects of M31 on collembola (*Folsomia candida*) in 28-day reproduction study

M31 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%] (day 28)	3.8	3.8	5.0	2.5	2.5	7.5	0.0
No. of juveniles (day 28)	830.5	824.5	880.5	812.3	871.5	805.5	857.0
Reproduction in [%] of control (day 28)	--	100.0	106.8	98.5	105.7	97.7	103.9
	Endpoints [mg M31/kg dry soil]						
NOEC (reproduction/mortality)	≥ 200						
LC_{50} (mortality)	> 200						
EC_{50} (reproduction)	> 200						

Conclusion

The study is acceptable.
Accepted endpoint: The EC_{50} and the LC_{50} could not be calculated, but it can be concluded that the EC_{50} and the LC_{50} are > 200.0 mg M31/kg dry soil. The NOEC for reproduction and mortality was determined to be 200 mg M31/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.2.1/3 Schulz, 2012a (new study, submitted with renewal dossier)

Author: Schulz, L.
Title: BAS 656 H (dimethenamid-P) - Effects of BAS 656 H (dimethenamid-P) on the reproduction of the predatory mite *Hypoaspis aculeifer*
Date: 05.11.2012
Doc ID: 12 10 48 097 S; BASF RegDoc# 2012/1129457
Guidelines: OECD 226 (2008)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item:	BAS 656 H, batch no. COD-001509 (BAS 656 H, Reg. No. 360 720): 95.9 % (±1.0 %).
Test species:	*Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference 2 days); source: in-house culture.
Test design:	14-day chronic laboratory test (according to OECD 226) on effects of BAS 656 H on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.
Endpoints:	Mortality and reproduction rate after 14 days.
Reference item:	Dimethoate EC 400 (411.7 g analysed). The effects of the reference item were investigated in a separate study.
Test rates:	Untreated control, solvent control (acetone), 62.5, 125, 250, 500 and 1000 mg BAS 656 H/kg dry soil.
Test conditions:	Artificial soil according to OECD 226; pH 5.5 – pH 5.7 at test initiation, pH 5.4 - 5.6 at test termination; water content at test initiation 45.99 % - 52.35 % of maximum water holding capacity (WHC) and 44.40 % - 52.19 % of maximum WHC at test termination; temperature: 19.5 °C - 21.4 °C; photoperiod: 16 h light : 8 h dark; light intensity: 470 lux, food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.
Statistics:	Descriptive statistics; Fisher Exact Binominal Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), William`s t-test for reproduction (α = 0.05, one-sided smaller).

Results and Discussion

Test item treatment groups had mortality rates of between 0.0 % - 5.0 %. The mortality rate in the untreated and the solvent control was 2.5 % and 0.0 %, respectively. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 262.9 and 258.1 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 184.5 and 257.8. BAS 656 H showed no statistically significantly adverse effects on reproduction up to and including 500 mg BAS 656 H/kg dry soil. At the highest concentration of the test item at 1000 mg BAS 656 H/kg dry soil, a statistically significant effect on reproduction compared to the solvent control was observed (William`s t-test, α = 0.05, one-sided smaller). The results are summarised in the following table.

Table B.9.4-8: Effects of BAS 656 H on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

BAS 656 H (dimethenamid-P) [mg/kg dry soil]	**Control**	**Solvent control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 14) [%]	2.5	0.0	5.0	0.0	2.5	5.0	5.0
No. of juveniles (day 14)	262.9	258.1	257.8	240.8	240.3	243.5	184.5 *
Reproduction [% of solvent control] (day 14)	--	100	100	93	93	94	72
Endpoint [mg BAS 656 H (dimethenamid-P)/kg dry soil]							
$NOEC_{mortality}$	1000						
$NOEC_{reproduction}$	500						
LC_{50}	> 1000						
EC_{50}	> 1000						

* statistically significantly different from the solvent control (William`s t-test, α = 0.05, one-sided smaller).

The reference item dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg as/kg dry soil. The EC_{50} (reproduction) for dimethoate EC 400 was calculated to be 6.87 mg as/kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

Conclusion

The study is acceptable. In a 14-day reproduction study with BAS 656 H on predatory soil mites (*Hypoaspis aculeifer*), the NOEC for reproduction was determined to be 500 mg BAS 656 H (dimethenamid-P)/kg dry soil. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.2.1/4 Friedrich, 2012a (new study, submitted with renewal dossier)

Author: Friedrich S.
Title: Reg. No. 360715 (metabolite of BAS 656 H, dimethenamid-P, M23) on the reproduction of the collembolan *Folsomia candida*
Date: 18.12.2012
Doc ID: 12 10 48 101 S; BASF RegDoc# 2012/1129536
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23, batch no. L81-76; analysed purity: 98.8 % (±1.0 %).

Test species: Collembola (*Folsomia candida*), juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day test in treated artificial soil according to OECD 232 and ISO 11267 (5 % peat); 5 different concentrations of the test item were homogenously mixed into artificial soil which was then filled in glass vessels before collembolans were introduced on top of the soil. 7 treatment groups (5 test item concentrations, untreated and solvent control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 juvenile collembolans. Assessment of adult mortality, reproduction rate (number of juveniles) and behavioural effects was carried out after 28 days.

Endpoints: Mortality, reproduction rate.

Reference item: Boric acid (100 %, analysed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (5 % peat); pH 6.11 – 6.20 at test initiation, pH 5.97 – 6.01 at test termination; water content at test initiation 58.5 % – 58.9 % of maximum water holding capacity (WHC) and 57.0 % – 57.7 % of maximum WHC at test termination; temperature: 18.5 °C – 20.7 °C; photoperiod: 16 h light : 8 h dark; light intensity: 640 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics. Fisher`s Exact Test with Bonferroni Correction for mortality data ($\alpha = 0.05$), Williams t-test for reproduction ($\alpha = 0.05$).

Results and Discussion

Mortalities of 8.8 % and 6.3 % were observed in the untreated control and the solvent control groups compared to 2.5 % to 7.5 % mortality in the test item treatment groups. No statistically significant effect on mortality was found in any test concentration (Fisher`s Exact Test with Bonferroni Correction, $\alpha = 0.05$). In the untreated and the solvent control group, mean numbers of 823 and 781 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 749 and 829 No statistically significant effect on the number of juveniles was found at any concentration tested (Williams t-test, $\alpha = 0.05$). The results are summarised in the following table.

Table B.9.4-9: Effect of Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23) on collembola (*Folsomia candida)* mortality and reproduction (28 d)

Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%]	8.8	6.3	7.5	7.5	7.5	5.0	2.5
No. of juveniles [28 d]	823	781	774	779	749	781	829
Reproduction (28 d) [% of control]	--	100.0	99.2	99.8	96.0	100.1	106.2
Endpoint [mg Reg. No. 360 715/kg dry soil]							
$NOEC_{mortality, reproduction}$	≥ 200						
LC_{50}	> 200						
EC_{50}	> 200						

Conclusion

The study is acceptable. In a 28-day collembolan reproduction study with Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23), the LC_{50} and EC_{50} was determined to be > 200 mg test item/kg dry soil. The NOEC for mortality and reproduction was determined to be 200 mg test item/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.2.1/5 Schulz, 2012b (new study, submitted with renewal dossier)

Author: Schulz L.
Title: Effects of Reg. No. 360715 (metabolite of BAS 656 H, dimethenamid-P, M23) on the reproduction of the predatory mite *Hypoaspis aculeifer*
Date: 20.12.2012
Doc ID: 12 10 48 101 S; BASF RegDoc# 2012/1129538

Guidelines: OECD 226 (2008)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23, batch no. L81-76; analysed purity: 98.8 % (±1.0 %).

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference 3 days); source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of Reg. No. 360 715 on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g analysed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 6.3 – pH 6.4 at test initiation, pH 6.3 – 6.4 at test termination; water content at test initiation 48.30 % – 51.85 % of maximum water holding capacity (WHC) and 48.12 % – 50.80 % of maximum WHC at test termination; temperature: 19.1 °C – 20.5 °C; photoperiod: 16 h light : 8 h dark; light intensity: 545 lux; food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.

Statistics: Descriptive statistics; Fisher Exact Binominal Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), William`s t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

Results and Discussion

Test item treatment groups had mortality rates of between 0.0 % - 2.5 %. No mortality could be observed in the untreated and the solvent control group, respectively. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 348.3 and 335.1 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 252.0 and 351.8. Reg. No. 360 715 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 100 mg/kg dry soil. At the highest concentration of the test item at 200 mg/kg dry soil, a statistically significant effect on reproduction compared to the solvent control was observed (William`s t-test, $\alpha = 0.05$, one-sided smaller). The results are summarised in the following table.

Table B.9.4-10: Effects of Reg. No. 360 715 on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%]	0.0	0.0	2.5	2.5	0.0	0.0	2.5
No. of juveniles [14 d]	348.3	335.1	346.8	351.8	339.5	297.0	252.0 *
Reproduction (14 d) [% of control]	--	100	103	105	101	89	75
Endpoint [mg Reg. No. 360 715/kg dry soil]							
$NOEC_{mortality}$	≥ 200						
$NOEC_{reproduction}$	100						
LC_{50}	> 200						
EC_{50}	> 200						

* statistically significantly different from the solvent control (William`s t-test, α = 0.05, one-sided smaller).

The reference item Dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg as/kg dry soil. The EC_{50} (reproduction) for Dimethoate EC 400 was calculated to be 6.87 mg as/kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

Conclusion

The study is acceptable. In a 14-day reproduction study with Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 200 mg/kg dry soil. The NOEC for mortality was determined to be 200 mg Reg. No. 360 715/kg dry soil. The NOEC for reproduction was determined to be 100 mg Reg. No. 360 715/kg dry soil. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.2.1/6 Friedrich, 2012b (new study, submitted with renewal dossier)

Author: Friedrich S.
Title: Effects of Reg. No. 360714 (metabolite of BAS 656 H, dimethenamid-P, M27) on the reproduction of the collembolans *Folsomia candida*
Date: 26.11.2012
Doc ID: 12 10 48 105 S; BASF RegDoc# 2012/1129537
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27, batch no. 1213-32; analysed purity: 97.4 % (±1.0 %).

Test species: Collembola (*Folsomia candida*), juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day test in treated artificial soil according to OECD 232 and ISO 11267 (5 % peat); 5 different concentrations of the test item were homogenously mixed into artificial soil which was then filled in glass vessels before collembolans were introduced on top of the soil. 7 treatment groups (5 test item concentrations, untreated and solvent control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 juvenile collembolans. Assessment of adult mortality,

reproduction rate (number of juveniles) and behavioural effects was carried out after 28 days.

Endpoints: Mortality, reproduction rate.

Reference item: Boric acid (100 %, analysed). The effects of the reference item (EC_{50} = 104 mg as/kg dry soil) were investigated in a separate study.

Test rates: Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27 /kg dry soil.

Test conditions: Artificial soil according to OECD 232 (5 % peat); pH 6.18 – 6.25 at test initiation, pH 6.00 – 6.04 at test termination; water content at test initiation 56.6 % – 57.0 % of maximum water holding capacity (WHC) and 55.4 % – 56.6 % of maximum WHC at test termination; temperature: 18.9 °C – 21.4 °C; photoperiod: 16 h light : 8 h dark; light intensity: 650 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics; Fisher`s Exact Binominal Test for mortality, $\alpha = 0.05$, one-sided greater data; Williams test for reproduction, $\alpha = 0.05$, one-sided smaller.

Results and Discussion

Mortalities of 3.8 % and 5.0 % were observed in the untreated control and the solvent control groups compared to 2.5 % to 5.0 % mortality in the test item treatment groups. No statistically significant effect on mortality was found in any test concentration (Fisher`s Exact Binominal Test, $\alpha = 0.05$, one-sided greater). In the untreated and the solvent control group, mean numbers of 890 and 948 juveniles were counted, respectively. In the treatment groups, the mean number of juveniles was between 897 and 970. No statistically significant effect on the number of juveniles was found at any concentration tested compared to the solvent control (Williams test, $\alpha = 0.05$, one-sided smaller). The results are summarised in the following table.

Table B.9.4-11: Effect of Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27) on collembola (*Folsomia candida)* mortality and reproduction (28 d)

Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%]	3.8	5.0	5.0	5.0	2.5	5.0	5.0
No. of juveniles [28 d]	890	948	897	921	952	906	970
Reproduction (28 d) [% of control]	--	100	94.6	97.2	100.5	95.6	102.4
Endpoint [mg Reg. No. 360 517/kg dry soil]							
$NOEC_{mortality, reproduction}$	≥ 200						
LC_{50}	> 200						
EC_{50}	> 200						

Conclusion

The study is acceptable. In a 28-day collembolan reproduction study with Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27), the LC_{50} and EC_{50} was determined to be > 200 mg test item/kg dry soil. The NOEC for mortality and reproduction was determined to be 200 mg test item/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.2.1/7 Schulz, 2012c (new study, submitted with renewal dossier)

Author:	Schulz L.
Title:	Effects of Reg. No. 360714 (metabolite of BAS 656 H, dimethenamid-P, M27) on the reproduction of the predatory mite *Hypoaspis aculeifer*
Date:	20.12.2012
Doc ID:	12 10 48 102 S; BASF RegDoc# 2012/1129539
Guidelines:	OECD 226 (2008)
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item:	Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27, batch no. 1213-32; analysed purity: 97.4 % (±1.0 %).
Test species:	*Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference 2 days); source: in-house culture.
Test design:	14-day chronic laboratory test (according to OECD 226) on effects of Reg. No. 360 714 on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.
Endpoints:	Mortality and reproduction rate after 14 days.
Reference item:	Dimethoate EC 400 (411.7 g analysed). The effects of the reference item were investigated in a separate study.
Test rates:	Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg/kg dry soil.
Test conditions:	Artificial soil according to OECD 226; pH 6.2 – pH 6.3 at test initiation, pH 6.2 – 6.3 at test termination; water content at test initiation 56.72 % – 57.01 % of maximum water holding capacity (WHC) and 54.29 % – 56.16 % of maximum WHC at test termination; temperature: 19.0 °C – 20.4 °C; photoperiod: 16 h light : 8 h dark; light intensity: 473 lux; food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.
Statistics:	Descriptive statistics; Fisher`s Exact Binominal Test with Bonferroni Correction for mortality ($\alpha$ = 0.05, one-sided greater), William`s t-test for reproduction (α = 0.05, one-sided smaller).

Results and Discussion

Test item treatment groups had mortality rates of between 2.5 % - 12.5 %. In the untreated control and the solvent control group, mortalities of 7.5 % and 1.3 % were observed. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group (Fisher`s Exact Binominal Test with Bonferroni Correction, α = 0.05, one-sided greater).

In the untreated and the solvent control group, mean numbers of 241.8 and 229.9 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between

217.8 and 268.8. Reg. No. 360 714 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 200 mg/kg dry soil, the highest concentration tested (William`s t-test, α = 0.05, one-sided smaller). The results are summarised in the following table.

Table B.9.4-12: Effects of Reg. No. 360 714 on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%]	7.5	1.3	5.0	7.5	2.5	2.5	12.5
No. of juveniles [14 d]	241.8	229.9	247.0	268.8	256.8	230.5	217.8
Reproduction (14 d) [% of control]	--	100	107	117	112	100	95
Endpoint [mg Reg. No. 360 714/kg dry soil]							
$NOEC_{mortality + reproduction}$	≥ 200						
LC_{50}	> 200						
EC_{50}	> 200						

The reference item Dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg as/kg dry soil. The EC_{50} (reproduction) for Dimethoate EC 400 was calculated to be 6.87 mg as/kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

Conclusion

The study is acceptable. In a 14-day reproduction study with Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 200 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be 200 mg Reg. No. 360 714/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

KCA 8.4.2.1/8 Schulz, 2014 (new study, submitted with renewal dossier)

Author: Schulz L.
Title: Effects of Reg.No. 360712 (Metabolite of BAS 656 H, dimethenamid-P) on the reproduction of the predatory mite *Hypoaspis aculeifer*
Date: 06.01.2014
Doc ID: 13 10 48 113 S; BASF RegDoc# 2013/1103674
Guidelines: OECD 226 (2008)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: Reg. No. 360 712 (metabolite of BAS 656 H, dimethenamid-P, M31, batch no. L81-46; analysed purity: 98.7 % (±1.0 %).

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult predatory mites (age difference 2 days); source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of Reg. No. 360 712 on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were

introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g analysed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 31.25, 62.5, 125, 250 and 500 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 5.8 – pH 5.9 at test initiation, pH 5.8 – 6.0 at test termination; water content at test initiation 50.49 % – 52.12 % of maximum water holding capacity (WHC) and 50.48 % – 52.32 % of maximum WHC at test termination; temperature: 19.5 °C – 21.4 °C; photoperiod: 16 h light : 8 h dark; light intensity: 522 lux; food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), Dunnett-t-test for reproduction (α = 0.05, one-sided smaller).

Results and Discussion

Test item treatment groups had mortality rates of between 0.0 % - 5.0 %. In the untreated control and the solvent control group, mortalities of 0.0 % and 3.8 % were observed. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group (Fisher's Exact Binominal Test with Bonferroni Correction, α = 0.05, one-sided greater).
In the untreated and the solvent control group, mean numbers of 249.6 and 215.0 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 183.3 and 256.5. Reg. No. 360 712 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 500 mg/kg dry soil, the highest concentration tested (Dunnett-t-test, α = 0.05, one-sided smaller). The results are summarised in the following table.

Table B.9.4-13: Effects of Reg. No. 360 712 on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

Reg. No. 360 712 [mg/kg dry soil]	**Control**	**Solvent control**	**31.25**	**62.5**	**125**	**250**	**500**
Mortality [%]	0.0	3.8	5.0	0.0	2.5	5.0	5.0
No. of juveniles (day 14)	249.6	215.0	256.5	183.3	197.3	186.0	209.8
Reproduction (day 14) [% of solvent control]	--	100	119	85	92	87	98
Endpoints [mg Reg. No. 360 712/kg dry soil]							
$NOEC_{mortality + reproduction}$	≥ 500						
LC_{50}	> 500						
EC_{50}	> 500						

The reference item Dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg as/kg dry soil. The EC_{50} (reproduction) for Dimethoate EC 400 was calculated to be 6.64 mg as/kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

Conclusion

The study is acceptable. In a 14-day reproduction study with Reg. No. 360 712 (metabolite of BAS 656 H, dimethenamid-P, M31) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 500 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be 500 mg Reg. No. 360 712/kg dry soil, the highest concentration tested. The test substance was incorporated in artificial soil (5 % peat).

B.9.5 Effects on soil nitrogen transformation

Three new studies on the effect of the dimethenamid-P and its metabolites M23, M27, and M31 to soil nitrogen transformation were submitted with the renewal dossier and are summarised below.

To increase the transparency and comprehensibility of the overall assessment, summaries of the studies assessed with the initial evaluation of dimethenamid-P were have been added by the RMS. No new evaluation of the previously submitted studies was performed.

KCA 8.5/1 Danneberg, 1991 (study evaluated in the initial monograph, 2000)

Author:	Danneberg, G.
Title:	Investigation on the effects of SAN 582 H on the activity of the microflora of soil
Date:	01.08.1991
Doc ID:	BE-S-7-91-01-DEH-01; BMF1999-42; BMF96-00042; BASF DocID# 91/11908
Guidelines:	Thalmann (1968); BBA-guideline 1-1, part VI, March 1990
GLP:	-/-
Validity:	C: additional information; N: not acceptable

Material and Methods

Test substance: SAN 582 H (dimethenamid-racemate)
Test concentrations: 2.4 mg/kg (1x), 12 mg/kg (5 x)
Test substrate: two different soils; sandy soil, 1.34 % org. C, pH 6.84; loamy soil, 3.91 % org. C., pH 5.5

Results and Discussion

The test was performed in plastic bags which is not acceptable according to current standards.

Table B.9.5-1: Effects of active substance dimethenamid on carbon conversion

type of soil	application rate in kg as/ha	in comparison with untreated in %	test duration in days	influence tolerable	Ref.
loamy sand	1.8	117.6	28	yes	1
	9.0	111.5	28	yes	1
clay silt	1.8	103.3	28	yes	1
	9.0	92.3	28	yes	1

The nitrogen mineralisation was tested with the active substance dimethenamid (0906) in two different soils. The part of the study about the nitrogen mineralisation with the active substance is not valid because in both of the soils no mineralisation occurred. Nevertheless, signs of effects at day 28 are given by the study (2.4 mg/kg: NH_4^+ +30.8 %, NO_3^- +42.9 %, total-N +36.4 %; 12 mg/kg: NH_4^+ +27.5 %, NO_3^- +49.6 %, total-N +37.6 %.

Conclusion

DMTA: In the study with the active substance dimethenamid-racemate (BMF9600042) no nitrification occurred. Therefore, the study is considered not valid with respect to the nitrogen turnover part.

KCA 8.5/2 Schulz, 2008a (new study, submitted with renewal dossier)

Author:	Schulz L.
Title:	Effects of Reg. No. 360715 (metabolite of BAS 656 H, M23) on the activity soil microflora (Nitrogen transformation test)
Date:	19.12.2008
Doc ID:	08 10 48 062 N; BASF RegDoc# 2008/1065117
Guidelines:	OECD 216 (2000)
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item:	M23 (metabolite of dimethenamid-P), batch no. L59-90, Reg. No. 360 715, purity: 98.4 %.
Test soil:	Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49 % C_{org}, 38.11 % water holding capacity (WHC).
Test design:	Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5 %). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyser II (Bran and Luebbe). Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to the measurement.
Endpoints:	Effects on the NO_3-nitrogen production 0, 7, 14 and 28 days after application.
Test concentrations:	Control, 0.2 mg M23/kg dry soil and 1.0 mg M23/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.
Reference item:	Dinoterb (purity: 98.0 ± 0.5 %). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.
Test conditions:	Soil moisture: approx. 45 % of its max. WHC; measured water content: 17.97 – 18.46 g/100 g dry soil; pH 6.3 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in glass flasks in the dark.
Statistics:	Descriptive statistics.

Results and Discussion

No adverse effects of M23 on nitrogen transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of -2.0 % (application rate 0.2 mg/kg dry soil) and -1.0 % (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarised in the following table.

Table B.9.5-2: Effects of M23 on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M23/kg dry soil		1.0 mg M23/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	10.3	10.9	+ 5.5	10.6	+ 2.6
Loamy sand soil (7 d)	30.8	30.4	- 1.3	30.9	+ 0.3
Loamy sand soil (14 d)	36.2	36.2	± 0.0	35.9	- 0.9
Loamy sand soil (28 d)	50.1	49.1	- 2.0	49.6	-1.0

1) Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of + 27.7 %, + 60.8 % and + 68.1 % at 6.8, 16.0 and 27.0 mg/kg dry soil.

Conclusion

The study is acceptable. Accepted endpoint: < 25 % effect at 1.0 mg as/kg dw.

KCA 8.5/3 Schulz, 2008b (new study, submitted with renewal dossier)

Author: Schulz L.
Title: Effects of Reg.No. 360 714 (metabolite of BAS 656 H, M27) on the activity of soil microflora (Nitrogen transformation test)
Date: 19.12.2008
Doc ID: 08 10 48 063 N; BASF RegDoc# 2008/1065119
Guidelines: OECD 216 (2000)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M27 (metabolite of dimethenamid-P), batch no. 01311-28, Reg. No. 360 714, purity: 97.1 %.

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49 % C_{org}, 38.11 % water holding capacity (WHC).

Test design: Determination of N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5 %). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyser II (Bran and Luebbe). Sampling scheme: 0, 7, 14, and 28 days after treatment. Sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M27/kg dry soil and 1.0 mg M27/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of

1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5 %). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45 % of its maximum water holding capacity; measured water content: 17.82 – 18.79 g/100 g dry soil; pH 6.3 – 6.5. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

Results and Discussion

No adverse effects of M27 on nitrogen transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +1.5 % (application rate 0.2 mg/kg dry soil) and +3.2 % (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarised in the following table.

Table B.9.5-3: Effects of M27 on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M27/kg dry soil		1.0 mg M27/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	11.3	11.2	- 1.5	11.7	+ 2.9
Loamy sand soil (7 d)	31.3	33.0	+ 5.4	31.7	+ 1.1
Loamy sand soil (14 d)	34.2	36.7	+ 7.3	37.6	+ 10.1
Loamy sand soil (28 d)	50.1	50.9	+ 1.5	51.7	+ 3.2

1) Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of + 27.7 %, + 60.8 % and + 68.1 % at 6.8, 16.0 and 27.0 mg/kg dry soil.

Conclusion

The study is acceptable. Accepted endpoint: < 25 % effect at 1.0 mg as/kg dw.

KCA 8.5/4 Schulz, 2008c (new study, submitted with renewal dossier)

Author: Schulz L.
Title: Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the activity of soil microflora (Nitrogen transformation test)
Date: 19.12.2008
Doc ID: 08 10 48 064 N; BASF RegDoc# 2008/1065115
Guidelines: OECD 216 (2000)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7 %.

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49 % C_{org}, 38.11 % water holding capacity (WHC).

Test design: Determination of N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5 %). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyser II (Bran and Luebbe). Sampling scheme: 0, 7, 14, and 28 days after treatment. Sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M31/kg dry soil and 1.0 mg M31/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5 %). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45 % of its max. WHC; measured water content: 16.00 – 18.70 g/100 g dry soil; pH 6.3 – 6.5. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

Results and Discussion

No adverse effects of M31 on nitrogen transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of + 2.9 % (application rate 0.2 mg/kg dry soil) and + 3.5 % (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarised in the following table.

Table B.9.5-4: Effects of M31 on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M31/kg dry soil		1.0 mg M31/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	16.3	16.2	- 0.6	15.9	- 2.3
Loamy sand soil (7 d)	36.7	36.2	- 1.3	37.1	+ 1.3
Loamy sand soil (14 d)	40.3	40.0	- 0.6	39.5	- 1.8
Loamy sand soil (28 d)	54.6	56.2	+ 2.9	56.5	+ 3.5

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of + 27.7 %, + 60.8 % and + 68.1 % at 6.8, 16.0 and 27.0 mg/kg dry soil.

Conclusion

The study is acceptable. Accepted endpoint: < 25 % effect at 1.0 mg as/kg dw.

B.9.6 Effects on terrestrial non-target higher plants

A new study testing the herbicidal activity the dimethenamid-P metabolite M31 in a pre-emergence greenhouse study according to OECD guideline 208 was submitted for the renewal assessment and is summarised below.
Additionally, a summary of a GLP-study on herbicidal activity of dimethenamid-P, several of its metabolites and the representative formulation BAS 656 12 H on 6 different plant species was provided by the applicant in document N4 of the renewal dossier. The study has not yet been submitted. Therefore the summary of the new study was included as additional information, subject to submission of the study.

To increase the transparency and comprehensibility of the overall assessment, summaries of the studies assessed with the initial evaluation of dimethenamid-P have been added by the RMS. No new evaluation of the previously submitted studies was performed.

B.9.6.1 Summary of screening data

No new studies were required or submitted for the renewal assessment. To increase the transparency and comprehensibility of the overall assessment, selected summaries of the studies assessed with the initial evaluation of dimethenamid-P have been added by the RMS.

KCA 8.6.1/1 Kaethner, 1995 (study evaluated in the addendum_02 of the initial mono graph, 2000)

Author:	Kaethner, M.
Title:	Screening Test on the Biological Efficacy of Dimethenamid Soil Metabolites M23 and M27 on Higher Plants.
Date:	1995
Doc ID:	PFL2002-227; PFL2002-228; BASF RegDoc.# 1995/11317
Guidelines:	
GLP:	No
Validity:	Additional information

Material and Methods

The metabolites M23 (Oxalamide) and M27 (Sulfonate) were tested for herbicidal activity on eight terrestrial higher plants species in screening tests (PFL2002-227). Four monocotyle (*Avena fatua, Bromus tectorum, Echinochloa crus-galli, Setaria viridis*) and four dicotyle species (*Abutilon theophrasti, Amaranthus retroflexus, Sinapis alba, Solanum nigrum*) were tested. Pre- and post-emergence tests (1-2 leaves) were conducted for both metabolites with an application rate of 250 and 1000 g/ha (the application rate intended usually for dimethenamid-P is 1.000 g as/ha).

Results and Discussion

Three weeks after application a visual assessment of damage, growth inhibition, chlorosis and burning did not show any herbicidal effect. The parent compound was not tested with the usual rate, but with the low rate of 0.16 g/ha corresponding to the total radiocarbon content in the leachate. At this concentration there was no effect on the plant species tested.

Additionally the herbicidal activity of the leachate and the metabolites M23 and M27 were tested using inhibition of growth, whitening of new grown fronds, antimetabolic activity, inhibition of

tetrapyrol biosythesis and light versus dark activity on the species *Lemna minor*. No herbicidal activity was observed at concentrations up to 50 µM, whereas with a solution of dimethenamid (0.3 µM and 0.03 µM) 80 % resp. 70 % growth inhibition was found.

In an additional document (PFL2002-228) the same results concerning the non-target terrestrial plants were described as in document PFL 2002-227.

Conclusion

In screening for herbicidal efficacy, both seedling emergence and in vegatative vigour tests, the metabolites M23 (oxalamide) and M27 (sulfonate) showed no herbicidal activity at 250 and 1000 g as/ha.

B.9.6.2 Testing on non-target plants

KCA 8.6.2/1 Dutillie & Sack, 2008 (new study, submitted with renewal dossier)

Author:	Dutillie H., Sack D
Title:	Effects of Reg.No. 360712 (M31, metabolite of BAS 656 H) on non-target plants in the greenhouse
Date:	26.09.2008
Doc ID:	353446; BASF RegDoc# 2008/1068011
Guidelines:	OECD 208 (2006)
GLP:	No
Validity:	Acceptable

Material and Methods

Test item:	M31 (metabolite of BAS 656 H; Reg. No 360 712), batch No. L81-46.
Reference items:	BAS 656 H (dimethenamid-P; Reg. No. 363 851), batch No. 6261B01BH; BAS 656 08 H, batch No. FRE-000484.
Test species:	Hairy crabgrass (*Digitaria sanguinalis*), Green foxtail (*Setaria viridis*), Italian ryegrass (*Lolium multiflorum*), Giant foxtail (*Setaria faberi*), Barnyard grass (*Echinocloa crus-galli*), Annual meadow grass (*Poa annua*), Shepherd´s purse (*Capsella bursa-pastoris*), Fat hen (*Chenopodium album*), Scentless mayweed (*Matricaria inodora*), Common chickweed (*Stellaria media*).
Test design:	5 treatment groups (2 rates for test item and both reference items, blank formulation, water treated control); 4 replicates/treatment; 1 pot/replicate, number of plants is related to standard sowing depending on species, pre-emergence applications using a laboratory spray cabin at a water rate of 750 L/ha. Following the application the plants were cultivated for 21 days in the greenhouse. Assessments for phytotoxicity (e.g. chlorosis, necrosis etc.) were done 7 and 21 days after application (DAA) for all plants. Shoot fresh weight was determined at study termination 21 DAA.
Endpoints:	Fresh weight and phytotoxicity.
Test rates:	Water control, blank formulation, 648 g as/ha and 1008 g as/ha for M31, BAS 656 H and BAS 656 08 H.
Test conditions:	Greenhouse conditions, average temperature: 14 °C - 31 °C, average humidity: about 80 %; photoperiod: 16 h light : 8 h dark; additional light when outdoor

illumination was less than 4500 lux.

Statistics: Descriptive statistics. Dunnett-test ($\alpha = 0.05$).

Results and Discussion

Phytotoxicity: BAS 656 08 H resulted in 83 % - 100 % plant damage in all tested species in the lower rate and in 98 % - 100 % plant damage in the higher rate. BAS 656 H caused plant damages up to 88 % - 100 % in the lower rate and 96 % - 100 % in the higher rate. No effects were observed in the plants treated with M31, metabolite of BAS 656 H, in both treatment rates. Also the water treated control and the blank formulation did not cause plant damages.

Biomass (fresh weight): BAS 656 08 H resulted in 100 % reduction of mean plant weight in all tested species in both tested rates, except for the species fat hen which showed a reduction of 76 % in the lower test rate and 96 % in the higher test rate. BAS 656 H resulted in 100 % reduction of mean plant weight in all tested species in both tested rates, except for the species fat hen which showed a reduction of 93 % in the lower test rate and 96 % in the higher test rate. The metabolite M31, tested at the lower rate, caused maximum 27 % reduction, in plant weight and also a maximum 43 % promotion. At the higher rate, the metabolite M31 caused maximum 12 % reduction, in plant weight and also a maximum 74 % promotion. Even though this reduction was statistically significant in one case in tests with the higher rate, it is not considered to be caused by the metabolite M31, as the same reduction was also observed by the blank formulation and it is well within the normal variability of the test system. Also at the lower test rate statistically significant differences were observed but it is not considered to be caused by the metabolite for the same reasons. The coincidence of significance of differences in plant biomass is supported by the fact that it was not observed at the higher test rate.

The results are summarised in the following table.

Table B.9.6-1: Effects of M31 (metabolite of BAS 656 H) on fresh weight and plant damage 21 DAA

Test substance	Test rate [g as/ha]	hairy crabgrass	green foxtail	italian ryegrass	giant foxtail	barnyard grass
Phytotoxicity [% chlorosis/necrosis/stunting/deformation]						
Control	--	0	0	0	0	0
Blank formulation	--	0	0	0	0	0
M31	648	0	0	0	0	0
	1008	0	0	0	0	0
BAS 656 H	648	100	100	100	100	100
	1008	100	100	100	100	100
BAS 656 08 H	648	100	100	100	100	100
	1008	100	100	100	100	100
Reduction of fresh weight [% of control]						
Control	--	0	0	0	0	0
Blank formulation	--	4.45	22.17*	-7.79	31.45*	-10.56
M31	648	14.94*	27.22*	-9.87	21.94*	1.52
	1008	-7.44	12.04	-31.58	11.74	-23.09
BAS 656 H	648	100*	100*	100*	100*	100*
	1008	100*	100*	100*	100*	100*
BAS 656 08 H	648	100*	100*	100*	100*	100*
	1008	100*	100*	100*	100*	100*
Test substance	**Test rate [g as/ha]**	**annual meadowgrass**	**shepherd´s purse**	**fat hen**	**scentless mayweed**	**chickweed**
Phytotoxicity [% chlorosis/necrosis/stunting/deformation]						
Control	--	0	0	0	0	0
Blank formulation	--	0	0	0	0	0
M31	648	0	0	0	0	0
	1008	0	0	0	0	0
BAS 656 H	648	100	100	88	99	95
	1008	100	100	96	100	100
BAS 656 08 H	648	100	100	83	98	99
	1008	100	100	98	100	99
Reduction of fresh weight [% of control]						
Control	--	0	0	0	0	0
Blank formulation	--	-2.84	-65.38	12.70*	14.07	-18.20
M31	648	14.18	-42.89	23.76*	29.95*	-15.77
	1008	-6.22	-74.01	12.38*	0.88	-35.90
BAS 656 H	648	100*	100*	92.85*	99.92*	99.80*
	1008	100*	100*	95.51*	100*	100*
BAS 656 08 H	648	100*	100*	75.81*	98.96*	99.95*
	1008	100*	100*	96.30*	100*	99.95*

* Statistically significant difference compared to the control (Dunnett-test, $\alpha = 0.05$).

Conclusion

The study is acceptable. In contrast to the statement of the authors the guideline OECD 227 (vegetative viguor test) is not covered by this study. Several replicates were excluded after Dixon's outlier test. Based on the results of this study it can be concluded that M31, metabolite of BAS 656 H, applied pre-emergence up to a rate of 1008 g/ha, showed inhibitory as well as promoting effects which were well within the effect range of formulation blank BAS 089 01 S, whereas the parent dimethenamid-P showed strong effects.

KCA 8.6.2/2 N.N., (new study, to be submitted with renewal dossier)

Author: unknown
Title: unknown
Date: unknown
Doc ID: unknown
Guidelines: unknown
GLP:
Validity: Additional information, subject to submission of the study ;
summary provided by the applicant in document N4 of the renewal dossier

Screening for biological activity

The soil metabolites M656PH030, M656PH023, M656PH030, M656PH031, M656PH032,M656PH043, M656PH045, M656PH047, M656PH054, M656H055, the Na^+-salt of M656PH027 and the ethylester derivate for M656PH062 have been screened for biological activity on plants in the greenhouse when applied in pre- and in post-emergence standard tests. The dose rate of the parent dimethenamid-P was the maximum defended dose rate of 864 g as/ha. This dose rate was adjusted for the metabolites based on their molar equivalent. The parent compound showed 21 days after application 99 % efficacy on the selected species of the grasses Bromus, Echinochloa, Setaria and Lolium and 90 % efficacy on the broadleaves Chenopodium and Geranium when applied in pre-emergence. The efficacy of the parent applied in post-emergence was 93 and 38 %. The broadleve weed Geranium was controlled with 75 %, Chenopodium was however not controlled in post emergence. All metabolites showed in pre- and in post enmergence not any sign of phytotoxicity. The data conclude that there is no biological activity for the metabolites.

Herbicidal efficacy of BAS 656 H metabolites (Glasshouse efficacy test) – nonGLP

Executive Summary

The biological activity of different metabolites of BAS 656 H was investigated in a plant assay in the glasshouse using monocot and dicot weed species. The results are summarised below. None of the tested metabolites did show biological activity at the tested rates.

Material and Methods

Test Material
The test compounds used were provided by BASF APR/DA:

BAS 656 12H	DMTA-p
LS 363851	DMTA-p (LS 363851)
M656PH030	LS 5296352
M656H031	LS 360712
M656H032	LS 395234
M656PH054	LS 5920718
M656H055	LS 5749263
M27 -Na salt	LS 360714
M656PH047	LS 5917260
M656PH023	LS 5886780
M656PH045	LS 5917261
M656PH043	LS 5917262
Ethylester derivative for M62	LS 5936274
Blank formulation	

The test compounds were all formulated in the same solvent containing standard formulation (5 %).

Experimental conditions:
Plants seeded in plastic posts were used for the experiment. The soil that was used for the experiment

was loamy sand with approximately 3 % of organic matter. The seeds of the test plants were sown separately for each species. 2 different application timings were used in the activity test, a pre-emergence treatment as well as a post-emergence treatment at a growth stage of GS12.
For the pre-emergence treatment, the active substances, were applied directly after sowing by means of finely distributing nozzles. The pots were irrigated gently to promote germination and growth and subsequently covered with transparent plastic hoods until the plants had rooted. This cover caused uniform germination of the test plants, unless this has been impaired by the active substances.
For the post-emergence treatment, the test plants were first grown to GS12-13 (corresponding to 2-3 leaves unfolded), and then treated with the active substances. For this purpose, the test plants were either sown directly or grown in the same pots, or they were first grown separately as seedlings and transplanted into the test containers a few days prior to treatment.
Each treatment was replicated 4-fold with several plants per treatment. After the spray application the plants were maintained inside a glasshouse.
The test period extended over 4 weeks.

The following plants were used in the experiment:

Code	**Scientific name**
BROIN	*Bromus inermis*
ECHCG	*Echinochloa crus-galli*
SETVI	*Setaria viridis*
LOLMU	*Lolium multiflorum*
GERDI	*Geranium dissectum*
CHEAL	*Chenopodium album*

An appropriate set non-treated plants served to check the development of the plants.

The parent and the metabolites were applied in 4 rates.
The rates were selected based on the average of recommended use rates of BAS 656 H in the field and to ensure a decrease in activity of the compounds within this range of test rates. It was sprayed with 375 L water/ha.

The blank formulation by itself was tested as a negative control (Leerformulierung LS 22).

Assessment
The response of the plants to the individual treatments was evaluated at 10 and 21 days after treatment. The evaluation was carried out using a scale from 0 to 100. 100 means no emergence of the plants, or complete destruction of at least the aerial moieties, and 0 means no damage, or normal course of growth. A good herbicidal activity is given at values of at least 70 and a very good herbicidal activity is given at values of at least 85.

Results and Discussion

The efficacy data of the compounds are summarised in Table B.9.6-2 (pre-emergence application) and Table B.9.6-3 (post-emergence application).

Table B.9.6-2: Activity of BAS 656 H and its metabolites following pre-emergence application

eval. dat		BROIN	BROIN	ECHCG	ECHCG	SETVI	SETVI	LOLMU	LOLMU	grasses Ø	grasses Ø	CHEAL	CHEAL	GERDI	GERDI	BLV Ø	BLV Ø
compound	g ai/ha	10	21	10	21	10	21	10	21	10	21	10	21	10	21	10	21
BAS 656 12H	864	90	97	93	100	95	100	98	98	94	99	45	76	53	100	74	88
720 g/l EC DMTA-p	172,8	86	97	90	98	91	100	95	98	91	98	28	48	40	96	66	72
	86,4	61	66	85	97	80	98	90	97	79	89	15	30	28	94	57	62
	43,2	35	15	75	88	66	96	81	80	64	70	0	20	0	83	37	51
LS 363851	864	91	98	95	100	93	100	97	98	94	99	50	83	53	98	75	90
	172,8	84	96	90	99	86	100	96	98	89	98	23	55	31	96	62	75
	86,4	60	68	80	95	75	98	91	98	77	89	10	30	18	86	54	58
	43,2	28	28	68	86	60	95	81	95	59	76	0	18	0	68	43	43
LS 5296352	1183	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	236,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	118,3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	59,15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 360712	1088	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	217,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	108,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	54,4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 395234	1038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	207,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	103,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	51,9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5920718	1007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	201,4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	100,7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50,35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5749263	781	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	156,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	78,1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	39,05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 360714	1076	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	215,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	107,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	53,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5917260	1101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	220,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	110,1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	55,05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5886780	850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	42,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5917261	944	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	188,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	94,4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	47,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5917262	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5936274	806	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	161,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	40,3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leerformulierung Lsg 22	5%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

None of the tested metabolites did show any notable activity on the tested weeds, whereas the parent BAS 656 H (dimethenamid-P; LS 363851) and the formulation BAS 656 H 12 did show high efficacy against grasses as well as broadleaf weeds. The activity level of BAS 656 H obtained in the test is on the average level that is expected for this glasshouse experiment conditions.
The blank formulation by itself did not show any significant biological efficacy.

Table B.9.6-3: Activity of BAS 656 H and its metabolites following post-emergence application

eval. dat		BROIN	BROIN	ECHCG	ECHCG	SETVI	SETVI	LOLMU	LOLMU	grasses Ø	grasses Ø	CHEAL	CHEAL	GERDI	GERDI	BLV Ø	BLV Ø
compound	g ai/ha	7	21	7	21	7	21	7	21	7	21	7	21	7	21	7	21
BAS 656 12H	864	40	95	70	94	61	95	61	95	58	95	0	0	49	68	24	34
720 g/l EC DMTA-p	172,8	15	50	68	93	40	90	44	86	42	80	0	0	35	55	18	28
	86,4	0	30	45	81	26	80	20	69	23	65	0	0	18	35	9	18
	43,2	0	5	18	70	5	66	0	28	6	42	0	0	0	18	0	9
LS 363851	864	43	94	71	95	68	93	60	91	60	93	0	0	59	75	29	38
	172,8	18	58	59	89	30	88	48	78	38	78	0	0	33	51	16	26
	86,4	5	29	40	79	25	80	28	54	24	60	0	0	20	25	10	13
	43,2	0	5	18	66	0	61	5	18	6	38	0	0	0	10	0	5
LS 5296352	1183	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	236,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	118,3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	59,15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 360712	1088	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	217,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	108,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	54,4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 395234	1038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	207,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	103,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	51,9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5920718	1007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	201,4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	100,7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50,35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5749263	781	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	156,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	78,1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	39,05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 360714	1076	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	215,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	107,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	53,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5917260	1101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	220,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	110,1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	55,05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5886780	850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	42,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5917261	944	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	188,8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	94,4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	47,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5917262	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LS 5936274	806	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	161,2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80,6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	40,3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leerformulierung Lsg	5%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Also in a post-emergence treatment, the efficacy of the metabolites at all rates tested was negligible, none of the tested metabolites did show any response. The active substance BAS 656 H (dimethenamid-P; LS 363851) and the formulation BAS 656 H 12 provided very good control. The blank formulation by itself did not show biological efficacy.

Conclusion

The study has not been submitted yet and could therefore not be evaluated in detail. Subject to submission of the study, the results of the study is used as additional information. Given the summary provided by the applicant in document N4 of the renewal dossier it can be concluded that the tested metabolites of BAS 656 H, applied pre- and post-emergence, show no biological activity on plants in any of the tested species up to the highest rate tested. In comparison, the parent compound BAS 656 H (dimethenamid-P) as well as the formulation BAS 656 12 H (720 g as/L) showed strong effects against various weed species, both indicating a rate-response relationship. Given the facts that 4 rates were tested with 4 replicates each, the test is deemed to give additional information which has to be considered in the risk assessment of the representative formulation BAS 656 12 H. Based on phytotoxicity, the calculated $ER_{50(\text{active substance})}$ = 93.3 g as/ha following post emergence application and ER_{50} < 43.2 g as/ha following post emergence application, both for *Lolium multiflorum*.

B.9.7 Effects on other terrestrial organisms (flora and fauna)

No new studies were required or submitted for the renewal assessment. Although according to current data requirements no studies on microbial carbon transformation are required for the renewal assessment, studies assessed with the initial evaluation of dimethenamid-P were provided by the applicant.
Therefore, only the study assessment from the initial dimethenamid-P monograph is quoted below for information, but no new evaluation was performed.

KCA 8.7/1 Schulz, 2008d (new study, submitted with renewal dossier)

Author:	Schulz L.
Title:	Effects of Reg. No. 360715 (metabolite of BAS 656 H, M23) on the activity of soil microflora (Carbon transformation test)
Date:	19.12.2008
Doc ID:	08 10 48 062 C; BASF RegDoc# 2008/1065116
Guidelines:	OECD 217 (2000)
GLP:	Yes
Validity:	Acceptable

Material and Methods

Test item:	M23 (metabolite of dimethenamid-P), batch no. L59-90, Reg. No. 360 715, purity: 98.4 %.
Test soil:	Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49 % C_{org}, 38.11 % water holding capacity (WHC).
Test design:	Determination of carbon transformation in soil after addition of glucose (concentration in soil 0.4 %). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.
Endpoints:	Effects on O_2 consumption 0, 7, 14 and 28 days after application.
Test concentrations:	Control, 0.2 mg M23/kg dry soil and 1.0 mg M23/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.
Reference item:	Dinoterb (purity: 98.0 ± 0.5 %). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.
Test conditions:	Soil moisture: approx. 45 % of its max. WHC; measured water content: 17.14 – 18.54 g/100 g dry soil; pH 6.2 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in steel vessels in the dark.
Statistics:	Descriptive statistics.

Results and Discussion

No adverse effects of M23 on carbon transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of -4.4 % (application rate 0.2 mg/kg dry soil) and -2.7 % (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarised in the

following table.

Table B.9.7-1: Effects of M23 on soil micro-organisms (carbon transformation) on days 0, 7, 14, and 28 of incubation

Soil (days)	Control	0.2 mg M23/kg dry soil		1.0 mg M23/kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	15.47	15.66	+ 1.3	15.24	- 1.5
Loamy sand soil (7 d)	14.85	14.80	- 0.3	14.25	- 4.0
Loamy sand soil (14 d)	13.82	13.49	- 2.4	13.57	- 1.8
Loamy sand soil (28 d)	12.77	12.21	- 4.4	12.42	- 2.7

1) Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of - 24.8 %, - 42.0 % and - 49.0 % at 6.8, 16.0 and 27.0 mg/kg dry soil.

Conclusion

The study is acceptable. Accepted endpoint: < 25 % effect at 1.0 mg as/kg dw.

KCA 8.7/2 Schulz, 2008e (new study, submitted with renewal dossier)

Author: Schulz L.
Title: Effects of Reg.No. 360714 (metabolite of BAS 656 H, M27) on the activity of soil microflora (Carbon transformation test)
Date: 19.12.2008
Doc ID: 08 10 48 063 C; BASF RegDoc# 2008/1065118
Guidelines: OECD 217 (2000)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M27 (metabolite of dimethenamid-P), batch no. 01311-28, Reg. No. 360 714, purity: 97.1 %.

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49 % C_{org}, 38.11 % water holding capacity (WHC).

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil 0.4 %). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on O_2 consumption 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M27/kg dry soil and 1.0 mg M27/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of

1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5 %). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45 % of its max. WHC; measured water content: 17.56 – 18.56 g/100 g dry soil; pH 6.3 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

Results and Discussion

No adverse effects of M27 on carbon transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +0.9 % (application rate 0.2 mg/kg dry soil) and -0.6 % (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarised in the following table.

Table B.9.7-2: Effects of M27 on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M27/kg dry soil		1.0 mg M27/kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	16.34	15.89	- 2.8	16.16	- 1.1
Loamy sand soil (7 d)	15.76	15.76	± 0.0	15.36	- 2.6
Loamy sand soil (14 d)	14.26	14.40	+ 1.0	14.13	- 0.9
Loamy sand soil (28 d)	13.65	13.76	+ 0.9	13.56	- 0.6

1) Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of - 24.8 %, - 42.0 % and - 49.0 % at 6.8, 16.0 and 27.0 mg/kg dry soil.

Conclusion

The study is acceptable. Accepted endpoint: < 25 % effect at 1.0 mg as/kg dw.

KCA 8.7/3 Schulz, 2008f (new study, submitted with renewal dossier)

Author: Schulz L.
Title: Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the activity of soil microflora (Carbon transformation test)
Date: 19.12.2008
Doc ID: 08 10 48 064 C; BASF RegDoc# 2008/1065109
Guidelines: OECD 217 (2000)
GLP: Yes
Validity: Acceptable

Material and Methods

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7 %.

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49 % C_{org}, 38.11 % water holding capacity (WHC).

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil 0.4 %). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on O_2 consumption 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M31/kg dry soil and 1.0 mg M31/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5 %). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45 % of its max. WHC; measured water content: 17.77 – 18.71 g/100 g dry soil; pH 6.3 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

Results and Discussion

No adverse effects of M31 on carbon transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of - 1.7 % (application rate 0.2 mg/kg dry soil) and - 2.7 % (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarised in the following table.

Table B.9.7-3: Effects of M31 on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M31/kg dry soil		1.0 mg M31/kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	16.23	16.28	+ 0.3	16.17	- 0.4
Loamy sand soil (7 d)	15.76	15.64	- 0.8	15.23	- 3.3
Loamy sand soil (14 d)	14.62	14.61	- 0.1	14.17	- 3.1
Loamy sand soil (28 d)	14.42	14.17	- 1.7	14.03	- 2.7

1) Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of - 24.8 %, - 42.0 % and - 49.0 % at 6.8, 16.0 and 27.0 mg/kg dry soil.

Conclusion

The study is acceptable. Accepted endpoint: < 25 % effect at 1.0 mg as/kg dw.

B.9.8 Effects on biological methods for sewage treatment

No new studies were required or submitted for the renewal assessment. To increase the transparency and comprehensibility of the overall assessment, selected summaries of the studies assessed with the initial evaluation of dimethenamid-P have been added by the RMS.

KCA 8.8/1 Scholtz, 1994 (study evaluated in the initial monograph, 2000)

Author:	Scholtz R.
Title:	Determination of the inhibitory effect on bacteria: Pseudomonas cell multiplication inhibition test
Date:	1994
Doc ID:	1994/11901; WAT1999-499; BASF RegDoc.# 94/11901
Guidelines:	ISO 38412-L8
GLP:	No
Validity:	Additional information

Conclusion

No indication of inhibitory effects on microbial activity up to a concentration of 400 mg/L.

KCA 8.8/2 Desmares-Koopmans, 1995 (study evaluated in the initial monograph, 2000)

Author:	Desmares-Koopmans M.
Title:	Activated sludge respiration inhibition test with dimethenamid technical
Date:	24.11.1995
Doc ID:	163136; WAT1999-500; BASF RegDoc.# 95/11327
Guidelines:	OECD 209
GLP:	Yes
Validity:	Additional information

Conclusion

No indication of inhibitory effects on microbial activity in steps up to a concentration of 100 mg/L.

B.9.9 Monitoring data

No studies submitted, not required.

B.9.10 Biological activity of metabolites potentially occurring in groundwater

No studies submitted, not required.

B.9.11 References relied on

A search for open literature which included papers in peer-reviewed journals and reports from government and other agencies in the EU and several other countries was performed by the applicant. The literature search was done via databases such as PubMed, Agricola, and SciFinder using the key-word “Dimethenamid” or “Dimethenamid-P” and the CAS Numbers 87674-68-8 and 163515-14-8, respectively. The initial search was a net cast as wide as possible to ensure complete coverage of the literature. The references were then reviewed and, on the basis of the title and the abstract, a subset was retained for use in the characterisation. Priority was given to papers published since 2003 and,

where possible, copies of these were obtained for more detailed review. No additional open-literature studies concerning ecotoxicology of dimethenamid-P were found helpful for risk assessment purposes.

The literature search strategy of the applicant is decribed in more detail in the Appendix to this document.

Data Point EU as of 2014	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner	Previously submitted Y/N If yes, old data point
KCA 8.1.1.1/1	[redacted]	1996	SAN 1289H technical: an acute oral toxicity study with the northern bobwhite [redacted] 131-187; AVS1999-58 BASF RegDoc.#96/5419 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.1.1 [8.1/01]
KCA 8.1.1.1/2	[redacted]	1988	SAN 582 H an acute oral toxicity study with the bobwhite [redacted] 131-124A, AVS9600045 BASF RegDoc.# 88/11373 GLP, unpublished	N	N	Not applicable	BASF	Y not relevant IIA. 8.8 [8.8/01]
KCA 8.1.1.2/1	[redacted]	1996	SAN 1289H Technical: A dietary LC_{50} study with the mallard [redacted] 131-186; AVS1999-61 BASF RegDoc.#96/5410 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.1.2 [8.1/02]
KCA 8.1.1.2/2	[redacted]	1996	SAN 1289H Technical: a dietary LC_{50} study with the northern bobwhite [redacted] 131-185; AVS1999-59 BASF RegDoc.#96/5412 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.1.2 [8.1/03]
KCA 8.1.1.2/3	[redacted]	1988	SAN 582H: A dietary LC_{50} study with bobwhite [redacted], 131-122, AVS9600042 BASF RegDoc.# 88/11370 GLP, unpublished	Y	N	Not applicable	BASF	Y not relevant IIA. 8.8 [8.8/02]
KCA 8.1.1.2/4	[redacted]	1988	SAN 582H: A dietary LC_{50} study with the mallard [redacted] 131-123, AVS9600043 BASF RegDoc.# 88/11369 GLP, unpublished	Y	N	Not applicable	BASF	Y not relevant IIA. 8.8 [8.8/03]
KCA 8.1.1.3/1	[redacted]	1994	SAN 582H Technical: A reproduction study with the mallard [redacted] 131-178, AVS9600047 BASF RegDoc.# 94/11899 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.1.3 [8.1/04]
KCA 8.1.1.3/2	[redacted]	[redacted]	SAN 582H Technical: a reproduction study with the northern bobwhite [redacted] 131-177; AVS9600046 BASF RegDoc.# 94/11900 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.1.3 [8.1/05]

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KCA 8.2.1	[redacted]	1996	SAN 1289H Technical: A 96-hour flow-through acute toxicity test with the rainbow trout (Oncorhhynchus mykiss) [redacted] 131A-163; WAT1999-481 BASF RegDoc.# 96/5417 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.2.1 [8.2/01]
KCA 8.2.1	[redacted]	1996	SAN 1289H Technical: A 96-hour flow-through acute toxicity test with the bluegill (Lepomis macrochirus) [redacted] 131A-162; WAT1999-482 BASF RegDoc.# 96/5414 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.2.1 [8.2/02]
KCA 8.2.1	[redacted]	1997	Dimethenamid metabolite (M3): 96-hour acute toxicity study in the rainbow trout [redacted] RCC 628986; WAT1999-483 BASF RegDoc.# 97/10271 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.2.1 [8.2/03]
KCA 8.2.1	[redacted]	1995	Dimethenamid oxalamide (M23): 96-hour static acute toxicity with the rainbow trout (Oncorhynchus mykiss) [redacted] 94-003-1018; WAT95-00674 BASF RegDoc.# 95/11318 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.2.1 [8.2/04]
KCA 8.2.1	[redacted]	1995	Dimethenamid sulfonate sodium salt (M27): 96-hour static acute toxicity test with the rainbow trout (Oncorhynchus mykiss) [redacted] 94-006-1018 BASF RegDoc.# 95/11330 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.2.1 [8.2/05]
KCA 8.2.1	[redacted]	1988	Acute toxicity of SAN-582-H to bluegill sunfish (lepomis macrochirus) [redacted] 36655; WAT95-00665 BASF RegDoc.# 88/11368 GLP, unpublished	Y	N	Not applicable	BASF	Y not relevant IIA. 8.8 [8.8/04]
KCA 8.2.1	[redacted]	1988	Acute toxicity of SAN-582-H to rainbow trout (salmo gairdneri) [redacted] 36656; WAT95-00664 BASF RegDoc.# 88/11366 GLP, unpublished	Y	N	Not applicable	BASF	Y not relevant IIA. 8.8 [8.8/05]
KCA 8.2.1/1	[redacted]	1996	SAN 1289H Technical: A 96-Hour Flow-Through Acute Toxicity Test With the Sheepshead Minnow (Cyprinodon variegatus) 1996/5416 [redacted] GLP, unpublished	Y	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.1

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KCA 8.2.1/2	[redacted]	2010	[redacted] - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) 2010/1123696 [redacted] Germany Fed.Rep. GLP, unpublished	Y	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.1
KCA 8.2.2	[redacted]	1991	DOZ 300H (SAN 582H): 21-day rainbow trout toxicity study under flow-through exposure conditions [redacted] 91/SAS047/0409; WAT95-00668 BASF RegDoc.# 91/11906 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.2.2 [8.2/06]
KCA 8.2.2.1	[redacted]	1992	SAN 582H Technical (K/E): An early life-stage toxicity test with the rainbow trout (Oncorhnchus mykiss) [redacted] 131A-130A BASF RegDoc.# 92/12456 GLP, unpublished	Y	N	Not applicable	BASF	Y relevant IIA. 8.2.2.2 [8.2/07]
KCA 8.2.2.3	[redacted]	1988	Accumulation of (^{14}C) SAN-582H in bluegill sunfish [redacted] N0958-2500; WAT95-00663 BASF RegDoc.# 88/11365 GLP, unpublished	Y	N	Not applicable	BASF	Y not relevant IIA. 8.2.3 [8.2/08]
KCA 8.2.2.3	Daum A.	1999	Determination of the octanol/water-partition of Reg. No. 360717 (BAS 656H -M3) by HPLC BASF Aktiengesellschaft, Limburgerhof, Germany BASF RegDoc.# 99/10261 GLP, unpublished	N	N	Not applicable	BASF	Y not relevant IIA. 8.2.3 [8.2/09]
KCA 8.2.2.3	Daum A.	1999	Determination of the octanol/water-partition coefficient of Reg. No. 360715 BASF Aktiengesellschaft, Limburgerhof, Germany BASF RegDoc.# 99/10264 GLP, unpublished	N	N	Not applicable	BASF	Y not relevant IIA. 8.2.3 [8.2/10]
KCA 8.2.2.3	Daum A.	1999	Determination of the octanol/water-partition coefficient of Reg. No. 360714 BASF Aktiengesellschaft, Limburgerhof, Germany BASF RegDoc.# 99/10307 GLP, unpublished	N	N	Not applicable	BASF	Y not relevant IIA. 8.2.3 [8.2/11]
KCA 8.2.4.1	Graves W., Swigert J.	1996	SAN 1289 H Technical: A 48-hour flow-through acute toxicity test with the cladoceran (Daphnia magna) Wildlife International Ltd., Easton, Maryland, USA 131A-164; WAT1999-487 BASF RegDoc.# 96/5415 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.4 [8.2/12]

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KCA 8.2.4.1	Gruetzner I.	1997	Dimethenamid metabolite M3: 48-hour acute toxicity to Daphnia magna RCC,Itingen, CH RCC 628964; WAT1999-488 BASF RegDoc.# 97/10272 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.4 [8.2/13]
KCA 8.2.4.1	van der Kolk J.	1995	Dimethenamid oxalamide (M23): 48-hour static acute immobilization toxicity test with daphids (Daphna magna) Springborn Laboratories (Europe) AG, Horn, Switzerland 94-004-1018; WAT95-00671 BASF RegDoc.# 95/11319 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.4 [8.2/14]
KCA 8.2.4.1	van der Kolk J.	1995	Dimethenamid sulfonate sodium salt (M27): 48-hour static acute immobilization toxicity test with daphnids (Daphnia magna) Springborn Laboratories (Europe) AG, Horn, Switzerland 94-007-1018; WAT95-00672 BASF RegDoc.# 95/11331 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.4 [8.2/15]
KCA 8.2.4.1	Frazier S.	1988	Acute toxicity of SAN-582-H to daphnia magna ABC Laboratories, Inc., Columbia, US 36657; WAT95-00680 BASF RegDoc.# 88/11367 GLP, unpublished	N	N	Not applicable	BASF	Y not relevant IIA. 8.8 [8.8/06]
KCA 8.2.4.1/1	Janson G.-M.	2008	Acute toxicity of Reg.No. 360 712 (metabolite of BAS 656 H) to Daphnia magna STRAUS in a 48 hour static test 2008/1042207 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.2.4
KCA 8.2.4.1/2	Salinas E.	2010	[redacted] - Acute toxicity (immobilisation) study in the water flea Daphnia magna STRAUS 2010/1212802 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.2.4
KCA 8.2.4.2/1	Graves W.,Swigert J.	1996	SAN 1289H Technical: A 96-Hour Flow-Through Acute Toxicity Test With The Saltwater Mysid (Mysidopsis bahia) 1996/5413 Wildlife International Ltd., Easton MD, United States of America GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.2.4
KCA 8.2.5.1	Holmes c., Swigert J.	1992	SAN 582H: A flow-through life-cycle toxicity test with the cladoceran (Daphnia magna) Wildlife International Ltd., Easton, Maryland, USA 131A-147A BASF RegDoc.# 92/12455 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.5 [8.2/16]

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KCA 8.2.5.1	Jenkins, C. A.	1991	DOZ 300 H (SAN 582 H): *Daphnia magna* 21 day juvenile production test under semistatic conditions 91/SAS048/0981 BASF RegDoc# 91/11952 GLP, unpunblished	N	N	Not applicable	BASF	Y
KCA 8.2.6.1	Hoberg J.	1997	SAN 1289H Technical - toxicity to the freshwater green alga, Selenastrum capricornutum Springborn Laboratories, Inc., Wareham, MA, USA 96-11-6778 BASF RegDoc.# 97/5170 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.6 [8.2/17]
KCA 8.2.6.1	Thompson S., Peters G.	1991	SAN 582H: A 5-day toxicity test with the freshwater alga (selenastrum capricornutum) Wildlife International Ltd. 131A-126; WAT95-00677 BASF RegDoc.# 91/11915 GLP, unpublished	N	N	Not applicable	BASF	Y not relevant IIA. 8.8 [8.8/07]
KCA 8.2.6.1/1	Backfisch K.	2013	Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Chlamydomonas reinhardtii 2013/1078084 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/3	Backfisch K.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Planktosphaeria botryoides 2013/1078081 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/5	Backfisch K.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Monopaphidium griffithii 2013/1078078 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/6	Backfisch K.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Schroederia setigera 2013/1078077 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/8	Backfisch K.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Pseudokirchneriella subcapitata 2013/1078075 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6

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KCA 8.2.6.1/9	Backfisch K.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Ankistrodesmus bibraianus 2012/1246639 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/10	Backfisch K.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Desmodesmus subspicatus 2012/1246638 BASF SE, Limburgerhof, Germany Fed.Rep. GLP; unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/11	Backfisch K.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Neochloris aquatica 2012/1246637 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/12	Backfisch K.	2014	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Pseudokirchneriella subcapitata after different exposure durations 2013/1299405 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/13	Backfisch K., Kubitza J.	2014	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the green alga Monoraphidium griffithii after different exposure durations 2013/1299407 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/14	Hoffmann F.	2008	Effect of Reg.No. 360 712 (M31, metabolite of dimethenamid-P) on the growth of the green alga Pseudokirchneriella subcapitata 2008/1035874 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/15	Salinas E.	2011	[redacted] of BAS 656-PH, DMTA-P) - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV 2010/1079231 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6

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KCA 8.2.6.1/16	Salinas E.	2011	[redacted] - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV 2010/1154437 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/17	Salinas E.	2011	[redacted] - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV 2011/1255812 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.1/18	Salinas E.	2011	[redacted] - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV 2010/1185631 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.2	Hoberg J.	1997	SAN 1289H Technical - toxicity to the freshwater diatom, Navicula pelliculosa Springborn Laboratories, Inc., Wareham, MA, USA 96-11-6782; WAT1999-491 BASF RegDoc.# 97/5171 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.6 [8.2/19]
KCA 8.2.6.2	Gruetzner I.	1997	Dimethenamid metabolite M3: Acute toxicity to Scenedesmus subspicatus RCC,Itingen, CH RCC 628975; WAT1999-501 BASF RegDoc.# 97/10274 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.6 [8.2/20]
KCA 8.2.6.2	van der Kolk J.	1995	Dimethenamid oxalamid (M23): 72-hour static acute toxicity test with freshwater alga Selenastrum capricornutum Springborn Laboratories (Europe) AG, Horn, Switzerland 94-005-1018; WAT95-00669 BASF RegDoc.# 95/11320 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.6 [8.2/21]
KCA 8.2.6.2	van der Kolk J.	1995	Dimethenamid sulfonate sodium salt (M27): 72-hour static acute toxicity test with the freshwater alga Selenastrum capricornutum Springborn Laboratories (Europe) AG, Horn, Switzerland 94-008-1018; WAT95-00670 BASF RegDoc.# 95/11332 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.6 [8.2/22]

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KCA 8.2.6.2/1	Kubitza J.	2005	Amendment to study BASF DocID 1997/10745: SAN 1289H technical - Toxicity to the freshwater diatom, Navicula pelliculosa 2005/1003999 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.6.2/3	Kubitza J.	2004	Amendment to study BASF DocID 1997/10746: SAN 1289H technical - Toxicity to the freshwater green alga Selenastrum capricornutum 2004/1025684 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.6
KCA 8.2.7	Hoberg J.	1997	SAN 1289 H Technical - toxicity to duckweed, Lemna gibba Springborn Laboratories, Inc., Wareham, MA, USA 96-11-6787; WAT1999-492 BASF RegDoc.# 97/10742 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.2.8 [8.2/23]
KCA 8.2.7/1	Hoffmann F.	2008	Effect of Reg.No. 360712 (M31, metabolite of dimethenamid-P) on the growth of Lemna gibba 2008/1035918 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/2	Hoffmann F., Grund S.	2012	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of Lemna gibba after different exposure scenarios 2012/1084264 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/3	Hoffmann F.	2012	Report Amendment No. 1: Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of Lemna gibba after different exposure scenarios 2012/1202274 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/4	Backfisch K., Kubitza J.	2012	Effect of dimethenamid-P (BAS 656 H, Reg.No. 363851) on the growth of Lemna gibba in presence and absence of sediment 2012/1215555 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/5	Kubitza J., Grund S.	2013	Effect of BAS 656P H (dimethenamid-P, Reg.No. 363851) on the growth of Lemna gibba in different peak exposure scenarios 2013/1291744 BASF SE, Limburgerhof, Germany Fed.Rep. Not GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8

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KCA 8.2.7/6	Janson G.-M.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the aquatic plant Ceratophyllum demersum after different exposure durations 2013/1286175 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/7	Janson G.-M.	2013	Effect of BAS 656 H (dimethenamid-P, Reg.No. 363851) on the growth of the aquatic plant Glyceria maxima 2013/1286172 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/8	Swierkot A.	2013	Reg.No. 5749263 (metabolite of BAS 656 H, dimethenamid-P M656H055, M55) - Lemna gibba CPCC 310 growth inhibition test 2013/1063800 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/9	Swierkot A.	2013	Reg.No. 403121 (metabolite of BAS 656 H, dimethenamid-P, M39) - Lemna gibba CPCC 310 growth inhibition test 2013/1191249 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/10	Swierkot A.	2013	Reg.No. 5917262 (metabolite of BAS 656 H, dimethenamid-P, M43) - Lemna gibba CPCC 310 growth inhibitation test 2013/1191248 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/11	Kubitza J.	2004	Amendment to study BASF DocID 1997/10742: SAN 1289H technical - Toxicity to duckweed; Lemna gibba 2004/1025686 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/12	Kubitza J.,Dohmen G.-P.	2003	Effect of dimethenamid-P - Tested as formulated product - BAS 656 08 H - On emergent aquatic plants 2002/1012788 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/13	Kubitza J.	2013	Report amendment no 1: Effect of dimethenamid-P - Tested as formulated product BAS 656 08 H - On emergent aquatic plants 2013/1361973 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8

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KCA 8.2.7/14	Kubitza J.	2014	Report amendment no 2: Effect of dimethenamid-P - Tested as formulated product - BAS 656 08 H - On emergent aquatic plants 2014/1082325 BASF SE, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.2.7/15	Kubitza J., Dohmen G.P.	2003	Effect of dimethenamid-P - Tested as formulated product BAS 656 08 H - on submersed aquatic plants 2002/1012789 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.2.8
KCA 8.3.1.1	Donat H.J.	1986	Laboratory studies on the acute oral contact and oral toxicities of SAN 582H (technical) to worker honeybees SANDOZ Ltd., Agro Research, Witterswil, Switzerland 66'583 BASF RegDoc.# 86/11170 Not GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.3.1.1 [8.3/01]
KCA 8.3.1.1	Donat H.J.	1990	Amendment to document: Laboratory studies on the acute oral contact and oral toxicities of SAN 582H (as pure compound) to worker honeybees SANDOZ Ltd., Agro Research, Witterswil, Switzerland BASF RegDoc.# 90/11149 Not GLP, unpublished	N	N	Not applicable	BASF	Y not relevant IIA. 8.3.1.1 [8.3/02]
KCA 8.3.1.1.1/1	Zenker K.	2011	Acute toxicity of BAS 656-H (Reg.No. 363 851, dimethenamid-P) to the honeybee Apis mellifera L. under laboratory conditions 2010/1126065 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.3.1.1
KCA 8.3.1.1.1/2	Roehlig U.	2014	Acute toxicity of BAS 656 H (dimethenamid-P) to the bumblebee Bombus terrestris L. under laboratory conditions 2013/1275562 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.3.1.1
KCA 8.3.1.1.2/1	Zenker K.	2011	Acute toxicity of BAS 656-H (Reg.No. 363 851, dimethenamid-P) to the honeybee Apis mellifera L. under laboratory conditions 2010/1126065 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.3.1.1

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KCA 8.3.1.1.2/2	Roehlig U.	2014	Acute toxicity of BAS 656 H (dimethenamid-P) to the bumblebee Bombus terrestris L. under laboratory conditions 2013/1275562 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.3.1.1
KCA 8.3.1.3/1	Kleebaum K.	2014	Acute toxicity of BAS 656 H (dimethenamid-P) to honeybee larvae (Apis melifera L.) under laboratory conditions (*in vitro*) 2013/1132510 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.3.1.2
KCA 8.4/1	Van Dijk A.	1988	Acute toxicity (LC_{50}) study of SAN 582 H to earthworms RCC,Itingen, CH RCC 204614; ARW96-00062 BASF RegDoc.# 88/11372 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.4.1 [8.4/01]
KCA 8.4/2	Krieg W.	1998	Effect of M23 (dimethenamid-metabolite) on the mortality of the earthworm Eisenia foetida BASF Aktiengesellschaft, Limburgerhof, Germany 47842; ARW1999-48 BASF RegDoc.# 98/10299 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.4.1 [8.4/02]
KCA 8.4/3	Krieg W.	1998	Effect of M27 (dimethenamid-metabolite) on the mortality of the earthworm Eisenia foetida BASF Aktiengesellschaft, Limburgerhof, Germany 47843; ARW1999-49 BASF RegDoc.# 98/10300 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.4.1 [8.4/03]
KCA 8.4/4	Krome K.	2008	Acute toxicity (14 days) of Reg.No. 360712 (Metabolite of BAS 656 H, M31) to the earthworm Eisenia fetida in artificial soil RRA 12620; 080818BO; BASF RegDoc# 2008/1052695 Dr. U. Noack - Laboratorium fuer angewandte Biologie, Sarstedt, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.6
KCA 8.4.1/1	Friedrich S.	2012	Sublethal toxicity of BAS 656 H (dimethenamid-P) to the earthworm Eisenia fetida in artificial soil with 5 % peat 12 10 48 093 S; BASF RegDoc# 2012/1129456 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.4.2

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KCA 8.4.1/2	Luehrs U.	2007	Effects of Reg.No. 360715 on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5 % peat 37431022; BASF RegDoc# 2007/1037731 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.4.2
KCA 8.4.1/3	Luehrs U.	2007	Effects of Reg.No. 360714 on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5 % peat 37421022; BASF RegDoc# 2007/1037732 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.4.2
KCA 8.4.1/4	Luehrs U.	2009	Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the reproduction and growth of earthworms Eisenia fetida in artificial soil with 5 % peat 46551022; BASF RegDoc# 2008/1070910 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.4.2
KCA 8.4.2.1/1	Friedrich S.	2011	Effects of BAS 656 H (Reg.No. 363 851, dimethenamid-P) on the reproduction of the collembolans Folsomia candida 11 10 48 015 S; BASF RegDoc# 2011/1000481 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N
KCA 8.4.2.1/2	Friedrich S.	2011	Effects of Reg.No. 360 712 (metabolite of BAS 656 H, M31) on the reproduction of the collembolans Folsomia candida 10 10 48 110 S; BASF RegDoc# 2011/1000222 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N
KCA 8.4.2.1/3	Schulz L.	2012	BAS 656 H (dimethenamid-P) - Effects of BAS 656 H (dimethenamid-P) on the reproduction of the predatory mite Hypoaspis aculeifer 12 10 48 097 S; BASF RegDoc# 2012/1129457 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N

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KCA 8.4.2.1/4	Friedrich S.	2012	Reg.No. 360715 (metabolite of BAS 656 H, dimethenamid-P, M23) on the reproduction of the collembolan Folsomia candida 12 10 48 101 S; BASF RegDoc# 2012/1129536 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N
KCA 8.4.2.1/5	Schulz L.	2012	Effects of Reg.No. 360715 (metabolite of BAS 656 H, dimethenamid-P, M23) on the reproduction of the predatory mite Hypoaspis aculeifer 12 10 48 101 S; BASF RegDoc# 2012/1129538 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N
KCA 8.4.2.1/6	Friedrich S.	2012	Effects of Reg.No. 360714 (metabolite of BAS 656 H, dimethenamid-P, M27) on the reproduction of the collembolans Folsomia candida 12 10 48 105 S; BASF RegDoc# 2012/1129537 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N
KCA 8.4.2.1/7	Schulz L	2012	Effects of Reg.No. 360714 (metabolite of BAS 656 H, dimethenamid-P, M27) on the reproduction of the predatory mite Hypoaspis aculeifer 12 10 48 102 S; BASF RegDoc# 2012/1129539 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N
KCA 8.4.2.1/8	Schulz L.	2014	Effects of Reg.No. 360712 (Metabolite of BAS 656 H, dimethenamid-P) on the reproduction of the predatory mite Hypoaspis aculeifer 13 10 48 113 S; BASF RegDoc# 2013/1103674 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N
KCA 8.5	Danneberg G.	1991	Investigation on the effects of SAN 582 H on the activity of the microflora of soil Battelle Institute,Frankfurt, Germany BE-S-7-91-01-DEH-01; BMF1999-42; BASF RegDoc.# 91/11908 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.5 [8.5/01]

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KCA 8.5/1	Schulz L.	2008	Effects of Reg.No. 360715 (metabolite of BAS 656 H, M23) on the activity soil microflora (Nitrogen transformation test) 08 10 48 062 N; BASF RegDoc# 2008/1065117 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.5
KCA 8.5/2	Schulz L.	2008	Effects of Reg.No. 360 714 (metabolite of BAS 656 H, M27) on the activity of soil microflora (Nitrogen transformation test) 08 10 48 063 N; BASF RegDoc# 2008/1065119 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.5
KCA 8.5/3	Schulz L.	2008	Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the activity of soil microflora (Nitrogen transformation test) 08 10 48 064 N; BASF RegDoc# 2008/1065115 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N IIA. 8.5
KCA 8.6	Hoberg J.	1997	SAN 1289H formulation - Determination of effects on seedling emergence and vegetative vigor of ten plant species Springborn Laboratories, Inc., Wareham, MA, USA 96-12-6810; PFL 1999-17; BASF RegDoc.# 97/5175 GLP, unpublished	N	N	Not applicable	BASF	Y not relevant II A 8.6 [8.6/01]
KCA 8.6.2/1	Dutillie H.,Sack D.	2008	Effects of Reg.No. 360712 (M31, metabolite of BAS 656 H) on non-target plants in the greenhouse 353446; BASF RegDoc# 2008/1068011 BASF SE, Limburgerhof, Germany Fed.Rep. Not GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.6
KCA 8.6	Kaethner M.	1995	Screening test on the biological efficacy of dimethenamid soil metabolites M23 and M27 on higher plants SANDOZ Agro, Ltd., Basle, CH BASF RegDoc.# 95/11317 Not GLP, unpublished	N	N	Not applicable	BASF	Y Not relevant IIA. 3.5.2 [3.5/01]
KCA 8.6.2/1	N.N.		Title unknown Study summary provided by the applicant in document N4 of the renewal dossier	N		New data for AIR3 renewal	BASF	N

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KCA 8.7/1	Schulz L.	2008	Effects of Reg.No. 360715 (metabolite of BAS 656 H, M23) on the activity of soil microflora (Carbon transformation test) 08 10 48 062 C; BASF RegDoc# 2008/1065116 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.6
KCA 8.7/2	Schulz L.	2008	Effects of Reg.No. 360714 (metabolite of BAS 656 H, M27) on the activity of soil microflora (Carbon transformation test) 08 10 48 063 C; BASF RegDoc# 2008/1065118 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.6
KCA 8.7/3	Schulz L.	2008	Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the activity of soil microflora (Carbon transformation test) 08 10 48 064 C; BASF RegDoc# 2008/1065109 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, unpublished	N	Y	New data for AIR3 renewal	BASF	N II A 8.6
KCA 8.8	Scholtz R.	1994	Dimethenamid: Determination of the inhibitory effect on bacteria: Pseudomonas cell multiplication inhibition test MBT Umwelttechnik AG, Zurich, Switzerland 1994/11901; WAT1999-499; BASF RegDoc.# 94/11901 Not GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.7 [8.7/01]
KCA 8.8	Desmares-Koopmans M	1996	Activated sludge respiraion inhibition test with dimethenamid technical Notox B.V., s-Hertogenbosch, Netherlands 163136; WAT1999-500 BASF RegDoc.# 95/11327 GLP, unpublished	N	N	Not applicable	BASF	Y relevant IIA. 8.7 [8.7/02]