Volume 3

Annex B

Mandestrobin

B.9 Ecotoxicology

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B.9.1 Effects on birds (Annex II A 8.1, III A 10.1)

B.9.1.1 Acute oral toxicity (Annex II A 8.1.1)

Reference:	**S-2200 TG: An Acute Oral Toxicity Study with the Northern Bobwhite**
Author(s), year:	[redacted] 2009
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0001
Guideline(s):	US EPA OPPTS Guideline 850.2100; FIFRA Subdivision E, Section 71-1; JMAFF Test Guideline 12 Nohsan No. 8147
GLP:	Yes (self-certification)
Deviations:	The birds remaining at termination of the test were not subjected to gross necropsy due to scheduling oversight. This is not considered to have affected the validity of integrity of the study. Determination of treatment-related effects for birds surviving until test termination were based upon observations, body weight and feed consumption data. Sufficient data was available to assess the impact upon birds.
Validity:	Acceptable

Test substance:	S-2200 TG; Lot number ST-0811G, purity: 93.4%
Material and methods:	
Test species:	Northern bobwhite quail (*Colinus virginianus*)
Number of organisms:	5 females and 5 males per group
Weight, age:	172 – 232 g at test initiation, 22 weeks
Type of test:	Acute oral toxicity
Medication:	Beginning two days after their arrival in the test facility, test birds were given soluble antibiotics in their drinking water for seven consecutive days. The birds received no form of antibiotic medication during the test
Applied concentrations:	0 (corn oil vehicle only), 292, 486, 810, 1350 and 2250 mg ai/kg bw, dosage volume: dosage volume: 6 mL/kg body weight
Type of application:	Oral intubation directly into the crop or proventriculus of each bird using a stainless steel 14 gauge cannula, diluent: corn oil
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: 24.4 ± 0.3 °C (SD), relative humidity: 31 ± 9% (SD), lighting: 8L:(164 lux):16D, Feed was provided ad libitum during acclimation and during the test, except during periods of fasting prior to testing.
Test parameter:	
Observations:	During acclimation, all birds were observed daily. Following dosing, multiple observations were performed on Day 0 of the test, with particular attention being paid for signs of regurgitation. From test initiation until termination, observations of mortality, signs of toxicity and abnormal behaviour were performed at least twice daily.
Body weight:	Body weights were measured individually at test initiation and on days 3, 7 and 14 of the test.
Feed consumption:	Average feed consumption was determined by pen for each dosage group and the control group for Days 0-3, 4-7 and 8-14.
Necropsy:	No birds died. The birds that were remaining at the termination of the test were not subjected to gross necropsy, see deviations above.
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform

	the calculation of an LD_{50} using the computer program of C.E. Stephan. The LD_{50} value was determined to be greater than the highest dosage tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.
Findings:	
Mortalities:	No mortalities occurred in the control group in the 292, 486, 810, 1350 and 2250 mg ai/kg treatment groups.
Clinical signs:	All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.
Body weight, feed consumption:	Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the 292, 486, 810, 1350 or 2250 mg ai/kg treatment groups.
Conclusion:	LD_{50} > 2250 mg ai/kg bw, NOEL: 2250 mg ai/kg bw
Validity criteria:	Mortality in the control did not exceed 10%.

B.9.1.2 Dietary toxicity (Annex II A 8.1.2)

Reference:	**S-2200 TG: A Dietary LC50 Study with the Northern Bobwhite**
Author(s), year:	[redacted] 2009a
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report no.: ROW-0004
Guideline(s):	US EPA OPPTS Guideline 850.2200; FIFRA Subdivision E, Section 71-2; ASTM Standard E857-87; OECD Guideline 205
GLP:	Yes (self-certification)
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	S-2200 Technical Grade, purity: 93.4 %, Lot No.: ST-0811G
Material and methods:	
Test species:	Northern bobwhite quail (*Colinus virginianus*)
Number of organisms, Age, weight:	30 birds in six pens each for the control group, 10 birds in two pens for each of the test item groups, sex undetermined, 10 days old, 20 to 25 grams in weight at test initiation
Type of test:	Dietary toxicity
Medication:	The birds received no form of antibiotic medication during acclimation or the test.
Applied concentrations:	Diets were prepared by mixing the test substance with the basal ration fortified with 2% w/w corn oil. Dietary test concentrations were corrected to 100% active ingredient based upon the reported purity of the test substance. Nominal dietary test concentrations used in this study were 0, 562, 1000, 1780, 3160 and 5620 ppm ai S-2200 TG, corresponding to 0, 106, 192, 368, 635 and 1136 mg ai/kg bw/d.
Analytics:	Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.
Acclimatisation:	Birds were acclimated to the caging and facilities from the day of hatch until initiation of the test
Time of exposure:	5 days administration period and 3 days post-exposure period
Test conditions:	Test temperature: 27.9 ± 0.5°C (SD), relative humidity: 43 ± 7% (SD), lighting: 16L (194 lux) : 8D
Test parameter:	
Observations:	Birds were observed four times on day 0 and at least twice daily thereafter. All

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signs of toxicity and abnormal behaviour were recorded.

Body weight: Body weights were measured at test initiation and on days 3, on Day 5 and at termination of the test on Day 8.

Feed consumption: Average feed consumption values were determined daily during the exposure period (Days 0-5) and for the entire post-exposure observation period (Days 6-8) for each treatment and control group.

Necropsy: As no mortalities were observed in the study, necropsy was not deemed necessary.

Statistics: There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD50 using the computer program of C.E. Stephan. The LD50 value was determined to be greater than the highest concentration tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

Findings:

Mortalities: No mortalities occurred in the control group and in the 562, 1000, 1780, 3160 or 5620 ppm ai treatment groups.

Clinical signs: One control bird was observed limping on the morning of day 3. On Day 4, 5, 6 and the afternoon of Day 8 the bird and an additional bird in the same housing unit were noted with picked toes and as limping. One bird of the 1780 ppm ai treatment group was noted as limping with picked toes on Days 7 and 8 of the test. All other control and treatment birds were normal in appearance and behaviour throughout the test.

Body weight: Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the 562, 1000, 1780, 3160 and 5620 ppm ai test treatment groups

Feed consumption: No effects on feed consumption at any of the concentrations tested were noted.

Table B.9.1.2-1: Bodyweights and food consumption of bobwhite quail following dietary exposure to S-2200

S-2200 concentration (mg ai/kg diet)	Mean body weight [g/bird] (SD)			Body weight change [g/bird] (SD)		Mean food consumption [g/bird/day]	
	Day 0	Day 5	Day 8	Day 0-5	Day 0-8	Days 0-5	Days 6-8
Control	23 (3)	35 (4)	45 (4)	12 (3)	22 (3)	6	8
562	24 (2)	37 (3)	48 (3)	14 (2)	25 (2)	6	8
1000	22 (1)	34 (2)	43(3)	12 (1)	21 (3)	5	7
1780	23 (4)	35 (5)	45 (6)	12 (4)	22 (5)	6	11
3160	22 (2)	35 (3)	45 (3)	13 (2)	23 (2)	6	7
5620	22 (2)	34 (2)	45 (3)	13 (1)	24 (3)	6	8

Conclusion: LC_{50} > 5620 ppm, corresponding to > 1136 mg ai/kg bw/day;
No mortality level and no-observed-effect concentration = 5620 ppm, corresponding to 1136 mg ai/kg bw/day

Validity criteria: Mortality in the control did not exceed 10%. The test substance in the diet has been satisfactorily maintained in the diet (>80% of nominal) and no compound-related mortality or observable toxic effects were noted in the lowest treatment level.

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Reference:	**Analytical Method Verification for the Determination of S-2200 TG in Avian Diet**
Author(s), year:	Martin, K.H. and Nixon, W.B. (2009)
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0003
Guideline(s):	European Commission Working Document SANCO/3029/99 rev.4
GLP:	Yes (self-certification)
Deviations:	None of relevance
Validity:	Acceptable

Test material: Avian diet

Material and methods:

Analyte: S-2167 (*R*-isomer of Mandestrobin)
S-2354 (*S*-isomer of Mandestrobin)

Principle of the method: Active substance content is determined after extraction with acetonitrile and dilution to the appropriate concentration with acetonitrile/water (50:50 v/v) by HPLC with UV detection (variable wavelength detector at 230 nm).

Mobile phase: water / acetonitrile; gradient mode

Column: ChiralPak AD-RH, 5 µm, 150 x 4.6 mm

Findings:

Specifity: Due to the chiral nature of the column the method is very specific to the 2 isomers of Mandestrobin. No interference was observed in chromatograms of standard, control, reagent blank and matrix blank samples.

Calibration (Linearity): The linearity was checked with 6 standard solutions of different concentrations. The calibration is found to be linear in the range 0.2 – 10.0 mg/L with a correlation coefficient R > 0.9999 for both isomers.

Accuracy (recovery): The mean recovery was determined from the sum of both isomers found in fortified samples at 2 different levels. A known amount of Mandestrobin (0.1 g/kg and 6 g/kg) was added to the avian diet samples (5 samples each). The high fortified samples were diluted 1:100 the low fortified ones 1:5. The mean recovery is between 70 and 110 % for both fortifications. The results are listed below.

Precision: The repeatability of the method, expressed as relative standard deviation (RSD), was ≤ 20 % for each fortification level (see table below). No outliers were identified and/or discarded.

LOQ: The LOQ was set at the lowest fortification level i.e. 0.05 mg/L

Table B.9.1.2-2: Analytical determination of S-2200 TG in Avian diet

Analyte	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Sum of S-2167 and S-2354	100	100	103	2	5
		6000	101	1	5

Conclusion: The method is acceptable and validated successfully according to SANCO 3029/99 rev.4 with an LOQ for Mandestrobin (S-2200) of 100 mg/kg.

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Reference:	**S-2200 TG: A Dietary LC50 Study with the Mallard**
Author(s), year:	[redacted] (2009b)
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report no.: ROW-0005
Guideline(s):	Guideline: US EPA OPPTS 850.2200, US EPA FIFRA 71-2, ASTM Standard E857- 87, OECD 205
GLP:	Yes (self-certification)
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	S-2200 Technical Grade; Lot No.: ST-0811G, purity: 93.4%
Material and methods:	
Test species:	Mallard duck (*Anas platyrhynchos*)
Number of organisms, Age, weight	30 birds in six pens each for the control group, 10 birds in two pens for each of the test item groups, sex undetermined, 10 days old, 110 to 178 grams in weight at test initiation
Type of test:	Dietary toxicity
Medication:	The birds received no form of antibiotic medication during acclimation or the test.
Applied concentrations:	Diets were prepared by mixing the test substance with the basal ration fortified with 2% w/w corn oil. Dietary test concentrations were corrected to 100% active ingredient based upon the reported purity of the test substance. Nominal dietary test concentrations used in this study were 0, 562, 1000, 1780, 3160 and 5620 ppm ai S-2200 TG, corresponding to 0, 248, 436, 795, 1459 and 2460 mg ai/kg bw/d.
Analytics:	Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.
Acclimatisation:	All birds were acclimated to the caging and facilities from the day of receipt until initiation of the test, 8 days.
Time of exposure:	5 days administration period and 3 days post-exposure period
Test conditions:	Test temperature 24.3 ± 0.6°C (SD), relative humidity: 73 ± 6% (SD), lighting: 16L (220 lux):8D
Test parameter:	
Observations:	Birds were observed four times on day 0 and at least twice daily thereafter. All signs of toxicity and abnormal behaviour were recorded
Body weight:	Body weights were measured at test initiation and on days 3, on Day 5 and at termination of the test on Day 8.
Feed consumption:	Average feed consumption values were determined daily during the exposure period (Days 0-5) and for the entire post-exposure observation period (Days 6-8) for each treatment and control group.
Necropsy:	As no mortalities were observed in the study, necropsy was not deemed necessary.
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD_{50} using the computer program of C.E. Stephan. The LD_{50} value was determined to be greater than the highest concentration tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.
Findings:	
Mortalities:	No mortalities occurred in the control group and in the 562, 1000, 1780, 3160 or 5620 ppm ai treatment groups.
Clinical signs:	All birds in the control group and all treatment birds were normal in appearance

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and behaviour throughout the test.

Body weight: Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the 562, 1000, 1780, 3160 and 5620 ppm ai test treatment groups

Feed consumption: No effects on feed consumption at any of the concentrations tested were noted.

Table B.9.1.2-3: Bodyweights and food consumption of mallard duck following dietary exposure to S-2200

S-2200 concentration (mg ai/kg diet)	Mean body weight [g/bird] (SD)			Body weight change [g/bird] (SD)		Mean food consumption [g/bird/day]	
	Day 0	Day 5	Day 8	Day 0-5	Day 0-8	Days 0-5	Days 6-8
Control	138 (14)	279 (29)	395 (42)	141 (20)	116 (23)	83	122
562	142 (17)	291 (24)	395 (26)	149 (10)	105 (8)	95	149
1000	142 (19)	267 (27)	380 (33)	125 (12)	113 (14)	89	117
1780	141 (13)	271 (33)	392 (42)	130 (25)	121 (15)	92	136
3160	146 (15)	271 (22)	387 (29)	126 (14)	115 (15)	96	131
5620	140 (11)	265 (21)	375 (30)	125 (18)	110 (14)	89	109

Conclusion: LC_{50} >5620 ppm, corresponding to >2460 mg ai/kg bw/day;
No mortality level and no-observed-effect concentration = 5620 ppm, corresponding to 2460 mg ai/kg bw/day

Validity criteria: Mortality in the control did not exceed 10%. The test substance in the diet has been satisfactorily maintained in the diet (>80% of nominal) and no compound-related mortality or observable toxic effects were noted in the lowest treatment level.

B.9.1.3 Reproductive toxicity (Annex IIA 8.1.3)

Reference:	**S-2200: A Reproduction Study with the Northern Bobwhite**
Author(s), year:	[redacted] (2011a)
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0031
Guideline(s):	US EPA OPPTS Guideline 850.2300; FIFRA Subdivision E, Section 71-4; ASTM Standard E1062-86; OECD Guideline 206
GLP:	Yes (self-certification)
Deviations:	Two offspring, one from the 250 ppm ai treatment group and one from the 500 ppm ai treatment group could not be accounted for at 14-day old body weight measurements, as they could not be located. These offspring were assumed to have died and not included as 14-day old survivors. This deviation caused no adverse impact on the outcome of the study.
Validity:	Acceptable

Test substance: S-2200 Technical Grade, Lot No.: ST-0811G, purity: 93.4%

Material and methods:

Test species: Northern bobwhite quail (*Colinus virginianus*)

Number of organisms: 16 pens with one male and one female per test group

Weight, age: 168 - 212 g at test initiation, 17 weeks

Type of test: Reproductive toxicity

Applied concentrations: Control (untreated diet), 250, 500 and 1000 ppm ai

Analytics: Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.

Type of application: Test substance mixed in the diet, prepared weekly

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Phases of the study:	Acclimation: 3 weeks; Pre-photostimulation: 7 weeks; Pre-egg laying (with photostimulation): 3 weeks; Egg-laying: approximately 11 weeks; Post-adult termination (final incubation, hatching, and 14-day offspring rearing period): 6 weeks
Time of exposure:	Approximately 21 weeks (pre-photostimulation until termination)
Test conditions:	
Temperature and relative humidity:	Adult housing: 20.3 ± 1.5°C (SD), 33 ± 12% (SD) Storage room for eggs until incubation: 14.1 ± 0.1°C (SD), 70 ± 5% (SD) Incubator: 37.4 ± 0.0°C (SD), 55 ± 0% (SD) Hatching compartment: 37.3 ± 0.0°C (SD), 57 ± 1% (SD) Hatchling housing: 27.4 ± 1.2°C (SD), 20 ± 8% (SD)
Lightning:	Weeks 1-7: 8L:16D Week 8 onwards: photoperiod increased to 17 hours of light per day and was maintained at that length until the adult birds were euthanized. Hatchlings: 16L:8D Illuminance: 262 lux
Feeding	All adult birds and their offspring were given feed and water ad libitum during acclimation and testing. The basal diet contained at least 27% protein and 2.5% crude fat, and no more than 3.8% crude fibre. Additional 5% (w/w) of limestone (approximately 38.5% Ca) was added to the basal diet for the adults.
Test parameter:	
Observations:	During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained of all mortalities and clinical observations.
Adult Body weight:	Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8, and at adult termination.
Adult feed consumption:	Feed consumption for each pen was measured weekly throughout the test. Attempts were made to minimize feed wastage.
Egg parameter:	Eggs were collected daily from all pens, when available. At weekly intervals all eggs were candled and those with cracks or other abnormalities were recorded and discarded. Apart from those sacrificed for eggshell measurements, intact eggs were transferred to an incubator. Eggs were candled on Day 11-12 of incubation to determine embryo viability and on Day 21 to determine embryo survival. Egg shell thickness measurements were performed weekly throughout the egg laying period. The average thickness of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a micrometer. Measurements were made to the nearest 0.002 mm.
Hatchling parameter:	On day 25 or 26 of incubation body weights of surviving hatchlings were recorded and the group body weight by pen was determined. At 14 days of age, the body weights of surviving hatchlings were recorded and the average body weight by parental pen of all surviving chicks was determined.
Necropsy:	Adult birds that died or were euthanized during the course of the study were subjected to a gross necropsy. At the end of the exposure period, all surviving adult birds were euthanized with carbon dioxide gas and necropsied.
Statistics:	Analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the

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individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation for reproductive parameters.

Findings:

Mortalities: No mortalities occurred in the 250 and 500 ppm treatment groups. One incidental mortality occurred in the control group and one in the 1000 ppm group. No other mortalities occurred during the course of the study. Due to the nature of the lesions observed at necropsy, the mortalities were not considered to be related to treatment (control bird: feather loss, bruising on the back, severe head and neck laceration, pale kidneys, spleen and liver, pasty cecal contents; 1000ppm bird: lacerations of the head, neck and neck muscle, thin, mottled liver, pale spleen and kidneys).

Clinical signs: No overt signs of toxicity were observed at any of the concentrations tested. Incidental clinical observations noted during the test included those that normally are associated with injuries and penwear.

Body weight: No effects.

Feed consumption: There were no apparent treatment-related effects upon feed consumption at the 250, 500 and 1000 ppm ai test concentrations. No statistically significant differences between the control group and the 500 or 1000 ppm ai treatment groups were observed at any of the feed consumption intervals. At the 250 ppm ai test concentration there was a slight increase in mean feed consumption during Week 11 of the test that was statistically significant at $p < 0.01$. The increase was slight, not concentration responsive or consistent over time and was therefore not considered to be treatment-related.

Gross necropsy: The finding observed included feather loss, foot lesions, nodules on head, head lesions, pale liver, intra-abdominal egg remnants, egg yolk peritonitis, ovary regressing and small testes. No dose-response regime was noted and the findings were therefore not considered to be treatment related.

Reproductive results: There were no treatment-related effects upon reproductive performance at any of the concentrations tested including egg shell thickness and offspring body weights. Two pairs were separated and were not included in the reproductive parameters due to aggression to their penmates.

Table B.9.1.3-1: Summary of reproductive results in the bobwhite quail

Reproductive parameter	Test concentration (mg ai/kg diet)			
	control	250	500	1000
Number of Replicates	14	15	15	15
Parameter without statistical analysis				
Total eggs laid [a]	736	800	728	789
Eggs cracked	19	15	31	22
Eggs set	646	703	627	686
Viable embryos	528	642	594	579
Live 3-week embryos	523	638	593	573
Hatchlings	494	588	51	539
14-day old survivors	476	540	508	514
Eggs laid/hen	53	53	49	53
Eggs laid/hen/day	0.54	0.54	0.5	0.54
14-day old survivors/hen	34	36	34	34
Parameter statistically analysed [b]				
Mean eggshell thickness measurements [mm]	0.224	0.229	0.232	0.235

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Reproductive parameter	Test concentration (mg ai/kg diet)			
	control	250	500	1000
Mean hatchling bodyweight [g]	6	6	6	6
Mean 14-day old survivor bodyweight [g]	27	27	25	28
Eggs laid/maximum laid [%]	72	73	66	72
Eggs cracked/eggs laid [%]	3	3	4	3
Viable embryos/eggs set [%]	83	91	95	86
Live 3-week embryos/viable embryos [%]	99	99	100	99
Hatchlings/live 3-week embryos [%]	94	93	92	94
14-day old survivors/hatchlings [%]	95	92	92	95
Hatchlings/eggs set [%]	76	84	88	80
14-day old survivors/eggs set [%]	73	77	80	76
Hatchlings/maximum set [%]	54	55	56	54
14-day old survivors/maximum set [%]	52	55	51	52

[a] Represents the total number of eggs laid in each group
[b] Values represent pen means for experimental group

Conclusion: NOEC = 1000 ppm ai, the highest concentration tested, as no treatment effects on the adult birds or on reproductive parameters were observed, equivalent to 91.1 mg ai/kg bw/d, based on a mean bw of 198.0 g and a mean feed consumption of 18 g/bird/d.

Validity criteria: Mortality in the control did not exceed 10% at the end of the test. The mean eggshell thickness was 0.224 mm. The number of 14-day old survivors/hen in the control was 34. The test substance has been satisfactorily maintained (>80% of nominal) throughout the test period.

Reference: **S-2200: A Reproduction Study with the Mallard**

Author(s), year: [redacted] (2011b)

Report/Doc. number: Sumitomo Chemical Co. Ltd Report No.: ROW-0032

Guideline(s): US EPA OPPTS Guideline 850.2300; FIFRA Subdivision E, Section 71-4; OECD Guideline 206

GLP: Yes (self-certification)

Deviations: Two offspring from the 500 ppm ai treatment group could not be accounted for at 14-day old body weight measurements, as they could not be located. These offspring were assumed to have died and not included as 14-day old survivors. This deviation caused no adverse impact on the outcome of the study.

Validity: Acceptable

Test substance: S-2200 Technical Grade, Lot No.: ST-0811G, purity: 93.4%

Material and methods:

Test species: Mallard (*Anas platyrhynchos*)

Number of organisms: 16 pens with one male and one female per test group

Weight, age: 895 - 1343 g at test initiation, 18 weeks

Type of test: Reproductive toxicity

Applied concentrations: Control (untreated diet), 250, 500 and 1000 ppm ai

Analytics: Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.

Type of application: Test substance mixed in the diet, prepared weekly

Phases of the study: Acclimation: 3 weeks; Pre-photostimulation: 8 weeks; Pre-egg laying (with photostimulation): 2 weeks; Egg-laying: approximately 10 weeks; Post-adult termination (final incubation, hatching, and 14-day offspring rearing period): 6

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	weeks
Time of exposure:	Approximately 18 weeks (pre-photostimulation until termination)
Test conditions:	
Temperature and relative humidity:	Adult housing: 21.1 ± 0.7°C (SD), 36 ± 10% (SD) Storage room for eggs until incubation: 14.1 ± 0.1°C (SD), 77 ± 7% (SD) Incubator: 37.4 ± 0.0°C (SD), 55 ± 0% (SD) Hatching compartment: 37.3 ± 0.0°C (SD), 60 ± 0% (SD) Hatchling housing: 24.9 ± 1.1°C (SD), 52 ± 18% (SD
Lightning:	Weeks 1-8: 8L:16D Week 9 onwards: photoperiod increased to 17 hours of light per day and was maintained at that length until the adult birds were euthanized. Hatchlings: 16L:8D Illuminance: 235 lux
Feeding	All adult birds and their offspring were given feed and water ad libitum during acclimation and testing. The basal diet contained at least 27% protein and 2.5% crude fat, and no more than 3.8% crude fibre. Additional 5% (w/w) of limestone (approximately 38.5% Ca) was added to the basal diet for the adults.
Test parameter:	
Observations:	During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained of all mortalities and clinical observations.
Adult Body weight:	Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8, and at adult termination.
Adult feed consumption:	Feed consumption for each pen was measured weekly throughout the test.
Egg parameter:	Eggs were collected daily from all pens, when available. At weekly intervals all eggs were candled and those with cracks or other abnormalities were recorded and discarded. Apart from those sacrificed for eggshell measurements, intact eggs were transferred to an incubator. Eggs were candled on Day 14 of incubation to determine embryo viability and on Day 21 to determine embryo survival. Egg shell thickness measurements were performed weekly throughout the egg laying period. The average thickness of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a micrometer. Measurements were made to the nearest 0.002 mm.
Hatchling parameter:	On day 27 or 28 of incubation body weights of surviving hatchlings were recorded and the group body weight by pen was determined. At 14 days of age, the body weights of surviving hatchlings were recorded and the average body weight by parental pen of all surviving chicks was determined.
Necropsy:	Adult birds that died or were euthanized during the course of the study were subjected to a gross necropsy. At the end of the exposure period, all surviving adult birds were euthanized by cervical dislocation and necropsied.
Statistics:	Analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation for reproductive parameters.

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Findings:

Mortalities: One female bird died in the 500 ppm treatment group. The hen was noted as depressed, with a reduced reaction to external stimuli, lower limb weakness and wing droop on Day 0 of Week 20. The bird was euthanized and external examination indicated that the bird had a body weight of 879 g, foot lesions and some loss of muscle mass. Internally the female had lesions of egg yolk peritonitis in the abdominal cavity with milky off-white fluid throughout and the ovary was regressing. Additionally, the bird's spleen was enlarged and pale, kidneys pale, gizzard contents bile stained, the small intestines were distended with gas, the upper gastrointestinal tract was mostly empty with areas of hyperemia and intestinal wall thickening. Due to the nature of the lesions, the mortality that occurred was not considered to be related to treatment.
No other adult mortalities occurred during the test.

Clinical signs: No overt signs of toxicity were observed at any of the concentrations tested. Incidental clinical observations noted during the test included those that normally are associated with injuries and penwear.

Body weight: No effects.

Feed consumption: There were no apparent treatment-related effects upon feed consumption at the 250, 500 and 1000 ppm ai test concentrations. At the 1000 ppm test concentration there was a slight increase in mean feed consumption during Week 12 of the test that was statistically significant at $p < 0.05$. However, the increase was slight and not consistent over time and was therefore not considered to be treatment-related.

Gross necropsy: The finding observed included feather loss, foot lesions, grossly enlarged spleen, liver with small white foci or flecks, liver cyst, nodules in the abdominal cavity, slight, egg yolk peritonitis, hemorrhagic follicle, ovaries regressing and regressed and small testes. No clear dose-response regime was noted and the findings were therefore not considered to be treatment related.

Reproductive results: There were no treatment-related effects upon reproductive performance at any of the concentrations tested. At the 500 ppm test concentration, a slight reduction in egg production was evidenced. However, the slight reduction observed was attributable to two pens that produced no eggs. When data from these two pens were eliminated, the mean number of eggs laid by the 500 ppm treatment group was 35±11 eggs/hen, comparable to the control group value of 41 ± 13 eggs/hen.

Table B.9.1.3-2: Summary of reproductive results in the mallard

Reproductive parameter	Test concentration (mg ai/kg diet)			
	control	250	500	1000
Number of Replicates	16	16	15	16
Parameter without statistical analysis				
Total eggs laid [a]	653	616	457	555
Eggs cracked	1	2	0	3
Eggs set	585	538	404	498
Viable embryos	509	490	320	472
Live 3-week embryos	502	474	319	468
Hatchlings	415	380	267	390
14-day old survivors	410	375	265	389
Eggs laid/hen	41	39	30	35
Eggs laid/hen/day	0.5	0.47	0.37	0.42
14-day old survivors/hen	26	23	18	24
Parameter statistically analysed [b]				
Mean eggshell thickness measurements [mm]	0.396	0.383	0.389	0.396

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Reproductive parameter	Test concentration (mg ai/kg diet)			
	control	250	500	1000
Mean hatchling bodyweight [g]	34	34	35	35
Mean 14-day old survivor bodyweight [g]	294	284	297	300
Eggs laid/maximum laid [%]	65	61	48	55
Eggs cracked/eggs laid [%]	0	0	0	1
Viable embryos/eggs set [%]	88	88	78	94
Live 3-week embryos/viable embryos [%]	98	97	100	99
Hatchlings/live 3-week embryos [%]	80	80	82	82
14-day old survivors/hatchlings [%]	97	96	100	100
Hatchlings/eggs set [%]	70	68	62	75
14-day old survivors/eggs set [%]	68	67	62	75
Hatchlings/maximum set [%]	45	41	31	42
14-day old survivors/maximum set [%]	44	41	30	42

[a] Represents the total number of eggs laid in each group
[b] Values represent pen means for experimental group

Conclusion: NOEC = 1000 ppm ai, the highest concentration tested, as no treatment effects on the adult birds or on reproductive parameters were observed, equivalent to 129.1 mg ai/kg bw/d, based on a mean bw of 1100 g and a mean feed consumption of 142 g/bird/d.

Validity criteria: Mortality in the control did not exceed 10% at the end of the test. The mean eggshell thickness was 0.396 mm. The number of 14-day old survivors/hen in the control was 26. The test substance has been satisfactorily maintained (>80% of nominal) throughout the test period.

Comment RMS:

As stated in the study report the number of eggs laid in the 500 ppm treatment group was reduced in comparison to the control mainly due to two replicates without any egg production. However, the number of eggs laid in the 1000 ppm treatment group also was considerably lower than in the control. As indicated in the table above count-data were not statistically analysed. The notifier submitted a statement by the laboratory on request. Regarding the analyses of the data the following was stated [redacted], 2013): "[…] The number of eggs laid per pen is inherently non-normally distributed. A salient characteristic of the normal distribution is that it has long tails that extend to infinity in both directions. However, the distribution of eggs laid per pen per day is bounded at the lower end by 0 and by ca. 70 at the maximum, as it is extremely rare for a female to lay more than 1 egg a day, and egg laying goes on for ca. 70 days. The distribution of other counts (number of live embryos on day 21, number hatched, hatchling 14-day survival) that are dependent on the distribution of the number of eggs laid will also tend toward non-normality, as they are also bonded by 0 and ca. 70. An example of a truncated distribution that is commonly observed in statistical literature is the distribution of proportions that are bounded at 0 and 1. For such proportions, the most widely used normalizing transformation is the arcsine (square-root) transformation. To allow this transformation to be applied to the study data raw count data was converted to a proportion (i.e. a percentage) bounded by 0 and 1 by dividing the counts by the maximum possible value (i.e. the maximum number of eggs set from any pen). The conversion to a proportion is indicated in the name of the endpoint by adding "of maximum laid" or "of maximum set", so that analysing "hatchlings of maximum set" is exactly equivalent to analysing "number hatched". All data that was inherently a proportion or was converted to a proportion was transformed to meet assumption of normality, and no tests of normality or homogeneity of variance were performed."

The statement of the notifier is considered to be conclusive and no further analysis of the data is deemed necessary. The notifier further submitted egg production data from the control groups of the 21 mallard reproduction studies conducted closest in time to the study above. The mean values for eggs/hen were: 33, 50, 46, 54, 53, 39, 38, 48, 49, 57, 41, 49, 48, 32, 47, 53, 47, 50, 46, 32 and 44. The mean for all 21 studies was 46 (SD: 7). The control value of 41 eggs/hen is therefore below the mean of the reported 20 mallard studies. The mean for the 1000ppm treatment group lies in the range of the historical control data. In

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addition, of the 20 control groups, nine had at least one hen that was non-productive, and two of the control groups had two non-productive hens. Hence, non-productive hens are not unusual and could be eliminated from the analysis. This would shift the number of eggs/hen to 35 in the 500 ppm treatment group. In the 1000 ppm treatment group one hen only produced one egg and could also be classified as non-productive. Elimination of this replicate would shift the number of eggs/hen to 37, what is less than 10 % lower than in the control group.
In conclusion, the interpretation of the results should focus on the statistically analysed parameters of the study and the endpoint as stated in the original study report is considered to be acceptable.

B.9.1.4 Acute oral toxicity of the preparation to the more sensitive of the species identified in tests with the active substance (Annex IIIA 10.1.6)

No avian acute oral toxicity studies have been performed with S-2200 25SC. However, the acute LD_{50} value for S-2200 25SC to rats was greater than 2000 mg/kg bw and no mortalities or clinical signs occurred following administration of S-2200 25SC at 2000 mg/kg bw. There is consequently no evidence that the formulated product is more acutely toxic to mammals than the active substance and there are also no grounds to expect the formulated product to be more acutely toxic to birds than the technical active substance. Since S-2200 25SC is intended for spray application and contains a single active substance, the toxicity of the representative formulated product to birds is considered to be adequately predicted from information relating to the active substance without the need for additional studies.

B.9.1.5 Supervised cage or field trials

The Tier I risk assessment demonstrates that S-2200 poses a low risk to birds, and therefore further studies are not considered necessary.

B.9.1.6 Effects of secondary poisoning

No study required.

B.9.1.7 Summary of effects on birds

Table B.9.1.7-1: Summary of avian toxicity endpoints for S-2200

Study type	Test species	NOEL	LD_{50}	Reference
Acute oral	*Colinus virginianus*	2250 mg ai/kg bw	> 2250 mg ai/kg bw	[redacted] 2009
Short-term dietary toxicity	*Colinus virginianus*	1136 mg ai/kg bw/d	**> 1136 mg ai/kg bw/d**	[redacted] 2009a
	Anas platyrhynchos	2460 mg ai/kg bw/d	> 2460 mg ai/kg bw/d	[redacted] (2009b)
Long-term toxicity and reproduction	*Colinus virginianus*	**91.1 mg ai/kg bw/d**	/	[redacted] (2011a)
	Anas platyrhynchos	129.1 mg ai/kg bw/d	/	[redacted]. (2011b)

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B.9.1.8 Risk assessment for birds

Birds may be exposed to S-2200 by eating contaminated vegetation, seeds and fruits, invertebrate prey like arthropods (i.e. insects) or earthworms or vertebrate prey. Another possible route of uptake is via drinking water.

The first tier risk assessment was conducted as recommended in the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).
The calculations are based on a single application rate of 200 g ai/ha in oilseed rape. Possible other food items, for which no respective risk assessment is performed, are covered by the calculations presented because residues in these items are expected to be significantly lower.
According to the guidance document an acute and a reproductive risk assessment should be performed starting with a screening step which is based on an "indicator species" in order to identify all those substances that clearly pose a low risk for birds. For oilseed rape the indicator species is small omnivorous bird with a shortcut value for the acute assessment of 158.8 and for the reproductive assessment of 64.8. The daily dietary dose for acute exposure is calculated by multiplication of the application rate with the shortcut value. For the reproductive assessment a time weighted average factor (twa) can additionally be applied. If the toxic effect is considered to be caused by long term exposure the twa is 0.53 (based on an averaging interval of 21 days and a default DT_{50} of 10 days). As the NOEC values obtained from the long-term studies with birds were the highest tested concentration, there are no indications that any effects might have been caused by short-term exposure and it is justified to apply a twa of 0.53. A multiple application factor in order to account for the possible build-up of residues was not applied as the intended use only includes only application.
A short-term toxicity test with birds generally is no longer part of the core data package, but two short-term studies with quails and mallards are available for S-2200. According to the guidance document (EFSA, 2009) the dietary LD_{50} should be used where it is lower than the acute LD_{50}. In order to comply with this recommendation the LD_{50} of >1136 mg ai/kg bwd is used for the acute risk assessment. However, it should be noted that neither in the dietary nor in the acute studies any treatment related mortalities were observed.

The calculations of TER-values (toxicity exposure ratio) for the acute and the long-term exposure are presented in the tables below.

B.9.1.8.1 Acute toxicity exposure ratio (TER_A) for birds (OECD IIIA 10.1.1)

Table B.9.1.8.1-1: Acute exposure of birds to S-2200 - calculated toxicity exposure ratios, Screening step

Time scale	Crop, application rate	Indicator species	Endpoint [mg ai/kg bw]	Shortcut value	MAF	f_{twa}	DDD [mg/kg bw]	TER
acute	Oilseed rape 0.2 kg ai/ha	Small omnivorous bird	> 1136	158.8	-	-	31.76	> 36

MAF…multiple application factor; f_{twa}…time weighted average factor; DDD…daily dietary dose; TER…toxicity exposure ratio

The acute TER exceeds the trigger of 10 as specified in Commission Regulation (EU) No 546/2011, indicating a low risk to birds if S-2200 is used according to GAP.

B.9.1.8.2 Short-term (TER_{ST}) and long-term (TER_{LT}) toxicity exposure ratio for birds (OECD IIIA 10.1.2)

No short-term risk assessment is required under EFSA's Bird and Mammals Guidance Document (2009), as this is assumed to be covered by the acute and long-term risk assessments.

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Table B.9.1.8.2-1: Long-term exposure of birds to S-2200 - calculated toxicity exposure ratios, Screening step

Time scale	Crop, application rate	Indicator species	Endpoint [mg ai/kg bw]	Shortcut value	MAF	f_{twa}	DDD [mg/kg bw]	TER
Long-term	Oilseed rape 0.2 kg ai/ha	Small omnivorous bird	91.1	64.8	-	0.53	6.87	13

MAF…multiple application factor; f_{twa}…time weighted average factor; DDD…daily dietary dose; TER…toxicity exposure ratio

The long-term TER exceeds the trigger of 5 as specified in Commission Regulation (EU) No 546/2011, indicating a low long-term risk to birds if S-2200 is used according to GAP.

B.9.1.8.3 Higher tier risk assessment

No refined risk assessment is required.

B.9.1.8.4 Assessment of the risk from metabolites formed in potential food items

Plant metabolism studies have shown 4-OH-S-2200 (free and conjugated), 2-CH2OH-S-2200 (free and conjugated) and De-Xy-S-2200 to be major metabolites in crops, as they are found at levels exceeding 10% TRR. However, residue studies using S-2200 25 SC (S-2200, 250 g/L) confirmed that residue levels of 4-OH-S-2200 (free and conjugated), 2-CH2OH-S-2200 (free and conjugated) and De-Xy-S-2200 in oilseed rape seed are non-detectable.

B.9.1.8.5 Risk to birds from secondary poisoning

As the log P_{ow} of S-2200 is greater than 3 (3.51) the risk to birds and mammals from secondary poisoning was assessed according to Guidance of EFSA (2009)
The major metabolites in soil and water were determined to be 2-COOH-S-2200, 5-COOH-S-2200, S-2200-OR and S-2200-ORC with an estimated (according to KOWWIN, v.1.67 estimate) log P_{ow} of 2.53, 2.88, 3.30 and 4.02, respectively. Hence, the potential of the metabolites to bioaccumulate in the food chain is considered to be low for the soil metabolites 2-COOH-S-2200 and 5-COOH-S-2200. However, the potential of the metabolites S-2200-OR and S-2200-ORC to have an effect on fish eating birds has to be addressed. As no long-term studies with the metabolites are available, as a conservative approach they are assumed to be 10 times more toxic than the parent compound.

B.9.1.8.5.1 Food chain from earthworm to earthworm-eating birds

As the log P_{ow} of the metabolites in soil is below the trigger of 3, no assessment for earthworm eating birds is required. An assessment for birds exposed to the parent compound is presented below in accordance with the Guidance Document (EFSA, 2009).

$$TER = \frac{NOEL_{long-term}}{PEC_{worm} \times 1.05}$$

The factor of 1.05 is used to convert the residues in worms to a daily dose based on a bird of 100 g eating 104.6 g worms per day, according to Smit. (2005).

$$PECworm = PEC_{soil} \times BCF$$

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$$BCF(C_{worm}/C_{soil}) = \frac{0.84 + 0.012 \times K_{ow}}{f_{oc} \times K_{oc}}$$

The risk assessment was performed for the single application in oilseed rape.

Table B.9.1.8.5.1-1: Parameters and calculations for the assessment of the long-term risk to earthworm-eating birds in oilseed rape

Parameter	S-2200
$NOEL_{long\text{-}term}$ [mg ai/kg bw/d]	91.1
K_{oc} (Organic carbon adsorption coefficient)	449
K_{ow} (Octanol water partition coefficient)	3236
f_{oc} (Organic carbon content of soil)	default value: 0.02
PEC_{soil} (max) [mg ai/kg]	0.067
BCF_{worm}	4.42
PEC_{worm} [mg a.s./kg]	0.30
TER	289

The TER-value following use in oilseed rape is above the trigger of 5 for long-term risk, indicating that the use of S-2200 poses a low risk to earthworm-eating birds.

B.9.1.8.5.2 Food chain from fish to fish-eating birds

The risk to fish-eating birds from bioaccumulation of S-2200 is calculated with the following equations in accordance with the Guidance Document (EFSA, 2009). Not only the BCF but also the toxicity for the metabolites is assumed to be 10 times higher than for the parent compound as a worst case approach.

$$TER = \frac{NOEL_{long\text{-}term}}{PEC_{fish} \times 0.159}$$

The factor of 0.159 is used to convert the residues in fish to a daily dose based on a bird of 1000 g eating 159 g per day, according to Smit (2005).

$$PEC_{fish} = PEC_{water} \times BCF$$

The risk assessment is based on the use in oilseed rape. As a worst-case approach the highest PEC_{water} without an ftwa was used.

Table B.9.1.8.5.2-1: Parameters and calculations for the assessment of the long-term risk to fish-eating birds in oilseed rape

Parameter	S-2200	S-2200-OR	S-2200-ORC
$NOEL_{long\text{-}term}$ [mg ai/kg bw/d]	91.1	9.1[a]	9.1[a]
PEC_{water} (Maximum according FOCUS step 1) [mg ai/L]	0.04354	0.00104	0.0008
BCF_{fish}	26	260[b]	260[b]
PEC_{fish} [mg ai/kg]	1.13	0.27	0.21
TER	506	212	273

[a] assumed to be 10 times more toxic than the parent compound
[b] BCF for the metabolites is assumed to be 10 times higher than for the parent compound

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The TER-values following use in oilseed rape are above the long-term trigger of 5, indicating that the use of S-2200 poses a low risk to fish-eating birds.

B.9.1.8.5.3 Biomagnification in terrestrial food chains

Although no residue studies are available for S-2200 in birds the ADME studies conducted with rats and a bioaccumulation study in fish demonstrate that S-2200 has a low potential to bioaccumulate and biomagnify. According to the ADME studies in rats S-2200 is widely distributed throughout the body. After single oral administration there was no major difference in distribution between high and low doses (1000 and 5 mg/kg bw, respectively) or between sexes. The major tissue residues were seen in the gastrointestinal tract, and in liver and kidney, as well as in uterus and ovaries at 168 hours after dosing. There was no evidence of accumulation into tissues. S-2200 was extensively metabolised to numerous metabolites (unchanged parent formed <0.2% of administered dose at low dose). The primary routes of metabolism were by oxidation and subsequent conjugation with glucuronic acid, demethylation with subsequent oxidation, or oxidation with subsequent demethylation.

In addition, metabolism studies in laying hens and lactating goats showed that the metabolic pathways in livestock were similar to that found in the rat. S-2200 is extensively metabolised and readily excreted in the hen and goat. Residue levels were low in eggs, milk, muscle and fat, and higher in liver and kidney. Parent S-2200 was the main component of the residue in eggs, milk fat, muscle (goat) and fat (hen and goat), and the main metabolites were 5-COOH-S-2200 (goat kidney and liver), 4-OH-S-2200 (in hen liver and as the glucuronide in goat kidney) and De-Xy-S-2200 (hen liver). S-2200 has a log Pow of 3.51, however as S-2200 is extensively metabolised, no significant accumulation of residues in tissues, particularly fatty tissues, was seen in the livestock metabolism studies.

B.9.1.8.6 Risk to birds from consumption of contaminated drinking water

The EFSA guidance document recommends two scenarios to assess the risk for birds via the uptake of contaminated drinking water. Both scenarios refer to small and smallest water reservoirs, namely pools in leaf whorls and puddles on soil.

Leaf scenario: Birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation.
Puddle scenario: Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil.

A leaf scenario is clearly the worst-case situation. It is relevant for spray applications only and should be considered for the following crop types and growth stages:

Leaf vegetables (forming heads) at principal growth stage 4 until harvest (classification according to BBCH52).
Other leaf vegetables (e.g. cauliflower) at principal growth stage 4 or later, with a morphology that facilitates collection of rain/irrigation water in reservoirs that are large enough and easily accessible to attract birds and sufficiently stable over some hours.

According to the recommendation given above the puddle scenario is considered to be relevant for the intended use in oilseed rape.
However, due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals (see below), no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the

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case of less sorptive substances (K_{oc} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500 L/kg).
A comparison of the relevant endpoints with the effective application rate for S-2200 is presented below.

Table B.9.1.8.6-1: Parameters and calculations for the assessment of the risk to birds drinking contaminated water

Crop	Exposure Scenario	Effective Application Rate [g ai/ha]	K_{oc} [L/kg]	LD_{50} / NOEL [mg ai/kg bw]	Ratio Application Rate:Endpoint
Oilseed rape	Acute	200	449	> 1136	< 0.18
	Long-term	200		91.1	< 2.2

As the ratio for S-2200, a less sorptive substance, is below 50, the risk is indicated to be acceptable. The risk from metabolites formed in the water is likely to be covered by the safety margins for the active ingredient.

B.9.1.8.7 Overall conclusions

The available data on avian toxicity of the active substance S-2200 and the risk assessment indicate an acceptable risk to birds via dietary exposure. In addition, the risk to birds from metabolites formed in potential food items, bioaccumulation and via drinking water is also considered to be low.

B.9.2 Effects on aquatic organisms (Annex IIA 8.2, Annex IIIA 10.2)

The results of the aquatic toxicity studies are based on mean measured concentrations irrespective of whether or not the mean measured concentrations were in the range of 80 – 120% of nominal concentrations. This approach was accepted by the RMS as the results based on mean measured concentrations are considered to be worst-case and more realistic than nominal concentrations.

B.9.2.1 Acute toxicity of active substance, metabolites and formulations to aquatic organisms

B.9.2.1.1 Acute toxicity to fish (Annex IIA 8.2.1, IIIA 10.2.1)

Active substance

Reference:	**S-2200 Technical Grade – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**
Author(s), year:	[redacted] 2009a
Report/Doc. number:	Report No. ROW-0007, Study No. 13048.6622
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	The protocol states that the average total length and weight of fish used for testing will be 4 to 6 cm and 0.5 to 2.0 g, respectively. The fish used during this study were an average length and weight of 3.4 cm and 0.39 g, respectively. Since the fish used for the preliminary exposure were larger in size than those used for the definitive testing and the biological response of the definitive exposure was consistent with preliminary results, this deviation is not considered to have had a negative impact on the results or interpretation of the study.
Validity:	Acceptable

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Material and methods:

Test substance:	S-2200 technical grade, purity: 93.4%, batch: ST-0811G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 60020652
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length (mean):	0.39 g (range 0.29 – 0.51 g) and 34 mm (range 30 – 38 mm), n = 30
Loading:	0.26 g fish/L solution
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.31, 0.63, 1.3, 2.5 and 5.0 mg ai/L
Measured (mean):	- (control and solvent control), 0.33, 0.57, 1.3, 2.6 and 5.3 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Well water, total hardness: 46 mg/L as $CaCO_3$, total alkalinity: 22 mg/L as $CaCO_3$
Temperature:	13 - 15 °C (recommended 13 – 17 °C)
pH:	6.8 – 6.9 (0 h, new solution), 7.2 – 7.5 (96 h, aged solution)
O_2 content:	6.4 – 11.0 mg O_2/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2200 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Binominal probability, NOEC: Directly from raw data

Findings:

Analytical data: Since S-2200 is a mixture of two isomers, S-2354 and S-2167, the mean recovery of S-2200 was calculated by the addition of the results of the analysis for S-2354 and S-2167 from aqueous solutions.
Over the whole test period the mean measured concentrations were in the range from 91 – 110% of nominal. See Table B.9.2.1.1-1

Table B.9.2.1.1-1 Concentrations measured during the 96 h static acute exposure of fish to S-2200 technical grade

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]							Percent of nominal [%]
	0-hour			96-hour			Mean	
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.021	< 0.021	< 0.042	< 0.021	< 0.021	< 0.042	n.a.	n.a.
Solvent control	< 0.021	< 0.021	< 0.042	< 0.021	< 0.021	< 0.042	n.a.	n.a.
0.31	0.16	0.21	0.37	0.11	0.18	0.30	0.33	110
0.63	0.32	0.31	0.63	0.24	0.30	0.54	0.57	91
1.3	0.70	0.65	1.4	0.65	0.62	1.3	1.3	100
2.5	1.4	1.2	2.6	1.3	1.3	2.6	2.6	110
5.0	2.6	2.4	5.0	2.8	2.6	5.5	5.3	110

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Behavioural effects: Controls and concentration levels up to 0.57 mg ai/L: No sublethal effects were

reported over the whole test period. At test concentration 1.3 mg ai/L following symptoms were noted after 6 hours: Lethargic behaviour, partial and complete loss of equilibrium, fish on bottom of the test vessel. After 24 hours first appearance of fish mortality. At test concentration 2.6 and 5.3 mg ai/L: 100% fish mortality after 6 hours exposure.
Thus the NOEC was 0.57 mg ai/L based on sublethal effects.

Mortality: See Table B.9.2.1.1-2

Table B.9.2.1.1-2: Effects on rainbow trout (*O. mykiss*) exposed to technical S-2200

S-2200 [mg ai/L] (mean measured)	Cumulative mean mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.33	0	0	0	0	0
0.57	0	0	0	0	0
1.3	0 [ab]	25 [bcd]	75 [bcd]	90 [a]	90 [ac]
2.6	100	100	100	100	100
5.3	100	100	100	100	100
NOEC = 0.57 mg ai/L					
LC_{50} (96 h) = 0.94 mg ai/L (95 % C.I. 0.57 – 1.3 mg ai/L)					

[a] lethargic behaviour
[b] complete loss of equilibrium
[c] fish on bottom of the test vessel
[d] partial loss of equilibrium

Conclusion: 96 h LC_{50} = 0.94 mg ai/L
96 h NOEC = 0.57 mg ai/L
based on mean measured concentrations.

Validity criteria: The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. Additionally, the dissolved oxygen concentration was measured to be above 60% air saturation (recommended at least 60%).

Reference: **S-2200 Technical Grade – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**

Author(s), year: [redacted] 2009d
Report/Doc. number: Report No. ROW-0008, Study No. 13048.6624
Guideline(s): OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP: Yes
Deviations: The protocol states that the average total length and weight of fish used for testing will be 1 to 3 cm and 0.5 to 3.0 g, respectively. The fish used during this study were an average length and weight of 2.8 cm and 0.36 g, respectively. Since the fish used for the preliminary exposure were within the ranges specified in the protocol and the biological response of the definitive exposure was consistent with preliminary results, this deviation is not considered to have had a negative impact on the results or interpretation of the study.

The protocol states that the test substance used is S-2200 TG (batch ST-0811G). This material was used on all occasions except the QC (quality control) preparation at the 0-hour interval. The material used for QC preparation at 0-hour

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	only was S-2200 PAI (batch 081103G, purity 100%). Since all QC samples were adjusted for purity and QC recoveries were acceptable, this interval was not negatively impacted by this deviation.
Validity:	Acceptable

Material and methods:

Test substance:	S-2200 technical grade, purity: 93.4%, batch: ST-0811G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 60020652
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length (mean):	0.36 g (range 0.15 – 0.54 g) and 28 mm (range 20 – 33 mm), n = 30
Loading:	0.24 g fish/L solution
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.31, 0.63, 1.3, 2.5 and 5.0 mg ai/L
Measured (mean):	- (control and solvent control), 0.29, 0.56, 1.2, 2.2 and 4.3 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Well water, total hardness: 64 mg/L as $CaCO_3$, total alkalinity: 20 mg/L as $CaCO_3$
Temperature:	21 - 22 °C (recommended 21 – 25 °C, OECD 203)
pH:	6.7 – 7.0 (0 h, new solution), 7.2 – 7.4 (96 h, aged solution)
O_2 content:	5.5 – 8.5 mg O_2/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2200 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Binominal probability, probit analysis, NOEC: Directly from raw data

Findings:

Analytical data:	Since S-2200 is a mixture of two isomers, S-2354 and S-2167, the mean recovery of S-2200 was calculated by the addition of the results of the analysis for S-2354 and S-2167 from aqueous solutions. Over the whole test period the mean measured concentrations were in the range from 86 – 94% of nominal. See Table B.9.2.1.1-3

Table B.9.2.1.1-3 Concentrations measured during the 96 h static acute exposure of fish to S-2200 technical grade

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]							
	0-hour			96-hour			Mean	Percent of nominal [%]
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.01	< 0.01	< 0.02	< 0.01	< 0.01	< 0.02	n.a.	n.a.
Solvent control	< 0.01	< 0.01	< 0.02	< 0.01	< 0.01	< 0.02	n.a.	n.a.
0.31	0.14	0.16	0.30	0.12	0.16	0.28	0.29	93
0.63	0.30	0.30	0.60	0.26	0.26	0.53	0.56	89
1.3	0.64	0.64	1.3	0.57	0.56	1.1	1.2	94
2.5	1.1	1.2	2.3	1.1	1.1	2.1	2.2	89
5.0	2.1	2.2	4.3	2.1	2.2	4.3	4.3	86

n.a…not applicable

WARNING: This document forms part of an EC evaluation and should not be used in isolation. Registration must not be granted on the basis of this document.

[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Behavioural effects: Control: At the end of the study (96 h) one fish died. No behavioural effects were observed.
Solvent control: At the end of the study one fish was observed to be at the bottom of the test vessel. No other behavioural effects were observed.
Concentration levels up to 0.56 mg ai/L: No sublethal effects were reported over the whole test period. At test concentration 1.2 mg ai/L two fish (10%) died at the end of the study. No behavioural effects were observed. At test concentration 2.2 following symptoms were noted after 6 hours: partial and complete loss of equilibrium, fish on bottom of the test vessel, fish at the surface of the solution, darkened pigmentation. After 6 hours first appearance of fish mortality. At the highest test concentration 100% fish mortality was observed after 6 hours exposure.
Thus the NOEC was 0.56 mg ai/L based on sublethal effects.

Mortality: See Table B.9.2.1.1-4

Table B.9.2.1.1-4: Effects on bluegill sunfish (*L. macrochirus*) exposed to technical S-2200

S-2200 [mg ai/L] (mean measured)	Cumulative mean mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	5
Solvent control	0	0	0	0	0 [b]
0.29	0	0	0	0	0
0.56	0	0	0	0	0
1.2	0	0	0	0	10
2.2	5	10 [abc]	30 [cd]	30 [c]	30 [ce]
4.3	100	100	100	100	100
NOEC = 0.56 mg ai/L					
LC_{50} (96 h) = 2.3 mg ai/L (95 % C.I. 1.9 – 2.8 mg ai/L)					

[a] fish at the surface of the solution
[b] fish on bottom of the test vessel
[c] partial loss of equilibrium
[d] complete loss of equilibrium
[e] darkened pigmentation

Conclusion: 96 h LC_{50} = 2.3 mg ai/L
96 h NOEC = 0.56 mg ai/L
based on mean measured concentrations.

Validity criteria: One fish (5%) died in the control group at the end of the test which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. Additionally, the dissolved oxygen concentration was measured to be above 60% air saturation (recommended at least 60%).

Reference: **S-2200 Technical Grade – Acute Toxicity to Fathead Minnow (*Pimephales promelas*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**

Author(s), year: [redacted] 2009e
Report/Doc. number: Report No. ROW-0009, Study No. 13048.6625
Guideline(s): OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP: Yes

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Deviations:	The protocol states that the average total length of fish used for testing will be 1 to 3 cm. The fish used during this study were an average length of 3.6 cm. Since loading rate and control performance were within protocol requirements, this deviation is not considered to have had a negative impact on the results or interpretation of the study. The protocol states that the test temperature will be maintained at 22 ± 1 °C. During this exposure, the minimum/maximum temperature ranged from 23 to 24 °C. This deviation did not have a negative impact on the results or interpretation of the study. The protocol states that the total dissolved oxygen concentrations will not be allowed to drop below 60% of saturation during the test. During the 24 hour observation interval, the dissolved oxygen levels ranged from 36 to 111% of saturation. Gentle, oil-free aeration was initiated to raise and maintain appropriated dissolved oxygen levels. During the 48 hour observation interval, dissolved oxygen levels in control replicate A dropped to 55% of saturation. The aeration was increased slightly in this replicate to correct the brief drop in dissolved oxygen. Since control performance exceeded protocol requirements, this slight deviation did not have a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	S-2200 technical grade, purity: 93.4%, batch: ST-0811G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 60020652
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length (mean):	0.71 g (range 0.52 – 1.1 g) and 36 mm (range 32 – 41 mm), n = 30
Loading:	0.47 g fish/L solution
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.19, 0.38, 0.75, 1.5 and 3.0 mg ai/L
Measured (mean):	- (control and solvent control), 0.15, 0.36, 0.72, 1.5 and 3.0 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Well water, total hardness: 46 mg/L as $CaCO_3$, total alkalinity: 22 mg/L as $CaCO_3$
Temperature:	22 – 23 °C
pH:	6.7 – 6.8 (0 h, new solution), 7.3 – 7.4 (96 h, aged solution)
O_2 content:	3.1 – 8.4 mg O_2/L (< 60% air saturation) After 24 hours of exposure a decrease of air saturation below 60 % was observed in the controls (negative and solvent) and the treatment groups up to a test concentration of 0.75 mg ai/L. Therefore, aeration with gentle, oil-free air was initiated at 24 hours of exposure to maintain dissolved oxygen concentration at or above 60% saturation.
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2200 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was

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continuously monitored throughout the study in one replicate.

Statistics: LC_{50}: Binominal probability, NOEC: Directly from raw data

Findings:

Analytical data: Since S-2200 is a mixture of two isomers, S-2354 and S-2167, the mean recovery of S-2200 was calculated by the addition of the results of the analysis for S-2354 and S-2167 from aqueous solutions.
Over the whole test period the mean measured concentrations were in the range from 81 – 100% of nominal. See Table B.9.2.1.1-5

Table B.9.2.1.1-5 Concentrations measured during the 96 h static acute exposure of fish to S-2200 technical grade

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]						Mean	Percent of nominal [%]
	0-hour			96-hour				
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.013	< 0.013	< 0.025	< 0.013	< 0.013	< 0.025	n.a.	n.a.
Solvent control	< 0.013	< 0.013	< 0.025	< 0.013	< 0.013	< 0.025	n.a.	n.a.
0.19	0.083	0.075	0.16	0.067	0.082	0.15	0.15	81
0.38	0.19	0.18	0.37	0.16	0.19	0.35	0.36	95
0.75	0.39	0.37	0.76	0.31	0.38	0.69	0.72	97
1.5	0.71	0.66	1.4	0.71	0.84	1.5	1.5	97
3.0	1.4	1.3	2.7	1.8	1.6	3.4	3.0	100

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Behavioural effects: Controls and concentration levels up to 0.36 mg ai/L: No sublethal effects were reported over the whole test period. At test concentration 0.72 mg ai/L following symptoms were noted after 6 hours: lethargic behaviour. At test concentration 1.5 mg ai/L following symptoms were noted after 6 hours: lethargic behaviour, partial and complete loss of equilibrium, fish on bottom of the test vessel. After 48 hours 100% fish mortality was observed.
Thus the NOEC was 0.36 mg ai/L based on sublethal effects.

Mortality: See Table B.9.2.1.1-6

Table B.9.2.1.1-6: Effects on fathead minnow (*P. promelas*) exposed to technical S-2200

S-2200 [mg ai/L] (mean measured)	Cumulative mean mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.15	0	0	0	0	0
0.36	0	0	0	0	0
0.72	0 [a]	0	0	0	0
1.5	30 [abcd]	95	100	100	100
3.0	100	100	100	100	100
NOEC = 0.36 mg ai/L					
LC_{50} (96 h) = 1.0 mg ai/L (95 % C.I. 0.72 – 1.5 mg ai/L)					

[a] lethargic behaviour
[b] fish on bottom of the test vessel
[c] partial loss of equilibrium
[d] complete loss of equilibrium

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Conclusion:	96 h LC_{50} = 1.0 mg ai/L 96 h NOEC = 0.36 mg ai/L based on mean measured concentrations.
Validity criteria:	The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. The dissolved oxygen concentration was measured to be below 60% air saturation (recommended at least 60%). After 24 hours of exposure a decrease of air saturation was observed in the controls and in the treatment groups up to a test concentration of 0.72 mg ai/L. Therefore gentle, oil-free aeration was intiated to raise and maintain dissolved oxygen levels at or above 60 % saturation. The study is considered acceptable. The decrease of air saturation after 24 hours of exposure is considered not relevant for the interpretation of the study as no adverse effects on fish were observed. In the highest test concentration the air saturation was above 60 % over the whole exposure period.

Reference:	**S-2354 (S-Isomer of S-2200) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**
Author(s), year:	[redacted] 2009b
Report/Doc. number:	Report No. ROW-0011, Study No. 13048.6621
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	The protocol states that the average total length and weight of fish used for testing will be 4 to 6 cm and 0.5 to 2.0 g, respectively. The fish used during this study were an average length and weight of 3.4 cm and 0.39 g, respectively. Since the water quality measurements, loading rate, and control performance were within protocol requirement, this deviation is not considered to have had a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7 %, batch: 60020653
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length (mean):	0.39 g (range 0.29 – 0.51 g) and 34 mm (range 30 – 38 mm), n = 30
Loading:	0.26 g fish/L solution
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 1.9, 3.8, 7.5, 15.0 and 30.0 mg ai/L
Measured (mean):	- (control and solvent control), 1.7, 3.1, 6.5, 12.0 and 9.6 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Well water, total hardness: 50 mg/L as $CaCO_3$, total alkalinity: 22 mg/L as $CaCO_3$
Temperature:	14 - 15 °C
pH:	6.7 – 6.8 (0 h, new solution), 7.1 – 7.3 (96 h, aged solution)
O_2 content:	6.4 – 10 mg O_2/L (> 60 % air saturation)
Light regime:	16 hours light / 8 hours darkness

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must be granted on the basis of this document.

Test parameters: Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2354 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.

Statistics: LC_{50}: Empirically estimated (no concentration tested resulted in ≥ 50 % mortality), NOEC: Directly from raw data

Findings:

Analytical data: Over the whole test period the mean measured concentrations were in the range from 32 – 88 % of nominal concentrations.
Since there was undissolved test substance present at the highest treatment level tested (i.e., 30 mg ai/L nominal, 9.6 mg ai/L measured), the water accommodated fraction was siphoned and used for the exposure solution. The analytical recovery for this solution was not consistent with the range of concentrations due to the functional limit of solubility.

Behavioural effects: Controls and concentration levels up to 3.1 mg ai/L: No sublethal effects were reported over the whole test period. At test concentration between 6.5 and 12.0 mg ai/L following symptoms were noted after 6 hours: partial and complete loss of equilibrium, lethargic behaviour, only slight gill movement, fish on bottom of test vessel.
Thus the NOEC was 3.1 mg ai/L based on sublethal effects.

Mortality: See Table B.9.2.1.1-7

Table B.9.2.1.1-7: Effects on rainbow trout (*O. mykiss*) exposed to S-2354 (S-2200 *S*-isomer)

S-2354 [mg ai/L] (mean measured)	Cumulative mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
1.7	0	0	0	0	0
3.1	0	0	0	0	5 [b]
6.5	0 [a]	0 [bcd]	0 [bcd]	0 [bd]	0 [bd]
12.0	0 [b]	10 [bc]	10 [bce]	10 [bc]	20 [bc]
9.6	0 [bc]	0 [bc]	0 [bc]	0 [bc]	0 [b]
NOEC = 1.7 mg ai/L					
LC_{50} (96 h) > 12 mg ai/L					

[a] lethargic behaviour
[b] fish on the bottom of the test vessel
[c] complete loss of equilibrium
[d] partial loss of equilibrium
[e] only slight gill movement

Conclusion: 96 h LC_{50} > 12.0 mg ai/L
96 h NOEC = 1.7 mg ai/L
based on mean measured concentrations.

Validity criteria: The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. Additionally, the dissolved oxygen concentration was measured to be above 60% air saturation (recommended at least 60%).

Comment RMS: The solubility of the active substance in water is 15.8 mg/L at 20°C (100% radiochemical purity) which is below the highest tested concentration of 30 mg/L S-2354. Considering the observed treatment related effects on mortality, the study is considered acceptable. In addition, the study is not used in the risk assessment

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as the risk assessment for the isomers is based on the results of the laboratory studies with the technical active substance (racemic mixture).

Reference:	**S-2167 (*R*-Isomer of S-2200) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**
Author(s), year:	[redacted] 2009c
Report/Doc. number:	Report No. ROW-0010, Study No. 13048.6623
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	S-2167 (S-2200 *R*-isomer), purity: 100 %, batch: 60020652, CAS No.: 394657-24-0
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length (mean):	0.39 g (range 0.29 – 0.51 g) and 34 mm (range 30 – 38 mm), n = 30
Loading:	0.26 g fish/L solution
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.19, 0.38, 0.75, 1.5 and 3.0 mg ai/L
Measured (mean):	- (control and solvent control), 0.17, 0.34, 0.72, 1.4 and 2.9 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Well water, total hardness: 50 mg/L as $CaCO_3$, total alkalinity: 22 mg/L as $CaCO_3$
Temperature:	14 – 15 °C
pH:	6.7 – 6.8 (0 h, new solution), 6.8 – 7.0 (96 h, aged solution)
O_2 content:	7.5 – 9.2 mg O_2/L (> 60 % air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2176 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Binominal probability, NOEC: Directly from raw data

Findings:

Analytical data:	Over the whole test period the mean measured concentrations were in the range from 89 – 96 % of nominal concentrations.
Behavioural effects:	Controls and concentration levels up to 0.34 mg ai/L: No sublethal effects were reported over the whole test period. At test concentration 0.72 mg ai/L following symptoms were noted after 6 hours: Partial and complete loss of equilibrium, lethargic behaviour, darkened pigmentation, fish on bottom of test vessel. At test concentration 1.4 and 2.9 mg ai/L: 100% fish mortality after 6 hours exposure.

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Thus the NOEC was 0.34 mg ai/L based on sublethal effects.

Mortality: See Table B.9.2.1.1-8

Table B.9.2.1.1-8: Effects on rainbow trout (*O. mykiss*) exposed to S-2167 (S-2200 *R*-isomer)

S-2176 [mg ai/L] (mean measured)	Cumulative mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.17	0	0	0	0	0
0.34	0	0	0	0	0
0.72	0 [abcde]	5 [abd]	25	30	30
1.4	100	100	100	100	100
2.9	100	100	100	100	100
NOEC = 0.34 mg ai/L					
LC_{50} (96 h) = 0.84 mg ai/L (95 % C.I. 0.34 – 1.4 mg ai/L)					

[a] lethargic behaviour
[b] fish on the bottom of the test vessel
[c] complete loss of equilibrium
[d] partial loss of equilibrium
[e] darkened pigmentation

Conclusion: 96 h LC_{50} = 0.84 mg ai/L
96 h NOEC = 0.34 mg ai/L
based on mean measured concentrations.

Validity criteria: The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. Additionally, the dissolved oxygen concentration was measured to be above 60% air saturation (recommended at least 60%).

Metabolites

Reference: **2-COOH-S-2200 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**

Author(s), year: [redacted] 2012a

Report/Doc. number: Report No. ROW-0033, Study No. 13048.6681

Guideline(s): OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1

GLP: Yes

Deviations: The protocol states that the fish biomass to solution ration ("loading") should not exceed 1.0 g/L solution. During the exposure the fish biomass to solution ratio was 1.1 g/L solution (mean). Since the control survival exceed the acceptable criteria and the dissolved oxygen levels were maintained above 60 % of saturation during the 96 h exposure, this deviation is not considered to have had a negative impact on the study.

The protocol states that water temperature of the test solutions will be maintained at 14 ± 1 °C by maintaining the aquaria in a water bath at the appropriate test temperature. Based on the continuous minimum and maximum temperature reading, at the 24- and 48-h intervals, the minimum temperature measurements reached 12 °C. The individual temperature measurements taken during the once daily water quality analyses were within the acceptable range. Since culture

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	temperatures for *O. mykiss* are 15 ± 2 °C, and there were no mortalities during the course of this study, this deviation is not considered to have had a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	2-COOH-S-2200 (metabolite of S-2200), purity: 99 %, batch: 317-001-47-1
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 3 replicates per treatment and control
Weight, length (mean):	1.6 g (range 0.72 – 2.3 g) and 53 mm (range 40 – 60 mm), n = 30
Loading:	1.1 g fish/L solution
Type of test, duration:	Static limit test, 96 hours
Applied concentrations:	
Nominal:	0 (control), 100 mg/L
Measured (mean):	- (control), 89 mg/L
Test conditions:	
Water quality:	Well water, total hardness: 60 mg/L as $CaCO_3$, total alkalinity: 23 mg/L as $CaCO_3$
Temperature:	13 – 15 °C
pH:	7.1 – 7.6 (0 h, new solution), 6.7 – 6.9 (96 h, aged solution)
O_2 content:	7.0 – 9.9 mg O_2/L (> 60 % air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of 2-COOH-S-2200 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Empirically estimated (concentration tested did not produce ≥ 50 % mortality), NOEC: Directly from raw data

Findings:

Analytical data:	Over the whole test period the mean measured concentrations were in the range from 89 % of nominal concentrations.
Behavioural effects:	Controls and test concentration: No sublethal effects were reported over the whole test period. Thus the NOEC was 89 mg/L based on sublethal effects.
Mortality:	See Table B9.2.1.1-9

Table B.9.2.1.1-9: Effects on rainbow trout (*O. mykiss*) exposed to 2-COOH-S-2200 (metabolite)

2-COOH-S-2200 [mg/L] (mean measured)	Cumulative mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
89	0	0	0	0	0
NOEC = 89 mg/L					
LC_{50} (96 h) > 89 mg/L					

Conclusion: 96 h LC_{50} > 89 mg/L
96 h NOEC = 89 mg/L
based on mean measured concentration.

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Validity criteria:	The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. Additionally, the dissolved oxygen concentration was measured to be above 60% air saturation (recommended at least 60%).

Reference:	**5-COOH-S-2200 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**
Author(s), year:	[redacted] 2012b
Report/Doc. number:	Report No. ROW-0034, Study No. 13048.6685
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	The protocol states that the fish biomass to solution ration ("loading") should not exceed 1.0 g/L solution. During the exposure the fish biomass to solution ratio was 1.13 g/L solution (mean). Since the control survival exceed the acceptable criteria and the dissolved oxygen levels were maintained above 60 % of saturation during the 96 h exposure, this deviation is not considered to have had a negative impact on the study. The protocol states that the fish used will be between 4 and 6 cm, in length. During definitive testing, the length of the fish ranged from 4.5 to 6.5 cm. Since the preliminary and definitive results were consistent, this deviation is not considered to have had a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	5-COOH-S-2200 (metabolite of S-2200), purity: 97.6 %, batch: 262-005-10-1
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 3 replicates per treatment and control
Weight, length (mean):	1.7 g (range 0.98 – 2.2 g) and 55 mm (range 45 – 65 mm), n = 30
Loading:	1.13 g fish/L solution
Type of test, duration:	Static limit test, 96 hours
Applied concentrations:	
Nominal:	0 (control), 100 mg/L
Measured (mean):	0 (control), 100 mg/L
Solvent:	None
Test conditions:	
Water quality:	Well water, total hardness: 68 mg/L as $CaCO_3$, total alkalinity: 22 mg/L as $CaCO_3$
Temperature:	14 – 15 °C
pH:	6.2 – 6.8 (0 h, new solution), 7.0 – 7.4 (96 h, aged solution)
O_2 content:	6.3 – 10 mg O_2/L (> 60 % air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of 5-COOH-S-2200 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Empirically estimated (concentration tested did not produce ≥ 50 %

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mortality), NOEC: Directly from raw data

Findings:

Analytical data: Over the whole test period the mean measured concentrations were in the range from 100 % of nominal concentrations.

Behavioural effects: Controls and test concentration: No sublethal effects were reported over the whole test period.
Thus the NOEC was 100 mg/L based on sublethal effects.

Mortality: See Table B.9.2.1.1-10

Table B.9.2.1.1-10: Effects on rainbow trout (*O. mykiss*) exposed to 5-COOH-S-2200 (metabolite)

5-COOH-S-2200 [mg/L] (mean measured)	Cumulative mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
89	0	0	0	0	0
NOEC = 100 mg/L					
LC_{50} (96 h) > 100 mg/L					

Conclusion: 96 h LC_{50} > 100 mg/L
96 h NOEC = 100 mg/L
based on mean measured concentration.

Validity criteria: The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. Additionally, the dissolved oxygen concentration was measured to be above 60% air saturation (recommended at least 60%).

Reference: **S-2200-OR – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**

Author(s), year: [redacted] 2012c

Report/Doc. number: Report No. ROW-0035, Study No. 13048.6689

Guideline(s): OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1

GLP: Yes

Deviations: The protocol states that total dissolved oxygen concentration will not be allowed to drop below 60 % of saturation during the test. At the 24 hour observation interval, the dissolved oxygen concentration in the 10 mg/L nominal treatment level dropped to 57% of saturation. Gentle, oil-free aeration was initiated at the 24 hour observation interval to raise and maintain dissolved oxygen concentrations in all test vessels for the remainder of the exposure. Since there were no adverse effects observed in this treatment, this deviation did not have a negative impact on the results or the interpretation of the study.

Validity: Acceptable

Material and methods:

Test substance: S-2200-OR (metabolite of S-2200), purity: 99.8 %, batch: 09SC8101007-1

Test species: Rainbow trout (*Oncorhynchus mykiss*)

Number of organisms: 10 fish per replicate, 1 replicate per treatment, solvent control and control

Weight, length (mean): 0.92 g (range 0.18 – 1.4 g) and 42 mm (range 39 – 45 mm), n = 30

Loading: 0.61 g fish/L solution

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Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control, solvent control), 0.63, 1.3, 2.5, 5.0 and 10 mg/L
Measured (mean):	- (control, solvent control), 0.48, 1.1, 2.1, 4.3 and 9.0 mg/L
Solvent:	None
Test conditions:	
Water quality:	Well water, total hardness: 84 mg/L as $CaCO_3$, total alkalinity: 28 mg/L as $CaCO_3$
Temperature:	14 – 15 °C
pH:	7.2 – 7.3 (0 h, new solution), 7.3 – 7.5 (96 h, aged solution)
O_2 content:	5.8 – 10 mg O_2/L (< 60 % air saturation) After 24 hours of exposure a decrease of air saturation below 60 % was observed. Therefore, aeration with gentle, oil-free air was initiated at 24 hours of exposure to maintain dissolved oxygen concentration at or above 60% saturation.
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2200-OR in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Empirically estimated (concentration tested did not produce ≥ 50 % mortality), NOEC: Directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range from 77 – 90 % of nominal concentrations.
Behavioural effects:	Controls and test concentrations: No sublethal effects were reported over the whole test period. Thus the NOEC was 9.0 mg/L based on sublethal effects.
Mortality:	See Table B.9.2.1.1-11

Table B.9.2.1.1-11: Effects on rainbow trout (*O. mykiss*) exposed to S-2200-OR (metabolite)

S-2200-OR [mg/L] (mean measured)	Cumulative mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.48	0	0	0	0	0
1.1	0	0	10	10	10 [a]
2.1	0	0	0	0	0
4.5	0	0	0	0	0
9.0	0	0	10	10	10 [a]
NOEC = 9.0 mg/L					
LC_{50} (96 h) > 9.0 mg/L					

[a] ASTM (2002) recognizes the limitations of acute toxicity testing, i.e., response less than or equal to 10 % is allowable in a control population and is considered within the expected range of naturally occurring variability. Therefore, the mortality (10%) observed in this treatment level is not considered an adverse response from exposure to the test substance.

Conclusion:	96 h LC_{50} > 9.0 mg/L 96 h NOEC = 9.0 mg/L based on mean measured concentration.

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Validity criteria:	The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. The dissolved oxygen concentration was measured to be below 60% air saturation (recommended at least 60%). After 24 hours of exposure a decrease of air saturation was observed in the controls and in the treatment groups. Therefore gentle, oil-free aeration was initiated to raise and maintain dissolved oxygen levels at or above 60 % saturation. The study is considered acceptable. The decrease of air saturation after 24 hours was only observed in the highest concentration level (9.0 mg/L). The observed effects at the test concentrations of 1.1 mg/L and 9.0 mg/L are not considered treatment related as no dose-response relationship was observed.
Comment RMS:	After 48 hours of exposure a mortality of 10% was observed in the 1.1 and 9.0 mg/L treatment group. This effect is not considered substance related as no mortalities were observed in the treatment groups between the test concentrations of 1.1 and 9.0 mg/L (2.1 and 4.5 mg/L). In addition, the observed effects of 10% are equal to the acceptable effects in a control population (validity criteria: less than 10% mortality). Hence, the RMS agrees with the NOEC of 9.0 mg/L proposed by the applicant.

Reference:	**S-2200-ORC – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline # 203, EC Guideline L383A, Method C.1 and OPPTS Daft Guideline 850.1075**
Author(s), year:	[redacted] 2012d
Report/Doc. number:	Report No. ROW-0036, Study No. 13048.6693
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	The protocol states that total dissolved oxygen concentration will not be allowed to drop below 60 % of saturation during the test. At the 24 hour observation interval, the dissolved oxygen concentration dropped to 36 to 57% of saturation. Gentle, oil-free aeration was initiated at the 24 hour observation interval to raise and maintain dissolved oxygen concentrations in all test vessels for the remainder of the exposure. Since there were no adverse effects observed in this treatment, this deviation did not have a negative impact on the results or the interpretation of the study. The protocol states that water temperature of the solutions will be maintained at 14 ± 1 °C. After the 24-, 48- and 72-hour intervals, the minimum temperature recorded was 12 °C. Since control survival exceeded acceptable criteria throughout this exposure, this deviation did not have a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	S-2200-ORC (metabolite of S-2200), purity: 100 %, batch: 09SC8101007-3
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 1 replicate per treatment, solvent control and control
Weight, length (mean):	1.6 g (range 0.72 – 2.3 g) and 53 mm (range 40 – 60 mm), n = 30
Loading:	1.0 g fish/L solution

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Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control, solvent control), 0.63, 1.3, 2.5, 5.0 and 10 mg/L
Measured (mean):	- (control, solvent control), 0.55, 1.4, 1.6, 4.2 and 3.8 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Well water, total hardness: 60 mg/L as $CaCO_3$, total alkalinity: 27 mg/L as $CaCO_3$
Temperature:	13 – 14 °C
pH:	6.9 – 7.0 (0 h, new solution), 6.8 – 7.4 (96 h, aged solution)
O_2 content:	3.8 – 11 mg O_2/L (< 60 % air saturation) After 24 hours of exposure a decrease of air saturation below 60 % was observed. Therefore, aeration with gentle, oil-free air was initiated at 24 hours of exposure to maintain dissolved oxygen concentration at or above 60% saturation.
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2200-ORC in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Trimmed Spearman Kräber Estimates, NOEC: Directly from raw data
Findings:	
Analytical data:	Based on the observation of undissolved test substance during the study, the practical limit of solubility for the test substance is estimated to be around 3.8 and 4.2 mg/L under the test conditions maintained. Over the whole test period the mean measured concentrations were in the range from 38 – 110 % of nominal concentrations.
Behavioural effects:	Controls and test concentrations up to 1.1 mg/L: No sublethal effects were reported over the whole test period. At test concentrations between 1.6 and 4.2 mg/L following symptoms were noted: Complete loss of equilibrium, lethargic behaviour, fish on bottom of test vessel. Thus the NOEC was 1.4 mg/L based on sublethal effects.
Mortality:	See Table B.9.2.1.1-12

Table B.9.2.1.1-12: Effects on rainbow trout (*O. mykiss*) exposed to S-2200-ORC (metabolite)

S-2200-ORC [mg/L] (mean measured)	Cumulative mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.55	0	0	0	0	0
1.4	0	0	0	0	0
1.6	0	0 [a]	0 [a]	0 [b]	0
4.2	0 [a]	80 [a]	80 [a]	80 [a]	80 [a]
3.8	0 [a]	0 [ad]	0 [ad]	0 [ad]	0 [acd]
NOEC = 1.4 mg/L					
LC_{50} (96 h) = 4.0 mg/L					

[a] bottom of the vessel
[b] lethargic behaviour
[c] complete loss of equilibrium
[d] undissolved test substance was observed

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Conclusion: 96 h LC_{50} = 4.0 mg/L
96 h NOEC = 1.4 mg/L
based on mean measured concentration.

Validity criteria: The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline.
The dissolved oxygen concentration was measured to be below 60% air saturation (recommended at least 60%). After 24 hours of exposure a decrease of air saturation was observed in the controls and in the treatment groups. Therefore gentle, oil-free aeration was initiated to raise and maintain dissolved oxygen levels at or above 60 % saturation.
The study is considered acceptable. The decrease of air saturation after 24 hours of exposure is considered not relevant for the interpretation of the study. A decrease of air saturation was observed in the controls and all treatment groups except for the 1.3 mg/L concentration level. However, only in the concentration levels between 1.6 and 4.2 mg/L adverse effects on fish (behavioural effects and mortality) were observed. Hence, it is assumed that the adverse effects are treatment related and not because of the decreased air saturation.

Comment RMS: The solubility of the metabolite in water is assumed to be between 3.8 and 4.2 mg/L. Considering the observed treatment related effects on mortality, the study is considered acceptable.

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Formulation

Reference:	**S-2200 25% SC – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions Following OECD Guideline #203, EC Guideline L383A, Method C.1, OPPTS Draft Guideline 850.1075, JMAFF 12 NohSan, No. 8147 Fish Acute Toxicity Test (2-7-1-1) and JMAFF 13 SeiSan No. 3986**
Author(s), year:	[redacted] 2011a
Report/Doc. number:	Report No. ROW-0024, Study No. 13048.6674
Guideline(s):	OECD Guideline 203, OPPTS 850.1075, EU Directive 92/69/EEC C.1, JMAFF Guideline No. 8147
GLP:	Yes
Deviations:	The protocol states that the fish will range in length from 4 to 6 cm. The fish used in this exposure had a mean total length of 3.9 cm (range 3.5 to 4.6 cm). However, this deviation is not considered to have a negative impact on the results since the mean value is with marginal range, the range of total length indicated in the JMAFF and OECD guidelines (5.0 ± 1.0 cm) is a recommendation, and the performance criteria (good health in acclimation and control groups) are fulfilled without abnormal circumstances.
Validity:	Acceptable

Material and methods:

Test substance:	S.2200 25% SC, purity: 24.96%, batch: C09-5F101G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 60020652
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 2 replicates per concentration and control
Weight, length:	0.51 g (range 0.26 – 0.70 g) and 3.9 cm (range 3.5 – 4.6 cm), n = 30
Loading:	0.34 g fish/L solution
Type of test, duration:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control), 0.12, 2.0, 3.7, 6.8 and 12 mg form./L equivalent to 0.29, 0.51, 0.93, 1.7 and 3.0 mg ai/L
Measured (mean):	- (control), 0.25, 0.45, 0.80, 1.6 and 2.9 mg ai/L
Solvent:	None
Test conditions:	
Water quality:	Well water, total hardness: 78 mg/L as $CaCO_3$, total alkalinity: 25 mg/L as $CaCO_3$, specific conductivity: 350 µmhos/cm
Temperature:	13 – 15 °C
pH:	6.9 – 7.1 (0 h, new solution), 6.7 – 7.0 (96 h, aged solution), range 6.7 – 7.4
O_2 content:	6.4 - 11 mg O_2/L (65 – 115% saturation)
Light regime:	16 hours light / 8 hours darkness (light intensity 720 – 900 lux)
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis (HPLC/UV) of S-2200 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment. Temperature was continuously monitored throughout the study in one replicate.
Statistics:	LC_{50}: Binominal probability, NOEC: Directly from raw data

Findings:

Analytical data:	S-2200 consists of a mixture of two isomers, S-2354 and S-2167. The final S-

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2200 concentration is derived from the addition of the concentration of two isomers.
Over the whole test period the mean measured concentrations of the active substance were in the range from 86 - 95% of nominal.
See Table B.9.2.1.1-13.

Table B.9.2.1.1-13: Concentrations measured during the 96 h static acute exposure of rainbow trout to S-2200 25 SC

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]							Percent of nominal [%]
	0-hour			96-hour			Mean	
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.01	< 0.01	< 0.02	< 0.01	< 0.01	< 0.02	n.a.	n.a.
0.25	0.13	0.13	0.27	0.11	0.12	0.23	0.25	86
0.45	0.23	0.23	0.46	0.21	0.23	0.44	0.45	89
0.80	0.43	0.43	0.86	0.36	0.38	0.74	0.80	86
1.60	0.79	0.79	1.60	0.79	0.79	1.60	1.60	93
2.90	1.4	1.4	2.80	1.50	1.50	2.90	2.90	95

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Behavioural effects: No behavioural effects were observed at concentration up to 0.8 mg ai/L. However, effects on survival (1 dead fish) were also observed at a concentration of 0.8 mg ai/L. Thus the NOEC was 0.45 mg ai/L based on mortality.

Mortality: See Table B.9.2.1.1-14

Table B.9.2.1.1-14: Effects on rainbow trout (*O. mykiss*) exposed to the formulation S-2200 25% SC

S-2200 [mg ai/L] (mean measured)	Cumulative mortality [%] (Number of dead fish)				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.45	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.80	0 (0)	5 (1)	5 (1)	5 (1)	5 (1)
1.60	35 (7) [a]	100 (20)	100 (20)	100 (20)	100 (20)
2.90	100 (20)	100 (20)	100 (20)	100 (20)	100 (20)
NOEC = 1.8 mg form./L (nominal) equivalent to 0.45 mg ai/L (mean measured)					
LC_{50} (96 h) = 4.4 mg form./L (nominal) equivalent to 1.1 mg ai/L (mean measured) (95 % C.I. 0.8 – 1.6 mg ai/L)					

[a] All surviving fish (13) were observed to be on bottom of the test vessel

Conclusion: 96 h LC_{50} = 1.1 mg ai/L
96 h NOEC = 0.45 mg ai/L
based on mean measured concentrations.

Validity criteria: The study is considered valid as no fish died in the controls which is in line with the recommended maximum control mortality of 10% according to the OECD guideline. Additionally, the dissolved oxygen concentration was measured to be above 60% air saturation (recommended at least 60%).

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B.9.2.1.2 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4, IIIA 10.2.1)

Active substance

Reference:	**S-2200 Technical Grade – Acute Toxicity to Water Fleas, (*Daphnia magna*), Under Static Conditions, Following OECD Guideline #202, OPPTS Draft Guideline 850.1010, The Official Journal of European Communities L383A, Method C.2 and JMAFF 12 NohSan, No. 8147 Daphnia Acute Immobilization Test (2-7-2-1) and JMAFF 13 SeiSan No. 3986**
Author(s), year:	Sayers, Lee E., 2010a
Report/Doc. number:	Report No. ROW-0013, Study No. 13048.6638
Guideline(s):	OECD Guideline 202, US EPA OPPTS 850.1010, JMAFF 12 NoHsan No. 8147, Daphnia Acute Immobilisation Test (2-7-2-1), EC Guideline Annex V - Method C.2
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	S-2200 technical grade, purity: 93.4%, batch: ST-0811G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 60020652
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates each with 5 daphnids per treatment, control and solvent control
Age:	First instar, ≤ 24 hours old
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.38, 0.75, 1.5, 3.0 and 6.0 mg ai/L
Measured (mean):	- (control and solvent control), 0.35, 0.70, 1.4, 2.9 and 6.0 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Fortified well water, total hardness: 180 mg/L as $CaCO_3$, total alkalinity: 80 mg/L as $CaCO_3$, specific conductivity: 600 µmhos/cm
Temperature:	20 - 21 °C
pH:	8.1 – 8.2 (0 - 48 h)
O_2 content:	8.3 – 9.0 mg O_2/L (96 – 106 % saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobility and sublethal effects were assessed after 0, 24 and 48 hours. For chemical analysis (LC/MS/MS) of S-2200 in the test media samples were taken at test initiation (0 h) and termination (48 h). Measurements of pH, temperature and dissolved oxygen concentrations were made at initiation and once daily. Total hardness, total alkalinity and specific conductance were measured at test initiation.
Statistics:	EC_{50}: Binominal probability, NOEC: Directly from the raw data

Findings:

Analytical data:	The overall mean measured concentration ranged from 93 – 100 % of nominal concentrations. See Table B.9.2.1.2-1.

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Table B.9.2.1.2-1 Concentrations measured during the 48 h static acute exposure of daphnids to S-2200 technical grade

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]							Percent of nominal [%]
	0-hour			96-hour			Mean	
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.013	< 0.013	< 0.025	< 0.013	< 0.013	< 0.025	n.a.	n.a.
Solvent control	< 0.013	< 0.013	< 0.025	< 0.013	< 0.013	< 0.025	n.a.	n.a.
0.38	0.17	0.18	0.35	0.71	0.18	0.35	0.35	93
0.75	0.36	0.37	0.73	0.32	0.35	0.67	0.70	94
1.5	0.73	0.69	1.4	0.67	0.72	1.4	1.4	94
3.0	1.4	1.5	2.9	1.3	1.5	2.9	2.9	97
6.0	3.1	3.2	6.3	2.8	2.9	5.7	6.0	100

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Effects: After 48 hours no immobilisation was observed in the control, solvent control and in test concentrations up to 0.7 mg/L. At 1.4 and 2.9 mg/L sublethal effects (lethargy) were observed after 24 h of exposure.
Thus the NOEC was determined to be 0.70 mg ai/L and the EC_{50} was 1.2 mg ai/L.
See Table B.9.2.1.2-2.

Table B.9.2.1.2-2: Effects on daphnids (*D. magna*) exposed to technical S-2200

S-2200 [mg ai/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
0.36	0	0
0.70	0	0
1.4	25 [a]	70 [a]
2.9	95 [a]	100
6.0	100	100
NOEC = 0.70 mg ai/L		
EC_{50} (48 h) = 1.2 mg ai/L (95 % C.I. 0.7 – 2.9 mg ai/L)		

[a] All surviving daphnids were observed to be lethargic.

Conclusion: 48 h EC_{50} = 1.2 mg ai/L
48 h NOEC = 0.7 mg ai/L
based on mean measured concentrations.

Validity criteria: No immobilization of daphnids was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum immobilisation of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 8.3 and 9.0 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.

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Reference:	**S-2354 (*S*-isomer of S-2200) – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of European Communities L 142/456, Method C.2**
Author(s), year:	[redacted] 2012f
Report/Doc. number:	Report No. ROW-0049, Study No. 13048.6706
Guideline(s):	OECD Guideline 202 and EC Guideline Annex V - Method C.2
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	S-2354 (*S*-isomer of S-2200), purity: 99.7%, batch: 060020653
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control
Age:	First instar, ≤ 24 hours old
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	0 (control), 0.0.94, 1.9, 3.8, 7.5 and 15 mg/L
Measured (mean):	- (control), 0.82, 1.7, 3.5, 7.3 and 14 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Fortified well water, total hardness: 190 mg/L as $CaCO_3$, total alkalinity: 96 mg/L as $CaCO_3$
Temperature:	20 - 21 °C
pH:	7.9 – 8.2 (0 – 48 h)
O_2 content:	8.4 – 9.2 mg O_2/L (99 – 107% saturation)
Light regime:	16 hours light / 8 hours darkness (light intensity 740 - 850 lux)
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. For chemical analysis (HPLC) of SS-2354 in the test media samples were taken at test initiation (0 h) and termination (48 h). Measurements of pH, temperature and dissolved oxygen concentrations were made at initiation and termination of exposure.
Statistics:	EC_{50}: Spearman-Kärber Estimates, NOEC: Directly from the raw data

Findings:

Analytical data:	The overall mean measured concentration ranged from 88 - 97% of nominal concentrations.
Effects:	After 48 hours no immobilisation was observed in the controls and in test concentrations up to 7.3 mg/L. Only 1 immobilized daphnid was observed at the highest test concentration. Sublethal effects (lethargic behaviour) were observed at the highest test concentration. Thus the NOEC was determined to be 7.3 mg/L and the EC_{50} was > 14 mg/L. See Table B.9.2.1.2-4.

Table B.9.2.1.2-4: Effects on daphnids (*D. magna*) exposed to the *S*-isomer S-2354

S-2354 [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
0.82	0	0
1.7	0	0
3.5	0	0

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

S-2354 [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
7.3	0	0
14	0 [a]	5 [a]
NOEC = 7.3 mg/L		
EC_{50} (48 h) > 14 mg/L		

[a] Several daphnids were observed to be lethargic.

Conclusion: 48 h EC_{50} > 14 mg/L
48 h NOEC = 7.3 mg/L
based on mean measured concentrations.

Validity criteria: No immobilization of daphnids was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum immobilisation of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 8.4 and 9.2 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.

Reference: **S-2167 (*R*-isomer of S-2200) – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of European Communities L 142/456, Method C.2**
Author(s), year: [redacted] 2012e
Report/Doc. number: Report No. ROW-0048, Study No. 13048.6704
Guideline(s): OECD Guideline 202 and EC Guideline Annex V - Method C.2
GLP: Yes
Deviations: None relevant
Validity: Acceptable

Material and methods:
Test substance: S-2167 (*R*-isomer of S-2200), purity: 100%, batch: 060020652
Test species: Water flea (*Daphnia magna*)
Number of organisms: 4 replicates each with 5 daphnids per treatment and control
Age: First instar, ≤ 24 hours old
Type of test, duration: Static test, 48 hours
Applied concentrations:
Nominal: 0 (control and solvent control), 0.078, 0.16, 0.31, 0.63, 1.3 and 2.5 mg/L
Measured (mean): - (control and solvent control), 0.062, 0.14, 0.29, 0.61, 1.2 and 2.5 mg/L
Solvent: Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:
Water quality: Fortified well water, total hardness: 190 mg/L as $CaCO_3$, total alkalinity: 95 mg/L as $CaCO_3$
Temperature: 20 - 21 °C
pH: 8.1 – 8.2 (0 – 48 h)
O_2 content: 8.3 – 9.2 mg O_2/L (98 – 107% saturation)
Light regime: 16 hours light / 8 hours darkness (light intensity 600 – 750 lux)
Test parameters: Immobility and sublethal effects were assessed after 24 and 48 hours. For chemical analysis (HPLC) of SS-2167 in the test media samples were taken at test initiation (0 h) and termination (48 h). Measurements of pH, temperature and dissolved oxygen concentrations were made at initiation and termination of exposure.

WARNING: This document forms part of an EC evaluation of data available and should not be read in isolation. Registration must not be granted on the basis of this document.

Statistics: EC_{50}: Spearman-Kärber Estimates, NOEC: Directly from the raw data

Findings:

Analytical data: The overall mean measured concentration ranged from 80 - 99 % of nominal concentrations.

Effects: After 48 hours no immobilisation was observed in the controls and in test concentrations up to 0.61 mg/L. At the highest test concentration of 2.5 mg/L the immobilisation was 100%. Sublethal effects (lethargic behaviour) were observed at 1.2 mg/L treatment group only.
Thus the NOEC was determined to be 0.61 mg/L (based on mortality and behavioural effects) and the EC_{50} was 0.92 mg/L.
See Table B.9.2.1.2-3.

Table B.9.2.1.2-3: Effects on daphnids (*D. magna*) exposed to the *R*-isomer S-2167

S-2167 [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
0.062	0	0
0.14	0	0
0.29	0	0
0.61	0	0
1.2	75 [a]	90 [a]
2.5	100	100
NOEC = 0.61 mg/L		
EC_{50} (48 h) = 0.92 mg/L (95 % C.I. 0.84 – 1.0 mg/L)		

[a] One daphnid was observed to be lethargic.

Conclusion: 48 h EC_{50} = 0.92 mg/L
48 h NOEC = 0.61 mg/L
based on mean measured concentrations.

Validity criteria: No immobilisation of daphnids was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum immobilization of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 8.3 and 9.2 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.

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Metabolites

Reference:	**2-COOH-S-2200 – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of European Communities L 142/456, Method C.2**
Author(s), year:	[redacted] 2012g
Report/Doc. number:	Report No. ROW-0037, Study No. 13048.6682
Guideline(s):	OECD Guideline 202, EC Guideline Annex V - Method C.2
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	2-COOH-S-2200, purity: 99.0%, batch: 317-001-47-1
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control
Age:	First instar ≤ 24 hours old
Type of test, duration:	Static limit test, 48 hours
Applied concentrations:	
Nominal:	0 (control), 6.3, 13, 25, 50 and 100 mg/L
Measured (mean):	- (control), 6.0, 13, 25, 50 and 100 mg/L
Solvent:	None
Test conditions:	
Water quality:	Fortified well water, total hardness: 170 mg/L as $CaCO_3$, total alkalinity: 86 mg/L as $CaCO_3$, specific conductivity: 730 µmhos/cm
Temperature:	21 °C
pH:	7.5 – 8.4 (test initiation), 7.8 – 8.2 (test termination)
O_2 content:	7.2 – 9.0 mg O_2/L (82 – 102% saturation)
Light regime:	16 hours light / 8 hours darkness (light intensity 750 – 880 lux)
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. For chemical analysis (HPLC) of 2-COOH-S-2200 in the test media samples were taken at test initiation (0 h) and termination (48 h). Measurements of pH, temperature and dissolved oxygen concentrations were made daily.
Statistics:	None, NOEC and EC_{50} were empirically estimated.

Findings:

Analytical data:	The overall mean measured concentrations are between 96 and 100% of nominal concentrations.
Effects:	After 48 hours no immobilisation and no sublethal effects were observed in the control and the test concentrations. Thus the NOEC was determined to be 100 mg/L and the EC_{50} was > 100 mg/L. See Table B.9.2.1.2-5

Table B.9.2.1.2-5: Effects on daphnids (*D. magna*) exposed to the metabolite 2-COOH-S-2200

2-COOH-S-2200 [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
Control	0	0
6.0	0	0
13	0	0
25	0	0
50	0	0
100	0	0

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2-COOH-S-2200 [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
NOEC = 100 mg/L		
EC_{50} (48 h) > 100 mg/L		

Conclusion: 48 h EC_{50} > 100 mg/L
48 h NOEC = 100 mg/L
based on mean measured concentrations.

Validity criteria: No immobilisation of daphnids was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum immobilization of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 7.2 and 9.0 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.

Reference: **5-COOH-S-2200 – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of European Communities L 142/456, Method C.2**

Author(s), year: [redacted] 2012h
Report/Doc. number: Report No. ROW-0038, Study No. 13048.6686
Guideline(s): OECD Guideline 202, EC Guideline Annex V - Method C.2
GLP: Yes
Deviations: None
Validity: Acceptable

Material and methods:
Test substance: 5-COOH-S-2200, purity: 97.6%, batch: 262-005-10-1
Test species: Water flea (*Daphnia magna*)
Number of organisms: 4 replicates each with 5 daphnids per treatment and control
Age: First instar ≤ 24 hours old
Type of test, duration: Static limit test, 48 hours
Applied concentrations:
Nominal: 0 (control), 6.3, 13, 25, 50 and 100 mg/L
Measured (mean): - (control), 6.0, 12, 24, 50 and 100 mg/L
Solvent: None
Test conditions:
Water quality: Fortified well water, total hardness: 160 mg/L as $CaCO_3$, total alkalinity: 92 mg/L as $CaCO_3$, specific conductivity: 670 µmhos/cm
Temperature: 21 °C
pH: 7.3 – 8.2 (test initiation), 8.0 – 8.2 (test termination)
O_2 content: 7.9 – 8.8 mg O_2/L (90 – 101% saturation)
Light regime: 16 hours light / 8 hours darkness (light intensity 320 - 1100 lux)
Test parameters: Immobility and sublethal effects were assessed after 24 and 48 hours. For chemical analysis (HPLC) of 5-COOH-S-2200 in the test media samples were taken at test initiation (0 h) and termination (48 h). Measurements of pH, temperature and dissolved oxygen concentrations were made daily.
Statistics: None, NOEC and EC_{50} were empirically estimated.
Findings:

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Analytical data: The overall mean measured concentrations are between 96 and 100% of nominal concentrations.

Effects: After 48 hours no immobilisation and sub-lethal effects were observed in the control and the test concentrations. In the second highest test concentration (50 mg/L) behavioural effects were observed in one test vessel. The NOEC was determined to be 100 mg/L and the EC_{50} was > 100 mg/L.
See Table B.9.2.1.2-6

Table B.9.2.1.2-6: Effects on daphnids (*D. magna*) exposed to the metabolite 5-COOH-S-2200

5-COOH-S-2200 [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
Control	0	0
6.0	0	0
12	0	0
24	0	0
50	0	0 [a]
100	0	0
NOEC = 100 mg/L		
EC_{50} (48 h) > 100 mg/L		

[a] Four daphnids were observed on the surface of the solution on one replicate vessel.

Conclusion: 48 h EC_{50} > 100 mg/L
48 h NOEC = 100 mg/L
based on mean measured concentrations.

Validity criteria: No immobilisation of daphnids was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum immobilisation of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 7.9 and 8.8 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.

Comment RMS: The behavioural effects observed in the second highest test concentration (50 mg/L) are considered not treatment-related. In no other test concentration (including the highest test concentration of 100 mg/L) adverse behavioural effects on the daphnids were observed. Hence, the NOEC is based on the highest tested concentration, 100 mg/L.

Reference: **S-2200-OR – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of European Communities L 142/456, Method C.2**
Author(s), year: [redacted] 2012i
Report/Doc. number: Report No. ROW-0039, Study No. 13048.6690
Guideline(s): OECD Guideline 202, EC Guideline Annex V - Method C.2
GLP: Yes
Deviations: None relevant
Validity: Acceptable

Material and methods:
Test substance: S-2200-OR, purity: 99.8%, batch: 09SC8101007
Test species: Water flea (*Daphnia magna*)
Number of organisms: 4 replicates each with 5 daphnids per treatment and control

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Age:	First instar ≤ 24 hours old
Type of test, duration:	Static limit test, 48 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 1.9, 3.8, 7.5, 15 and 30 mg/L
Measured (mean):	- (control and solvent control), 1.6, 3.3, 6.7, 14 and 9.4 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Fortified well water, total hardness: 160 mg/L as $CaCO_3$, total alkalinity: 90 mg/L as $CaCO_3$, specific conductivity: 710 µmhos/cm
Temperature:	20 - 21 °C
pH:	8.2 – 8.3 (test initiation), 7.9 – 8.1 (test termination)
O_2 content:	7.8 – 8.9 mg O_2/L (89 – 101% saturation)
Light regime:	16 hours light / 8 hours darkness (light intensity 680 - 900 lux)
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. For chemical analysis (HPLC) of S-2200-OR in the test media samples were taken at test initiation (0 h) and termination (48 h). Measurements of pH, temperature and dissolved oxygen concentrations were made daily.
Statistics:	None, NOEC and EC_{50} were empirically estimated.
Findings:	
Analytical data:	The overall mean measured concentrations are between 32 - 91% of nominal concentrations. For the 1.9 – 15 mg/L test solutions the measured concentrations were generally consistent between sampling intervals and maintained the expected concentration gradient. The highest test concentration tested, 30 mg/L, indicated a stable exposure concentration but lower recoveries due to the limit water solubility of the test substance.
Effects:	After 48 hours immobilisation was observed in the second highest test concentration (14 mg/L). Adverse effects on daphnids (lethargic behaviour) were observed in the two highest tests concentrations (9.4 and 14 mg/L, mean measured concentrations). No immobilisation and adverse sub-lethal effects were observed in the controls and in the lower treatment groups. The NOEC was determined to be 6.7 mg/L (based on sub-lethal effects and immobilisation) and the EC_{50} was > 14 mg/L. See Table B.9.2.1.2-7

Table B.9.2.1.2-7: Effects on daphnids (*D. magna*) exposed to the metabolite S-2200-OR

S-2200-OR [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
1.6	0	0
3.3	0	0
6.7	0	0
14	0	20 [a]
9.4	0	0 [ab]
NOEC = 6.7 mg/L		
EC_{50} (48 h) > 14 mg/L		

[a] Several daphnids were observed to be lethargic.
[b] Undissolved material was observed to be on the bottom of the vessels. Several daphnids were observed to be swimming, carrying undissolved material.

Conclusion:	48 h EC_{50} > 14 mg/L 48 h NOEC = 6.7 mg/L

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	based on mean measured concentrations.
Validity criteria:	No immobilisation of daphnids was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum immobilisation of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 7.8 and 8.9 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.
Comment RMS:	The overall mean measured concentrations are between 32 - 91% of nominal concentrations. In the highest test concentration (30 mg/L) undissolved test material was observed. The mean measured test concentration was 9.4 mg/L (32% of the nominal test concentration).

Reference:	**S-2200-ORC – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of European Communities L 142/456, Method C.2**
Author(s), year:	[redacted] 2012j
Report/Doc. number:	Report No. ROW-0040, Study No. 13048.6694
Guideline(s):	OECD Guideline 202, EC Guideline Annex V - Method C.2
GLP:	Yes
Deviations:	The protocol states that alkalinity and conductivity will be 110 to 130 mg $CaCO_3$/L and 400 to 600 µmhos/cm, respectively. Two weeks prior to testing, alkalinity was 90 to 96 mg $CaCO_3$/L and conductivity was 676 to 725 µmhos/cm. Since culture and control survival exceeded the acceptable criteria throughout this exposure, this deviation did not impact the results or the interpretation of this study.
Validity:	Acceptable

Material and methods:

Test substance:	S-2200-ORC, purity: 100%, batch: 09SC8101007-3
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control
Age:	First instar ≤ 24 hours old
Type of test, duration:	Static limit test, 48 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.63, 1.3, 2.5, 5.0 and 10 mg/L
Measured (mean):	– (control and solvent control), 0.62, 1.2, 2.5, 5.0 and 11 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Fortified well water, total hardness: 170 mg/L as $CaCO_3$, total alkalinity: 94 mg/L as $CaCO_3$, specific conductivity: 720 µmhos/cm
Temperature:	21 °C
pH:	8.2 (test initiation), 8.2 – 8.3 (test termination)
O_2 content:	8.2 – 8.6 mg O_2/L (93 – 98% saturation)
Light regime:	16 hours light / 8 hours darkness (light intensity 720 - 880 lux)
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. For chemical analysis (HPLC) of S-2200-ORC in the test media samples were taken at test initiation (0 h) and termination (48 h). Measurements of pH, temperature and dissolved oxygen concentrations were made daily.
Statistics:	EC_{50}: Spearman-Kärber Estimates, NOEC: Directly from the raw data

Findings:

WARNING: This document forms part of an EC evaluation data package and should not be used in isolation. Registration must not be granted on the basis of this document.

Analytical data: The overall mean measured concentrations are between 96 - 110% of nominal concentrations.

Effects: After 48 hours immobilisation and sub-lethal effects were observed in the two highest test concentration (5 and 11 mg/L). No immobilisation and adverse sub-lethal effects were observed in the controls and in the lower treatment groups. The NOEC was determined to be 2.5 mg/L (based on sub-lethal effects and immobilisation) and the EC_{50} was 9.6 mg/L.
See Table B.9.2.1.2-8

Table B.9.2.1.2-8 Effects on daphnids (*D. magna*) exposed to the metabolite S-2200-ORC

S-2200-ORC [mg/L] (mean measured)	Mean cumulative immobilised organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
0.62	0	0
1.2	0	0
2.5	0	0
5.0	0 [a]	25 [abc]
11	10 [ab]	55 [abcd]
NOEC = 2.5 mg/L		
EC_{50} (48 h) = 9.6 mg/L (95 % C.I. 5.9 - 16 mg/L)		

[a] Several daphnids were observed to be lethargic.
[b] Undissolved material was observed in the vessels.
[c] Several daphnids were observed to be swimming, carrying undissolved material.
[d] Several daphnids were observed to be on the bottom of the test vessel.

Conclusion: 48 h EC_{50} = 9.6 mg/L
48 h NOEC = 2.5 mg/L
based on mean measured concentrations.

Validity criteria: No immobilisation of daphnids was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum immobilisation of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 8.2 and 8.6 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.

Comment RMS: The test concentrations used in the study are between 0.63 and 10 mg/L (nominal). Under consideration of the results of the fish acute toxicity test a water solubility of the metabolite S-2200-ORC between 3.8 and 4.2 mg/L is predicted. Nevertheless, even at the two highest test concentrations (5.0 and 10 mg/L) no undissolved test material was observed. The mean measured test concentrations were in a range from 96 to 110%. The explanation of the difference in water solubility of the metabolite might be due to the different test conditions (i.e. hardness of the test water).

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Formulation

Reference:	**S-2200 25 SC – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline '202, OPPTS Draft Guideline 850.1010, The Official Journal of the European Communities L383A, Method C.2 and JMAFF 12 NohSan, No. 8147 Daphnia Acute Immobilization Test (2-7-2-1) and JMAFF 13 SeiSan No. 3986**
Author(s), year:	[redacted] 2011b
Report/Doc. number:	Report No. ROW-0025, Study No. 13048.6675
Guideline(s):	OECD Guideline 202, JMAFF No. 8147 and 3986, OPPTS 850.1010, EU Directive 92/69/EEC C.2
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	S-2200 25% SC, purity: 24.96%, batch: C09-5F101G
Reference substance:	S-2343 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 060020652
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control
Age:	First instar, ≤ 24 hours old
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	0 (control), 0.76, 1.5, 3.0, 6.0 and 12 mg form./L equivalent to 0.19, 0.38, 0.75, 1.5 and 3.0 mg ai/L
Measured (mean):	- (control), 0.19, 0.36, 0.70, 1.5 and 3.0 mg ai/L
Solvent:	None
Test conditions:	
Water quality:	Fortified well water, total hardness: 180 mg/L as $CaCO_3$, total alkalinity: 86 mg/L as $CaCO_3$, specific conductivity: 820 µmhos/cm
Temperature:	20 – 21 °C
pH:	8.2 (test initiation), 8.1 (test termination)
O_2 content:	7.7 – 8.7 mg O_2/L (86 – 99% saturation)
Light regime:	16 hours light / 8 hours darkness (light intensity: 670 – 880 lux)
Test parameters:	Immobility and sublethal effects were assessed after 0, 24 and 48 hours. For chemical analysis (HPLC/UV) of test media samples were taken at test initiation (0 h) and termination (48 h).
Statistics:	EC_{50}: Binominal probability, NOEC: Directly from the raw data
Findings:	
Analytical data:	S-2200 consists of a mixture of two isomers, S-2354 and S-2167. The final S-2200 concentration is derived from the addition of the concentration of two isomers. Over the whole test period the mean measured concentrations of the active substance were in the range from 93 to 100% of nominal concentrations. See Table B.9.2.1.2-9.

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Table B.9.2.1.2-9: Concentrations measured during the 48 h static acute exposure of daphnids to S-2200 25 SC

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]							Percent of nominal [%]
	0-hour			96-hour			Mean	
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.0057	< 0.0057	< 0.011	< 0.0057	< 0.0057	< 0.011	n.a.	n.a.
0.19	0.093	0.096	0.19	0.091	0.090	0.18	0.19	97
0.38	0.19	0.18	0.37	0.18	0.18	0.36	0.36	96
0.75	0.36	0.35	0.71	0.34	0.23	0.68	0.70	93
1.5	0.78	0.79	1.6	0.73	0.71	1.4	1.5	100
3.0	1.5	1.5	3.0	1.5	1.5	3.0	3.0	100

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Effects: After 48 hours no immobility was observed in the control and in test concentration up to 0.36 mg ai/L. At test concentrations 0.70, 1.5 and 3.0 mg ai/L the immobility was between 55 and 100%. Thus the NOEC was determined to be 0.36 mg ai/L. See Table B.9.2.1.2-10

Table B.9.2.1.2-10: Effects on daphnids (*D. magna*) exposed to S-2200 25% SC

S-2200 25% SC [mg form./L] (nominal)	S-2200 [mg ai/L] (mean measured)	Mean cumulative immobilised organisms [%]	
		24 hours	48 hours
Control	Control	0	5
0.76	0.19	0	0
1.5	0.36	0	0
3.0	0.70	0 [a]	55 [a]
6.0	1.5	75 [b]	100
12	3.0	100	100
NOEC = 1.5 mg form./L (nominal) equivalent to 0.36 mg ai/L (mean measured)			
EC_{50} (48 h) = 2.68 mg form./L (nominal) equivalent to 0.67 mg ai/L (mean measured) (95 % C.I. 0.36 – 1.5 mg ai/L)			

[a] Several daphnids were observed to be lethargic.
[b] Several daphnids were observed to be on the bottom of the test vessel.

Conclusion: 48 h EC_{50} = 0.67 mg ai/L
48 h NOEC = 0.36 mg ai/L
based on mean measured concentration.

Validity criteria: After 48 hours one out of 20 daphnids (5%) was observed to be immobile in the control which is in the line with the recommended maximum immobilisation of 10% according to the OECD guideline. The dissolved oxygen concentration at the end of the test was between 7.7 and 8.7 mg/L in control and test vessels and hence above the recommended dissolved oxygen concentration of at least 3 mg/L.

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B.9.2.1.3 Effects on algal growth and growth rate (Annex IIA 8.2.6, IIIA 10.2.1)

Reference:	**S-2200 Technical Grade – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following The Official Journal of the European Communities L383A, Method C.3 and JMAFF 12 Nohsan, No. 8147 Alga, Growth Inhibition Test 2-7-7**
Author(s), year:	Softcheck, Katherina A., 2012a (Amendment)
Report/Doc. number:	Report No. ROW-0015, Study No. 13048.6640
Guideline(s):	JMAFF 12 Nohsan Guideline No. 8147, EC Guideline L383A - C.3
GLP:	Yes
Deviations:	None
Validity:	Acceptability to be discussed

Material and methods:

Test substance:	S-2200 technical grade, purity: 93.4%, batch: ST-0811G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 060020652
Test species:	Green alga (*Pseudokirchneriella subcapitata)*, class Chlorophyceae
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment group and medium control and 6 replicates per solvent control
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium and solvent control), 0.078, 0.17, 0.38, 0.80, 1.8 and 4.0 mg ai/L
Measured (mean):	- (medium and solvent control), 0.067, 0.16, 0.35, 0.77, 1.7 and 3.6 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL/L
Test conditions:	
Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	22 - 23 °C
pH:	7.0 – 7.3 (0 h), 7.4 – 8.7 (72 h)
Conductivity:	80 µmhos/cm (0 – 72 h)
Incubation:	Continuous illumination at 4700 to 5800 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test Significance of effects compared to the control: Williams' Test

Findings:

Analytical data:	S-2200 consists of a mixture of two isomers, S-2354 and S-2167. The final S-2200 concentration was derived from the sum of the concentration of the two isomers. Mean measured concentrations were in the range of 86 - 96% of nominal concentrations over the whole test duration. See Table B.9.2.1.3-1.

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Table B.9.2.1.3-1: Concentrations measured during the 72 h static exposure of algae to S-2200

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]						Mean	Percent of nominal [%]
	0-hour			72-hour				
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.005	< 0.005	< 0.01	< 0.005	< 0.005	< 0.01	n.a.	n.a.
Solvent control	< 0.005	< 0.005	< 0.01	< 0.005	< 0.005	< 0.01	n.a.	n.a.
0.078	0.038	0.036	0.075	0.031	0.029	0.060	0.067	86
0.17	0.089	0.080	0.17	0.074	0.072	0.15	0.16	92
0.38	0.19	0.18	0.37	0.16/0.17[b]	0.16/0.17[b]	0.33/0.34[b]	0.35	91
0.80	0.42	0.39	0.82	0.37	0.36	0.72	0.77	96
1.8	0.93	0.84	1.8	0.79	0.81	1.6	1.7	94
4.0	2.1	1.9	3.9	1.7	1.7	3.4	3.6	91

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
[b] Results of the additional sample without algae present to determine biological uptake/degradation.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Morphological effects: No effects on the morphology and appearance of the cells were observed during the study period.

Biomass, growth rate and cell density: See Table B.9.2.1.3-2 and Table B.9.2.1.3-3.

Table B.9.2.1.3-2: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to S-2200 technical grade

S-2200 [mg/L] (mean measured)	Cell density x 10^4 cells/mL (SD)		
	24 h	48 h	72 h
Control	2.83 (0.38)	22.08 (5.51)	135.33 (28.81)
Solvent control	4.00 (0.85)	19.25 (4.76)	124.57 (17.44)
0.067	4.25 (0.87)	18.67 (4.37)	104.83 (14.40)
0.16	2.92 (1.66)	16.92 (6.69)	90.00 (25.81)
0.35	3.00 (1.30)	20.67 (6.63)	75.67 (12.22)
0.77	2.00 (0.43)	11.00 (2.88)	50.00 (15.59)
1.7	2.33 (0.80)	7.42 (1.13)	26.00 (6.24)
3.6	1.58 (1.01)	4.33 (1.04)	10.75 (1.95)

SD…Standard Deviation

Table B.9.2.1.3-3: Effects of technical S-2200 on the green alga *P. subcapitata*

S-2200 [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72 h)
Control	-	-
Solvent control	-	-
0.067	12	4
0.16	24 *	8 *
0.35	29 *	11 *
0.77	57 *	20 *
1.7	76 *	33 *
3.6	90 *	51 *
NOEC	0.067 mg ai/L	0.067 mg ai/L
EC_{50} (95 % C.I.)	0.67 mg ai/L (0.51 – 0.92 mg ai/L)	3.4 mg ai/L (3.1 – 3.6 mg ai/L)

* Significantly different compared to the solvent control, based on Williams' Test

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Conclusion:	72 h E_bC_{50} = 0.67 mg ai/L 72 h E_rC_{50} = 3.4 mg ai/L 72 h NOEC = 0.067 mg ai/L (biomass and growth rate) based on mean measured concentrations.
Validity criteria:	The biomass in the controls (medium and solvent control) should increase by a factor of greater than 16, as recommended in the test guideline. The mean coefficient of variation (CV) for section-by-section specific growth rates is 35.5% and 27.5% in the medium control and solvent control, respectively (recommended: < 35%). The CV of average specific growth rates during the whole test period is 4.5% and 3.2% in the medium control and solvent control, respectively (recommended < 7%). Hence, the validity criteria regarding the mean daily CV for growth rate for the medium control is not met. The results for the control exceed the required trigger value of 35%. All other acceptance criteria were within the validity criteria according to the OECD guideline. The results of this study (NOEC, EC_{50} values) were calculated as compared to the solvent control. The results of this study are therefore considered acceptable and appropriately define the toxicity of the active substance to *Pseudokirchneriella subcapitata*.
Comments RMS:	The coefficients of variation for growth rates in the medium control were calculated by the RMS using ToxRat. Based on the results of the statistical analyses the validity criteria of the study recommended in the test guidelines are not met. The RMS does not agree with the argumentation given by the applicant. According to the test guideline both controls, medium and solvent control have to meet the validity criteria, irrespective of whether the results of the study were calculated based on the solvent control only. The relevance of this validity criterion might be a point for further discussion.

Reference:	**S-2354 (*S*-Isomer of S-2200) – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following The Official Journal of the European Communities L383A, Method C.3**
Author(s), year:	Softcheck, Katherina A., 2012c
Report/Doc. number:	Report No. ROW-0051, Study No. 13048.6705
Guideline(s):	OECD Guideline 201, EC Guideline L383A - C.3
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 060020653
Test species:	Green alga (*Pseudokirchneriella subcapitata)*, class Chlorophyceae
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment group and medium control and 6 replicates per solvent control
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium and solvent control), 0.94, 1.9, 3.8, 7.5 and 15 mg/L
Measured (mean):	- (medium and solvent control), 0.79, 1.6, 3.5, 6.0 and 12 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL/L

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Test conditions:

Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	24 - 25 °C
pH:	7.1 – 7.3 (0 h), 7.7 – 8.6 (72 h)
Conductivity:	68 - 89 µmhos/cm (0 h), 67 - 89 µmhos/cm (72 h)
Incubation:	Continuous illumination at 4500 to 5900 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test Significance of effects compared to the control: Bonferroni's adjusted t-Test

Findings:

Analytical data:	Mean measured concentrations were in the range of 80 - 91% of nominal concentrations over the whole test duration.
Morphological effects:	No effects on the morphology and appearance of the cells were observed during the study period.
Biomass, growth rate and cell density:	See Table B.9.2.1.3-4 and Table B.9.2.1.3-5

Table B.9.2.1.3-4: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to S-2354

S-2354 [mg/L] (mean measured)	Cell density (x 10^4 cells/mL), (± SD)		
	24 h	48 h	72 h
0 (control)	8.00 (1.75)	40.75 (4.67)	138.33 (27.14)
0 (solvent control)	6.88 (1.81)	31.33 (5.52)	115.22 (21.91)
0.79	7.00 (1.25)	32.08 (14.54)	127.64 (23.23)
1.6	6.00 (0.66)	35.50 (7.47)	117.19 (20.41)
3.5	3.83 (0.72)	31.50 (5.27)	124.17 (27.25)
6.0	4.25 (0.90)	30.08 (5.91)	103.58 (14.75)
12	5.33 (2.13)	18.00 (2.22)	50.75 (5.06)

Table B.9.2.1.3-5: Effects of technical S-2354 on the green alga *P. subcapitata*

S-2354 [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72 h)
0 (control)	-	-
0 (solvent control)	-	-
0.79	- 8	- 2
1.6	- 4	- 1
3.5	- 2	- 2
6.0	10	2
12	51 *	17 *
NOEC	6.0 mg/L	6.0 mg/L
EC_{10}	5.2 mg/L (n.a. – 8.3 mg/L)	8.8 mg/L (6.0 - 10 mg/L)
EC_{20}	6.9 mg/L (n.a. – 9.1 mg/L)	> 12 mg/L (n.a.)
EC_{50} (95 % C.I.)	12 mg/L (9.1 – n.a. mg/L)	> 12 mg/L (n.a.)

* Significantly different compared to the solvent control, based on Bonferroni's adjusted t-Test
Negative values indicate an increase of algal growth
n.a… not applicable, corresponding 95% confidence limit could not be calculated

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Conclusion:	72 h E_bC_{50} = 12 mg/L 72 h E_rC_{50} = > 12 mg/L 72 h NOEC = 6.0 mg/L (biomass and growth rate) based on mean measured concentrations.
Validity criteria:	The biomass in the controls (medium and solvent control) should increase by a factor of greater than 16, as recommended in the test guideline. The mean coefficient of variation (CV) for section-by-section specific growth rates is 27% in the medium control and solvent control, respectively (recommended: < 35%). The CV of average specific growth rates during the whole test period is 4.2% and 4.4% in the medium control and solvent control, respectively (recommended < 7%).

Reference:	**S-2167 (*R*-Isomer of S-2200) – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following The Official Journal of the European Communities L383A, Method C.3**
Author(s), year:	Softcheck, Katherina A., 2012b
Report/Doc. number:	Report No. ROW-0050, Study No. 13048.6703
Guideline(s):	OECD Guideline 201, EC Guideline L383A - C.3
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 060020652
Test species:	Green alga (*Pseudokirchneriella subcapitata)*, class Chlorophyceae
Number of organisms:	1 x 10^4 cells/mL, 3 replicates per treatment group and medium control and 6 replicates per solvent control
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium and solvent control), 0.078, 0.16, 0.31, 1.3, 2.5 and 5.0 mg/L
Measured (mean):	- (medium and solvent control), 0.72, 0.13, 0.26, 0.54, 1.2, 2.3 and 4.4 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL/L
Test conditions:	
Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	23 - 24 °C
pH:	7.2 – 7.3 (0 h), 7.2 – 8.2 (72 h)
Conductivity:	84 - 120 µmhos/cm (0 h), 83 – 120 µmhos/cm (72 h)
Incubation:	Continuous illumination at 4500 to 5900 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Normal distribution and homogeneity of variance: Shapiro Wilks' Test and

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Bartlett's Test
Significance of effects compared to the control: Bonferroni's adjusted t-Test (biomass), Dunnett's Multiple Comparison Test (growth rate)

Findings:

Analytical data: Mean measured concentrations were in the range of 79 to 92% of nominal concentrations over the whole test duration.

Morphological effects: No effects on the morphology and appearance of the cells were observed during the study period.

Biomass, growth rate and cell density: See Table B.9.2.1.3-6 and B.9.2.1.3-7

Table B.9.2.1.3-6: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to S-2167

S-2167 [mg/L] (mean measured)	Cell density (x 10^4 cells/mL), (± SD)		
	24 h	48 h	72 h
Control	5.42 (1.61)	20.50 (6.71)	82.75 (19.52)
Solvent control	5.38 (1.92)	23.88 (5.22)	94.38 (30.17)
0.072	5.42 (1.04)	25.50 (6.06)	77.25 (14.32)
0.13	5.75 (1.09)	20.67 (10.02)	67.58 (12.50)
0.26	5.33 (1.81)	13.58 (4.06)	58.25 (17.35)
0.54	4.25 (0.90)	9.00 (1.75)	32.42 (5.48)
1.2	2.33 (0.88)	9.08 (1.70)	18.00 (3.28)
2.3	2.67 (1.59)	4.92 (1.18)	9.33 (2.04)
4.4	1.58 (0.58)	3.67 (1.01)	7.42 (2.50)

Table B.9.2.1.3-7: Effects of technical S-2167 on the green alga *P. subcapitata*

S-2167 [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72 h)
Control	-	-
Solvent control	-	-
0.072	9	3
0.13	22	7
0.26	38 **	11
0.54	64 **	23 *
1.2	76 **	36 *
2.3	87 **	51 *
4.4	91 **	56 *
NOEC	0.13 mg/L	0.26 mg/L
EC_{10}	0.074 mg/L (n.a. – 0.25 mg/L)	0.24 mg/L (n.a. – 0.48 mg/L)
EC_{20}	0.12 mg/L (n.a. – 0.41 mg/L)	0.47 mg/L (0.17 – 0.78 mg/L)
EC_{50} (95 % C.I.)	0.38 mg/L (0.06 – 0.60 mg/L)	2.2 mg/L (1.5 – n.a. mg/L)

* Significantly different compared to the solvent control, based on Dunnett's Multiple Comparison Test
** Significantly different compared to the solvent control, based on Bonferroni's adjusted t-Test
n.a… not applicable, corresponding 95% confidence limit could not be calculated.

Conclusion: 72 h E_bC_{50} = 0.38 mg/L
72 h E_rC_{50} = 2.2 mg/L
72 h NOEC = 0.13 mg/L (biomass)
72 h NOEC = 0.26 mg/L (growth rate)
based on mean measured concentrations.

Validity criteria: The biomass in the controls (medium and solvent control) should increase by a factor of greater than 16, as recommended in the test guideline.
The mean coefficient of variation (CV) for section-by-section specific growth rates is 28% in the medium control and solvent control, respectively (recommended: <

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35%).
The CV of average specific growth rates during the whole test period is 5% and 7% in the medium control and solvent control, respectively (recommended < 7%).

Metabolites

Reference:	**2-COOH-S-2200 – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986**
Author(s), year:	Softcheck, Katherina A., 2012d
Report/Doc. number:	Report No. ROW-0041, Study No. 13048.6683
Guideline(s):	JMAFF 12 Nohsan Guideline No. 8147,JMAFF 13 Seisan Guideline No. 3986, EC Guideline L383A - C.3
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	2-COOH-S-2200, purity: 99.0 %, batch: 317-001-47-1
Test species:	Green alga (*Pseudokirchneriella subcapitata)*
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment group and 6 replicates per control (medium)
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium control), 6.3, 13, 25, 50 and 100 mg/L
Measured (mean):	- (medium control), 5.7, 12, 23, 49 and 83 mg/L
Solvent:	None
Test conditions:	
Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	24 °C
pH:	4.6 – 7.3 (0 h), 4.5 – 8.0 (72 h)
Conductivity:	92 - 110 µmhos/cm (0 h), 89 - 120 µmhos/cm (72 h)
Incubation:	Continuous illumination at 4500 to 5300 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test Significance of effects compared to the control: Bonferroni's adjusted t-Test

Findings:

Analytical data:	Mean measured concentrations were in the range of 83 - 97% (0 – 72 h) of nominal concentrations.
Morphological effects:	No effects on the morphology and appearance of the cells were observed during

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the study period.

Biomass, cell density & growth rate: See Table B.9.2.1.3-8 and Table B.9.2.1.3-9

Table B.9.2.1.3-8: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to 2-COOH-S-2200

2-COOH-S-2200 [mg/L] (mean measured)	Cell density (x 10^4 cells/mL), (± SD)		
	24 h	48 h	72 h
0 (control)	4.73 (1.53)	21.17 (6.03)	71.58 (10.98)
5.7	6.58 (1.38)	27.58 (2.74)	82.25 (13.86)
12	4.08 (0.80)	24.17 (3.59)	89.00 (19.84)
23	7.25 (0.66)	24.33 (2.47)	84.42 (1.77)
49	3.25 (0.87)	16.67 (1.61)	65.33 (19.28)
83	1.92 (0.58)	1.75 (0.90)	1.17 (1.38)

Table B.9.2.1.3-9: Effects of 2-COOH-S-2200 on the green alga *P. subcapitata*

2-COOH-S-2200 [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72 h)
0 (control)	-	-
5.7	- 23	- 3
12	- 19	- 6
23	- 20	- 4
49	15	3
83	97 *	108 *
NOEC	49 mg/L	49 mg/L
EC_{50} (95 % C.I.)	58 mg/L (50 – 64 mg/L)	62 mg/L (56 – 74 mg/L)

* Significantly different compared to the solvent control, based on Bonferroni's Adjusted t-Test
Negative values indicate an increase of algal growth
n.a… not applicable, corresponding 95% confidence limit could not be calculated.

Conclusion: 72 h E_bC_{50} = 58 mg/L
72 h E_rC_{50} = 62 mg/L
72 h NOEC = 49 mg/L (biomass and growth rate)
based on mean measured concentrations.

Validity criteria: The biomass in the controls (medium control) should increase by a factor of greater than 16, as recommended in the test guideline.
The mean coefficient of variation (CV) for section-by-section specific growth rates is 30% in the medium control (recommended: < 35%).
The CV of average specific growth rates during the whole test period is 3.5% in the medium control (recommended < 7%).

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Reference:	**5-COOH-S-2200 – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986**
Author(s), year:	Softcheck, Katherina A., 2012e
Report/Doc. number:	Report No. ROW-0042, Study No. 13048.6687
Guideline(s):	JMAFF 12 Nohsan Guideline No. 8147, JMAFF 13 Seisan Guideline No. 3986, EC Guideline L383A - C.3
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	5-COOH-S-2200, purity: 97.6 %, batch: 262-005-10-1
Test species:	Green alga (*Pseudokirchneriella subcapitata)*
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment group and 6 replicates per control (medium)
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium control), 6.3, 13, 25, 50 and 100 mg/L
Measured (mean):	- (medium control), 3.3, 6.8, 13, 27 and 54 mg/L
Solvent:	None
Test conditions:	
Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	24 °C
pH:	5.9 – 7.4 (0 h), 7.2 – 7.6 (72 h)
Conductivity:	93 - 110 µmhos/cm (0 h), 100 - 110 µmhos/cm (72 h)
Incubation:	Continuous illumination at 4500 to 5800 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test EC_{50} and NOEC values were empirically estimated.

Findings:

Analytical data:	Mean measured concentrations were in the range of 52 - 55% (0 – 72 h) of nominal concentrations.
Morphological effects:	No effects on the morphology and appearance of the cells were observed during the study period.
Biomass, cell density & growth rate:	See Table B.9.2.1.3-10 and Table B.9.2.1.3-11

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Table B.9.2.1.3-10: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to 5-COOH-S-2200

5-COOH-S-2200 [mg/L] (mean measured)	Cell density (x 10^4 cells/mL), (± SD)		
	24 h	48 h	72 h
0 (control)	4.92 (0.72)	13.96 (3.34)	79.58 (11.98)
3.3	5.58 (0.76)	16.08 (3.76)	81.00 (4.75)
6.8	4.92 (1.42)	17.83 (2.88)	73.00 (6.56)
13	4.00 (1.75)	17.42 (6.39)	73.58 (9.40)
27	4.08 (1.38)	20.17 (1.18)	86.08 (16.93)
54	4.75 (2.41)	17.75 (5.34)	82.50 (20.22)

Table B.9.2.1.3-11: Effects of 5-COOH-S-2200 on the green alga *P. subcapitata*

5-COOH-S-2200 [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72 h)
0 (control)	-	-
3.3	- 6	- 1
6.8	- 1	1
13	1	1
27	- 15	- 2
54	- 9	-1
NOEC	54 mg/L	54 mg/L
EC_{50} (95 % C.I.)	> 54 mg/L (n.a.)	> 54 mg/L (n.a.)

Negative values indicate an increase of algal growth
n.a… not applicable, corresponding 95% confidence limit could not be calculated

Conclusion: 72 h E_bC_{50} > 54 mg/L
72 h E_rC_{50} > 54 mg/L
72 h NOEC = 54 mg/L (biomass and growth rate)
based on mean measured concentrations.

Validity criteria: The biomass in the controls (medium control) should increase by a factor of greater than 16, as recommended in the test guideline.
The mean coefficient of variation (CV) for section-by-section specific growth rates is 30% in the medium control (recommended: < 35%).
The CV of average specific growth rates during the whole test period is 3.4% in the medium control (recommended < 7%).

Reference: **S-2200-OR – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986**

Author(s), year: Softcheck, Katherina A., 2012f
Report/Doc. number: Report No. ROW-0043, Study No. 13048.6691
Guideline(s): JMAFF 12 Nohsan Guideline No. 8147, JMAFF 13 Seisan Guideline No. 3986, EC Guideline L383A - C.3
GLP: Yes
Deviations: None
Validity: Acceptability to be discussed

Material and methods:
Test substance: S-2200-OR, purity: 99.8 %, batch: 09SC8101007-1
Test species: Green alga (*Pseudokirchneriella subcapitata)*
Number of organisms: 1 x 10^4 cells/mL; 3 replicates per treatment group and medium control and 6

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	replicates per solvent control
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium control), 0.63, 1.3, 2.5, 5.0 and 10 mg/L
Measured (mean):	- (medium control), 0.59, 1.2, 2.3, 4.8 and 9.9 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL/L
Test conditions:	
Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	22 - 23 °C
pH:	7.1 – 7.5 (0 h), 7.6 – 7.8 (72 h)
Conductivity:	94 - 100 µmhos/cm (0 h), 89 - 98 µmhos/cm (72 h)
Incubation:	Continuous illumination at 4600 to 5400 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test EC_{50} and NOEC values were empirically estimated.
Findings:	
Analytical data:	Mean measured concentrations were in the range of 92 - 99% (0 – 72 h) of nominal concentrations.
Morphological effects:	No effects on the morphology and appearance of the cells were observed during the study period.
Biomass, cell density & growth rate:	See Table B.9.2.1.3-12 and Table B.9.2.1.3-13

Table B.9.2.1.3-12: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to S-2200-OR

S-2200-OR [mg/L] (mean measured)	Cell density (x 10^4 cells/mL), (± SD)		
	24 h	48 h	72 h
0 (control)	3.42 (0.38)	17.25 (2.95)	62.83 (26.74)
0 (solvent control)	4.08 (1.43)	14.17 (2.71)	68.33 (14.75)
0.59	3.83 (0.63)	13.75 (1.50)	59.08 (10.69)
1.2	2.50 (1.15)	11.50 (1.39)	69.83 (2.65)
2.3	4.58 (2.25)	12.33 (1.42)	67.50 (8.90)
4.8	4.75 (2.41)	10.42 (0.38)	50.00 (4.87)
9.9	4.33 (1.89) [a]	11.75 (0.66)	26.92 (1.18)

[a] An aggregation of cells were observed in the treatment level.

Table B.9.2.1.3-13: Effects of S-2200-OR on the green alga *P. subcapitata*

S-2200-OR [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72 h)
0 (control)	-	-
0 (solvent control)	-	-
0.59	11	3
1.2	7	- 1
2.3	3	0
4.8	25 *	7 *

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S-2200-OR [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72 h)
9.9	46 *	21 *
NOEC	2.3 mg/L	2.3 mg/L
EC_{50} (95 % C.I.)	> 9.9 mg/L (n.a.)	> 9.9 mg/L (n.a.)

Negative values indicate an increase of algal growth
* Significantly different compared to the solvent control, based on Bonferroni's adjusted t-Test
n.a… not applicable, corresponding 95% confidence limit could not be calculated

Conclusion: 72 h E_bC_{50} > 9.9 mg/L
72 h E_rC_{50} > 9.9 mg/L
72 h NOEC = 2.3 mg/L (biomass and growth rate)
based on mean measured concentrations.

Validity criteria: The cell density in the controls (medium and solvent control) increased by a factor of greater than 16, as recommended in the test guideline.
The mean coefficient of variation (CV) for section-by-section specific growth rates is 23% and 27% in the medium control and solvent control, respectively (recommended: < 35%).
The CV of average specific growth rates during the whole test period is 10.2% and 5.0% in the medium control and solvent control, respectively (recommended < 7%). Hence, the validity criteria regarding the CV for 0-hour to 72-hour growth rate for the medium control is not met. The results for the control exceed the required trigger value of 7%. All other acceptance criteria were within the validity criteria according to the OECD guideline. The results of this study (NOEC, EC_{50} values) were calculated as compared to the solvent control.
In addition, there are other aspects which indicate that the study should be considered valid.
The growth of *Pseudokirchneriella subcapitata* were observed in both controls (negative and solvent) is excellent; the toxicity level can be sufficiently evaluated using the data from the study. Moreover, the toxicity is clearly low (i.e., EC_{50} > highest test concentration).
In addition, the variation of CV in the negative control is marginal (10.2% vs. 7%) and CV has generally no influence on EC_{50} evaluation. The results of this study are therefore considered acceptable and appropriately define the toxicity of the metabolite S-2200-OR to *Pseudokirchneriella subcapitata*.

Comment RMS: According to the test guideline both controls, medium and solvent control have to meet the validity criteria, irrespective of whether the results of the study were calculated based on the solvent control only.
However, taken into account the argumentation given by the applicant the RMS can agree on the validity of the study.

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Reference:	**S-2200-ORC – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986**
Author(s), year:	Softcheck, Katherina A., 2012g
Report/Doc. number:	Report No. ROW-0044, Study No. 13048.6695
Guideline(s):	JMAFF 12 Nohsan Guideline No. 8147, JMAFF 13 Seisan Guideline No. 3986, EC Guideline L383A - C.3
GLP:	Yes
Deviations:	None
Validity:	Acceptability to be discussed

Material and methods:

Test substance:	S-2200-ORC, purity: 100 %, batch: 09SC8101007-3
Test species:	Green alga (*Pseudokirchneriella subcapitata)*
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment group and medium control and 6 replicates per solvent control
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium control), 0.63, 1.3, 2.5, 5.0 and 10 mg/L
Measured (mean):	- (medium control), 0.64, 1.3, 2.4, 3.4 and 5.0 mg/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL/L
Test conditions:	
Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	23 – 24 °C
pH:	7.2 – 7.4 (0 h), 7.9 – 8.0 (72 h)
Conductivity:	87 - 90 µmhos/cm (0 h), 90 - 110 µmhos/cm (72 h)
Incubation:	Continuous illumination at 4600 to 5900 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test EC_{50} and NOEC values were empirically estimated.

Findings:

Analytical data:	Mean measured concentrations were in the range of 50 – 100 % (0 – 72 h) of nominal concentrations.
Morphological effects:	No effects on the morphology and appearance of the cells were observed during the study period.
Biomass, cell density & growth rate:	See Table B.9.2.1.3-14 and Table B.9.2.1.3-15

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Table B.9.2.1.3-14: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to S-2200-ORC

S-2200-ORC [mg/L] (mean measured)	Cell density (x 10^4 cells/mL), (± SD)		
	24 h	48 h	72 h
0 (control)	5.00 (1.00)	17.25 (3.68)	48.67 (14.10)
0 (solvent control)	5.17 (1.86)	20.96 (5.03)	60.71 (6.67)
0.64	4.33 (0.52)	19.50 (0.25)	51.33 (3.88)
1.3	3.83 (1.91)	17.25 (5.48)	57.00 (10.64)
2.4	6.67 (3.02)	22.17 (2.36)	58.33 (11.41)
3.4	4.08 (1.42)	16.67 (5.30)	51.25 (6.77)
5.0	3.67 (0.38	15.00 (3.31)	33.17 (2.25)

Table B.9.2.1.3-15: Effects of S-2200-ORC on the green alga *P. subcapitata*

S-2200-ORC [mg/L] (mean measured)	Percent inhibition relative to the solvent control [%]	
	Biomass (0 – 72 h)	Growth rate (0 – 72)
0 (control)	-	-
0 (solvent control)	-	-
0.64	13	4
1.3	13	2
2.4	- 3	1
3.4	19	4
5.0	40 *	15 *
NOEC	3.4 mg/L	3.4 mg/L
EC_{50} (95 % C.I.)	> 5.0 mg/L (n.a.)	> 5.0 mg/L (n.a.)

Negative values indicate an increase of algal growth
* Significantly different compared to the solvent control, based on Bonferroni's adjusted t-Test
n.a… not applicable, corresponding 95% confidence limit could not be calculated

Conclusion: 72 h E_bC_{50} > 5.0 mg/L
72 h E_rC_{50} > 5.0 mg/L
72 h NOEC = 3.4 mg/L (biomass and growth rate)
based on mean measured concentrations.

Validity criteria: The biomass in the controls (medium control and solvent control) should increase by a factor of greater than 16, as recommended in the test guideline.
The mean coefficient of variation (CV) for section-by-section specific growth rates is 22% and 33% in the medium control and solvent control, respectively (recommended: < 35%).
The CV of average specific growth rates during the whole test period is 7.6% and 2.8% in the medium control and solvent control, respectively (recommended < 7%).
Hence, the validity criteria regarding the CV for 0-hour to 72-hour growth rate for the medium control is not met. The results for the control exceed the required trigger value of 7%. All other acceptance criteria were within the validity criteria according to the OECD guideline. The results of this study (NOEC, EC_{50} values) were calculated as compared to the solvent control.
In addition, there are other aspects which indicate that the study should be considered valid.
The growth of *Pseudokirchneriella subcapitata* were observed in both controls (negative and solvent) is excellent; the toxicity level can be sufficiently evaluated using the data from the study. Moreover, the toxicity is clearly low (i.e., EC_{50} > highest test concentration).
In addition, the variation of CV in the negative control is marginal (7.6% vs. 7%) and CV has generally no influence on EC_{50} evaluation.

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The results of this study are therefore considered acceptable and appropriately define the toxicity of the metabolite S-2200-ORC to *Pseudokirchneriella subcapitata*.

Comment RMS: According to the test guideline both controls, medium and solvent control have to meet the validity criteria, irrespective of whether the results of the study were calculated based on the solvent control only.
However, taken into account the argumentation given by the applicant the RMS can agree on the validity of the study.

Formulation

Reference:	**S-2200 25% SC – 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986**
Author(s), year:	Softcheck, Katherina A., 2011
Report/Doc. number:	Report No. ROW-0026, Study No. 13048.6676
Guideline(s):	JMAFF 12 Nohsan Guideline No. 8147, JMAFF 13 Seisan Guideline No. 3986, EC Guideline L383A - C.3
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	S-2200 25% SC, purity: 24.96%, batch: C09-5F101G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 060020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 060020652
Test species:	Green alga (*Pseudokirchneriella subcapitata)*
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment group, 6 replicates per control
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (medium control), 0.23, 0.52, 1.12, 2.48, 5.6 and 12 mg form./L equivalent to 0.058, 0.13, 0.28, 0.62, 1.4 and 3.0 mg ai/L
Measured (mean):	- (medium control), 0.55, 0.12, 0.26, 0.56, 1.3 and 2.8 mg ai/L
Solvent:	None
Test conditions:	
Test medium:	Algal Assay Procedure (AAP) medium (according to guideline), initial pH adjusted to 7.5 ± 0.1
Temperature:	23 - 24 °C
pH:	7.2 – 7.4 (0 h), 7.5 – 8.7 (72 h)
Conductivity:	86 - 89 µmhos/cm (0 h), 91 - 98 µmhos/cm (72 h)
Incubation:	Continuous illumination at 4500 to 5300 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope at 24-hour intervals. Observations of the health and morphology of the algal cells were made under the microscope at each 24-hour interval. For chemical analysis (HPLC/UV method) of test the substance, samples of test solution were taken at test initiation and at test termination. Measurements of pH and conductivity were made at initiation and at test termination; light intensity was measured at 0 hour and at each 24-hour interval.

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Statistics: Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test
Significance of effects compared to the control: Bonferroni's adjusted t-Test

Findings:

Analytical data: S-2200 consists of a mixture of two isomers, S-2354 and S-2167. The final S-2200 concentration was derived from the sum of the concentration of the two isomers.
Mean measured concentrations were in the range of 91 – 94 % of nominal concentrations over the whole test duration. See Table B.9.2.1.3-16.

Table B.9.2.1.3-16: Concentrations measured during the 72 h static exposure of algae to S-2200 technical grade

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]							Percent of nominal [%]
	0-hour			72-hour			Mean	
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.002	< 0.002	< 0.004	< 0.002	< 0.002	< 0.004	-	-
0.055	0.03	0.028	0.058	0.037	0.014	0.051	0.055	94
0.12	0.063	0.063	0.13	0.069	0.040	0.11	0.12	91
0.26	0.13	0.13	0.26	0.16/0.13 [b]	0.10/0.11 [b]	0.26/0.24 [b]	0.26	92
0.56	0.28	0.29	0.57	0.31	0.25	0.55	0.56	91
1.3	0.65	0.67	1.3	0.72	0.52	1.2	1.3	92
2.8	1.4	1.4	2.9	1.4	1.3	2.7	2.8	93

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
[b] Results of the additional sample without algae present to determine biological uptake/degradation.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Morphological effects: After 72 h of exposure cells were observed to be normal in all control and treatment groups.

Biomass, growth rate and cell density: See Table B.9.2.1.3-17 and Table B.9.2.1.3-18

Table B.9.2.1.3-17: Cell density of *P. subcapitata* after 24, 48 and 72 h of exposure to S-2200 25% SC

S-2200 [mg/L] (mean measured)	Cell density (x 10^4 cells/mL), (± SD)		
	24 h	48 h	72 h
0 (control)	5.71 (1.21)	26.33 (4.45)	95.13 (10.11)
0.055	4.75 (1.75)	20.92 (4.62)	93.67 (15.32)
0.12	7.17 (1.66)	38.33 (7.25)	109.75 (9.56)
0.26	6.50 (1.56)	26.00 (3.54)	86.75 (15.69)
0.56	5.58 (1.81)	20.17 (2.50)	68.25 (16.09)
1.3	4.67 (1.28)	13.67 (2.47)	43.42 (4.82)
2.8	2.67 (1.76)	10.75 (1.39)	27.08 (1.38)

Table B.9.2.1.3-18: Effects of the formulation S-2200 25% SC on the green alga *P. subcapitata*

S-2200 25% SC [mg/L] (nominal)	S-2200 [mg ai/L] (mean measured)	Percent inhibition relative to the control	
		Biomass (0 – 72 h)	Growth rate (0 – 72)
0 (control)	0 (control)	-	-
0.23	0.055	9	0
0.52	0.12	- 27	- 3
1.12	0.26	5	2
2.48	0.56	26 *	7 *
5.6	1.3	51 *	17 *

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S-2200 25% SC [mg/L] (nominal)	S-2200 [mg ai/L] (mean measured)	Percent inhibition relative to the control	
		Biomass (0 – 72 h)	Growth rate (0 – 72)
12	2.8	68 *	27 *
NOEC		0.26 mg ai/L	0.26 mg ai/L
EC_{50} (95 % C.I.)		1.2 mg ai/L (0.72 – 1.8 mg ai/L)	> 2.8 mg ai/L (n.a.)

Negative values indicate an increase of algal growth compared to the control.
* Significantly reduced compared to the control, based on Bonferroni's adjusted t-Test.
n.a… not applicable, corresponding 95% confidence limit could not be calculated

Conclusion: 72 h E_bC_{50} = 1.2 mg ai/L
72 h E_rC_{50} > 2.8 mg ai/L
72 h NOEC = 0.26 mg ai/L (biomass and growth rate)
based on mean measured concentrations.

Validity criteria: The cell density in the medium control increased by a factor of greater than 16, as recommended in the test guideline.
The mean coefficient of variation (CV) for section-by-section specific growth rates is 22% in the medium control (recommended: < 35%).
The CV of average specific growth rates during the whole test period is 2.6% in the medium control (recommended < 7%).

B.9.2.1.4 Effects on aquatic plants (Annex IIA 8.2.8)

According to the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev. 4 final) a test on higher aquatic plants (*Lemna sp.*) is required for herbicides only. The proposed use of S-2200 is as fungicide for control of white mould in winter oilseed rape, thus data on the effects on higher aquatic plants are not required.

B.9.2.1.5 Effects on marine or estuarine organisms (Annex IIA 8.11, IIIA 10.2.2.4, IIIA 10.2.2.5)

No toxicity studies with marine or estuarine organisms were submitted by the notifier.

B.9.2.1.6 Summary of acute toxicity

Table B.9.2.1.6-1: Summary of acute toxicity data for aquatic organisms (based on mean measured concentrations)

Test organism	Test condition	Time	Endpoint	NOEC [mg/L]	EC_{50}/LC_{50} [mg/L]	Reference
S-2200 technical grade						
Oncorhynchus mykiss Rainbow trout	static	96 h	Mortality	0.57	0.94	[redacted] 2009a
Lepomis macrochirus Bluegill sunfish	static	96 h	Mortality	0.56	2.3	[redacted] 2009d
Pimephales promelas Fathead minnow	static	96 h	Mortality	0.36	1.0	[redacted] 2009e
Daphnia magna Water flea	static	48 h	Immobility	0.70	1.2	Sayers, L.E., 2010a
P. subcapitata Green algae	static	72 h	Biomass Growth rate	0.067 0.067	0.67 3.4	Softcheck, K.A., 2012a
S-2354 (*S*-isomer of S-2200)						

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Test organism	Test condition	Time	Endpoint	NOEC [mg/L]	EC_{50}/LC_{50} [mg/L]	Reference
Oncorhynchus mykiss Rainbow trout	static	96 h	Mortality	1.7	> 12	[redacted]., 2009b
Daphnia magna Water flea	static	48 h	Immobility	7.3	> 14	[redacted] 2012f
P. subcapitata Green algae	static	72 h	Biomass Growth rate	6.0 6.0	12 > 12	Softcheck, K.A., 2012c
S-2167 (*R*-isomer of S-2200)						
Oncorhynchus mykiss Rainbow trout	static	96 h	Mortality	0.34	0.84	[redacted] 2009c
Daphnia magna Water flea	static	48 h	Immobility	0.61	0.92	[redacted] 2012e
P. subcapitata Green algae	static	72 h	Biomass Growth rate	0.13 0.26	0.38 2.2	Softcheck, K.A., 2012b
Metabolite 2-COOH-S-2200						
Oncorhynchus mykiss Rainbow trout	static [a]	96 h	Mortality	89	> 89	[redacted] 2012a
Daphnia magna Water flea	static	48 h	Immobility	100	> 100	[redacted] 2012g
P. subcapitata Green algae	static	72 h	Biomass Growth rate	49 49	58 62	Softcheck, K.A., 2012d
Metabolite 5-COOH-S-2200						
Oncorhynchus mykiss Rainbow trout	static [a]	96 h	Mortality	100	> 100	[redacted] 2012b
Daphnia magna Water flea	static	48 h	Immobility	100	> 100	[redacted] 2012h
P. subcapitata Green algae	static	72 h	Biomass Growth rate	54 54	> 54 > 54	Softcheck, K.A., 2012e
Metabolite S-2200-OR						
Oncorhynchus mykiss Rainbow trout	static	96 h	Mortality	9.0	> 9.0	[redacted] 2012c
Daphnia magna Water flea	static	48 h	Immobility	6.7	> 14	[redacted] 2012i
P. subcapitata Green algae	static	72 h	Biomass Growth rate	2.3 2.3	> 9.9 > 9.9	Softcheck, K.A., 2012f
Metabolite S-2200-ORC						
Oncorhynchus mykiss Rainbow trout	static	96 h	Mortality	1.4	4.0	[redacted] 2012d
Daphnia magna Water flea	static	48 h	Immobility	2.5	9.6	[redacted] 2012j
P. subcapitata Green algae	static	72 h	Biomass Growth rate	3.4 3.4	> 5.0 > 5.0	Softcheck, K.A., 2012g
Formulation – S-2200 25% SC						
Oncorhynchus mykiss Rainbow trout	static	96 h	Mortality	1.8 form. (0.45 ai)	4.4 form. (1.1 ai)	[redacted] 2011a
Daphnia magna Water flea	static	48 h	Immobility	1.5 form. (0.36 ai)	2.68 form. (0.67 ai)	[redacted] 2011b

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Test organism	Test condition	Time	Endpoint	NOEC [mg/L]	EC_{50}/LC_{50} [mg/L]	Reference
P. subcapitata Green algae	static	72 h	Biomass Growth rate	1.12 form. (0.26 ai) 1.12 form. (0.26 ai)	5.2 form. 1.2 ai > 12 form. (> 2.8 ai)	Softcheck, K.A., 2011

[a] Limit test

B.9.2.2 Chronic toxicity of active substance, metabolites and formulations to aquatic organisms

B.9.2.2.1 Prolonged toxicity (21 day exposure) to fish (Annex IIA 8.2.2.1, IIIA 10.2.4)

No study submitted. The requirement for data on the chronic effects of S-2200 on juvenile fish has been addressed by the submission of an early life stage toxicity test (ELS-test) with fathead minnow (*Pimephales promelas*).

B.9.2.2.2 Fish early life stage toxicity test (Annex IIA 8.2.2.2)

Active substance

Reference:	**S-2200 Technical Grade – Early Life-Stage Toxicity Test with Fathead Minnow, *Pimephales promelas*, Following OECD Guideline #210 and OPPTS Draft Guideline 850.1400**
Author(s), year:	[redacted] 2010
Report/Doc. number:	Report No. ROW-0019, Study No. 13048.6626
Guideline(s):	OECD Guideline 210, US EPA OPPTS 850.1400
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	S-2200 technical grade, purity: 93.4 %, batch: ST-0811G
Reference substances:	S-2354 (S-2200 *S*-isomer), purity: 99.7 %, batch: 060020653 S-2167 (S-2200 *R*-isomer), purity: 100 %, batch: 060020652
Test species:	Fathead minnow (*Pimephales promelas*)
Number of organisms:	2 replicates per test concentration, control and solvent control. 30 eggs per egg incubation cup, after completion of hatch larvae were thinned to 10 individuals per replicate, 40 individuals per treatment level or controls.
Age:	Freshly fertilized eggs, 2.5 hours old
Type of test, duration:	Flow-through test, 32 days (28 days post hatch)
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.018, 0.031, 0.052, 0.088 and 0.15 mg ai/L
Measured (mean):	- (control and solvent control), 0.021, 0.030, 0.051, 0.087 and 0.15 mg ai/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 3 µg/L
Test conditions:	
Water quality:	Well water, total hardness: 60 – 76 mg/L as $CaCO_3$, total alkalinity: 20 – 24 mg/L as $CaCO_3$, specific conductance: 420 – 450 µmhos/cm
Temperature:	24 – 26 °C
pH:	6.5 – 8.0 during the total test period

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O_2 content: 6.0 – 8.9 mg O_2/L (> 60 % saturation)

Light regime: 16 hours light / 8 hours darkness, sudden transitions were avoided

Feeding: Larvae were fed of live brine shrimp nauplii (*Artemia salina*) 3 times daily beginning on day 4 post-hatch. Larvae were not fed during the final 24 hours of the test.
Residual food and fecal matter were brushed and siphoned when necessary in order to minimise microbiological growth.

Test parameters: Abnormal appearance and behaviour of larvae were assessed daily. Number of surviving larvae was estimated at least twice a week. At test termination the length and the weight were determined.
Determined endpoints were: Hatching success, overall fry survival, mean length, wet and dry weight.
Temperature, pH and dissolved oxygen concentration were measured daily. Total hardness, alkalinity and specific conductance were measured weekly.
Analytical measurements (HPLC/UV) of S-2200 in test solutions samples were taken at 0, 4, 11, 18, 25 and 32 days.

Statistics: If control and solvent control can be pooled: t-Test
Hatching success, percentage normal larvae at hatch and percentage larval survival: Arscine square-root percentage formation
Testing for normal distribution: Shapiro Wilks' Test
Homogeneity of variance: Bartlett's Test, Levene's Test
Data met the assumptions for normal distribution and homogeneity of variance: Williams test

<u>Findings:</u>

Analytical data: Overall mean measured concentrations in test media were 97 - 120 % of nominal. Table B.9.2.2.2-1

Table B.9.2.2.2-1: Concentrations measured in exposure solution during the early life-stage exposure of fathead minnow to S-2200 technical grade

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a] Day 0 S-2167	Day 0 S-2354	Day 0 S-2200	Day 32 S-2167	Day 32 S-2354	Day 32 S-2200	Mean	Percent of nominal [%]
Control	< 0.0063	< 0.0063	< 0.013	< 0.0063 [a]	< 0.0063	< 0.013	n.a.	n.a.
Solvent control	< 0.0063	< 0.0063	< 0.013	< 0.0063 [a]	< 0.0063	< 0.013	n.a.	n.a.
0.018	0.011	0.010	0.021	0.011	0.011	0.022	0.021	120
0.031	0.016	0.015	0.031	0.015	0.016	0.031	0.030	98
0.052	0.026	0.025	0.051	0.026	0.026	0.052	0.051	97
0.088	0.043	0.043	0.086	0.045	0.046	0.090	0.087	99
0.15	0.078	0.074	0.15	0.078	0.076	0.15	0.15	98

n.a.…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).

Biological observation: Total length of larvae: No significant differences were observed in the mean total length of the larvae from the treatment groups (26.8 – 27.66 mm) and the solvent control groups (27.7 mm).
Dry weight of larvae: No significant differences were observed in the mean dry weight of the larvae from the treatment groups (0.0455 – 0.0488 g) and the pooled control groups (0.0492 g).
Time to hatch: In controls and all treatment levels hatching completed on day 4.
Morphological and behavioural effects: Over the total test period no morphological

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and behavioural effects were observed.

Effects: No significant effects on the hatching success and fry survival were observed.
See Table B.9.2.2.2-2 and Table B.9.2.2.2-3

Table B.9.2.2.2-2: Hatching success and fry survival

S-2200 [mg ai/L] (mean measured)	Mean embryo hatching success [%]	Normal larvae at hatch (mean) [%]	Mean larval survival after 28 days post-hatch [%]
Control	89	99	95
Solvent control	90	98	95
Pooled control [a]	90	99	95
0.021	83	100	90
0.030	88	93	95
0.051	91	100	95
0.087	91	93	88
0.15	90	100	93
NOEC = 0.15 mg ai/L			
LOEC > 0.15 mg ai/L			
MATC > 0.15 mg ai/L			

[a] No statistically significant difference between dilution control and solvent control (t-Test)

Table B.9.2.2.2-3: Length and Weight

S-2200 [mg a.s/L] (mean measured)	Mean length [mm] (SD) (28 d post-hatch)	Mean dry weight [g] (SD) (28 d post-hatch)
Control	28.2 (0.19)	0.0505 (0.0019)
Solvent control	27.7 (0.27)	0.0479 (0.0025)
Pooled control [a]	n.a. [b]	0.0492 (0.0025)
0.021	27.6 (1.64)	0.0472 (0.0063)
0.030	27.4 (0.70)	0.0471 (0.0039)
0.051	27.5 (0.46)	0.0488 (0.0021)
0.087	26.8 (2.58)	0.0478 (0.0076)
0.15	27.5 (0.79)	0.0455 (0.0025)
NOEC = 0.15 mg ai/L		
LOEC > 0.15 mg ai/L		
EC_{50} > 0.15 mg ai/L		

[a] No statistically significant difference between dilution control and solvent control.
[b] Not applicable. Total length data was compared to the solvent control data.

Conclusion: 32 d NOEC = 0.15 mg ai/L (growth, survival)
32 d LOEC > 0.15 mg ai/L
32 d EC_{50} > 0.15 mg ai/L
based on mean measured concentrations

Validity criteria: The dissolved oxygen concentration was above 60 % air saturation throughout the test (recommended: 60 – 100 %).
A solubilising agent (DMF) was used. No significant effects on survival nor on the early life-stages were revealed by the solvent-only control.
The overall survival of fertilised eggs in the controls and the solvent-only controls was greater than 60% for fathead minnow.
The water temperature did not differ by more than ± 1.5 °C between test chambers or between successive days at any time during the test and was within the temperature ranges species for fathead minnow (25 ± 2 °C)

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B.9.2.2.3 Fish life cycle test (Annex IIA 8.2.2.3)

No study was submitted. The BCF of the active substance S-2200 is 25 – 26 and thus clearly less than 1000. In addition there was more than 95 % elimination of S-2200 residues in fish in 14 days during the depuration phase of the fish bioaccumulation study.
The acute toxicity to fish was determined to be greater than 0.1 mg/L (LC_{50} = 0.94 mg ai/L). The active substance was identified to be persistent in water and sediment (DT_{90} > 100 d) based on a worst-case DT_{90} of 1725 days. However under consideration of the low toxicity to fish and the low potential of bioaccumulation, no FLC-study is considered required.

B.9.2.2.4 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5, IIIA 10.2.4)

Active substance

Reference:	**S-2200 Technical Grade – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna* Under Flow-Through Conditions, Following OPPTS Draft Guideline 850.1300, OECD Guideline #211, The Official Journal of the European Communities L225, Method C.20, JMAFF 12 NohSan, No. 8147 *Daphnia spp*. Reproduction Toxicity Studies (2-7-2-3) and JMAFF 13 SeiSan No. 3986**
Author(s), year:	Sayers, Lee E., 2010
Report/Doc. number:	Report No. ROW-0020, Study No. 13048.6639
Guideline(s):	OECD Guideline 211, OPPTS Draft Guideline 850.1300, EC Guideline L225, Method C.20, JMAFF 12 NohSan Guideline No. 8147, JMAFF 13 SeiSan Guideline No. 3986
GLP:	Yes
Deviations:	The protocol states that samples will be removed from each control and test concentration on days 0, 8, 10, 14, 16, 20 and 21. During this exposure, 100% immobilisation was observed in the 1.2 mg ai/L (nominal) treatment level as of day 3 of exposure. Samples were removed from the aged solution at this concentration at day 8 but additional solutions at this concentration were not prepared or analysed at subsequent intervals. This deviation did not have a negative impact on the results or interpretation of the study. The protocol states that dissolved oxygen levels will not be allowed to drop below 60% of saturation for the duration of the study. At several intervals during this study, dissolved oxygen levels in aged test solutions of the solvent control, 0.075, 0.15, 0.30 and 0.60 mg ai/L (nominal) treatment levels were found to be below 60% of saturation. Since these deviations were observed in the aged test solutions, which were renewed at 48-hour intervals, and no adverse effects were observed throughout the study at these concentrations, this deviation did not have a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	S-2200 technical grade, purity: 93.4 %, batch: ST-0811G
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.7%, batch: 60020653 S-2167 (S-2200 *R*-isomer), purity: 100%, batch: 60020652
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	10 replicates per treatment group and controls, each with 10 daphnids
Age:	First instar, < 24 hours old

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Type of test, duration:	Flow-through test, 21 d
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.075, 0.15, 0.30, 0.60 and 1.2 mg ai/L
Measured (mean):	- (control and solvent control), 0.076, 0.15, 0.31, 0.56 and 1.4 mg ai/L
Solvent:	Acetone (CAS No. 67-64-1)
Test conditions:	
Water quality:	Fortified well water, hardness: 180 – 190 mg/L as $CaCO_3$, alkalinity: 86 – 100 mg/L as $CaCO_3$, specific conductivity: 600 – 650 µmhos/cm
Temperature	19 – 21 °C
pH	7.5 – 9.0
O_2 content:	New solution: 8.4 – 11 mg O_2/L (> 60 % saturation) Old solution: 3.8 – 9.7 mg O_2/L (40 – 110 % saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding	Daphnids were fed daily with 200 µL of algal suspension (*Ankistrodesmus falcatus,* 4 x 10^7 cells/mL), and 50 µL mL of a yeast, cereal leaves and digested flaked fish food suspension (equivalent to 0.43 mg carbon/daphnid/day).
Test parameters:	At each renewal period (test initiation and at 48-hour intervals thereafter), freshly prepared test solution was added so a second set of clean beakers and daphnids were carefully transferred from the aged solution into the freshly prepared test solutions. Parent mobility, mortality and abnormal behaviour were observed daily. Reproduction (mean time to first brood, age at first brood, offspring per surviving parental) were observed on day 7 and three times per week through day 21. At test termination body length and parental body mass (dry weight) were reported. For chemical analysis (LC/MS/MS) of S-2200 in the newly prepared test media samples were taken on days 0, 8, 14 and 20 from each test concentration. Additionally, aged test solutions were sampled and analysed on test days 2, 10, 16 and 21. Measurements of pH, dissolved oxygen and temperature in each test and control solution were made at initiation and end of each renewal period. Total hardness, alkalinity and specific conductance were measured and recorded in the freshly prepared solutions of the highest available nominal test concentration and the control at test initiation and weekly thereafter.
Statistics:	Comparison of the number of surviving daphnids in the control to each mean measured concentration: Fisher's Exact Test Comparison of the performance of the control organisms with that of the solvent control organisms: Student's t-Test Chi-Square Test for normality was used to compare the observed sample distribution with a normal distribution for all endpoints. Check of homogeneity of variance (reproduction, length and weight): Bartlett's Test Normal distribution and homogeneity of variance (reproduction and growth): William's Test NOEC and EC_{50} were empirically estimated. TOXSTAT® version 3.5 was used to perform the statistical computations.
Findings:	
Analytical data:	The mean measured concentrations ranged from 94 - 120% of nominal concentrations. See Table B.9.2.2.4-1

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Table B.9.2.2.44-1: Concentrations measured in exposure solution during the 21-day chronic exposure of daphnids to S-2200 technical grade

S-2200 [mg ai/L] (nominal)	Measured concentration [mg ai/L] [a]							Percent of nominal [%]
	Day 0 (new)			Day 21 (aged)			Mean	
	S-2167	S-2354	S-2200	S-2167	S-2354	S-2200		
Control	< 0.005	< 0.005	< 0.01	< 0.005	< 0.005	< 0.01	n.a.	n.a.
Solvent control	< 0.005	< 0.005	< 0.01	< 0.005	< 0.005	< 0.01	n.a.	n.a.
0.075	0.039	0.042	0.082	0.038	0.036	0.074	0.076	100
0.15	0.077	0.079	0.016	0.073	0.076	0.15	0.15	98
0.30	0.16	0.17	0.32	0.16	0.17	0.33	0.31	100
0.60	0.20	0.21	0.41	0.36	0.37	0.73	0.56	94
1.2	0.70	0.75	1.4	[b]	[b]	[b]	1.4	120

n.a…not applicable
[a] Mean measured concentrations (as S-2200) and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
Concentrations expressed as less than values were below the minimum detectable limit (MDL).
[b] Samples were not analysed due to 100% immobilisation in this treatment group.

Biological observation: In the dilution water control and solvent control daphnids released their first brood of offspring on test day 9 and day 8, respectively. In the treatment groups first brood release occurred on test day 7, 7, 9 and 8 in the 0.076, 0.15, 0.31 and 0.56 mg ai/L treatment levels, respectively. Due to 100% immobilisation by test day 3, no offspring were released in the 1.4 mg ai/L treatment level.

Effects: See Table B.9.2.2.4-2

Table B.9.2.2.4-2: Summary of effects of long-term exposure of S-2200 on *Daphnia magna*

S-2200 [mg ai/L] (nom)	S-2200 techn. [mg ai/L] (mm)	Mean percent survival at day 21 [%]	Mean number of offspring per surviving female at day 21 (SD)	Mean (SD) Dry weight of parent after 21 d [mg]	Mean (SD) body length of parent after 21 d [mm]
Control	Control	90	135 (10)	1.09 (0.08)	4.86 (0.07)
Solvent control	Solvent control	90	139 (13)	1.14 (0.13)	4.81 (0.12)
Pooled control [a]	Pooled control	90	137 (11)	1.12 (0.11)	4.83 (0.10)
0.075	0.076	90	147 (16)	1.03 (0.13)	4.89 (0.06)
0.15	0.15	90	138 (9)	1.05 (0.07)	4.84 (0.06)
0.30	0.31	100	138 (11)	1.12 (0.08)	4.86 (0.07)
0.60	0.56	100	128 (21)	1.13 (0.09)	4.86 (0.11)
1.2	1.4	0 [b]	n.a.	n.a.	n.a.
NOEC (based on mean measured)		0.56 mg ai/L	0.56 mg ai/L	0.56 mg ai/L	0.56 mg ai/L
LOEC (based on mean measured)		1.4 mg ai/L	> 0.56 mg ai/L	> 0.56 mg ai/L	> 0.56 mg ai/L
EC_{50} (based on mean measured)		0.97 mg ai/L	> 0.56 mg ai/L	> 0.56 mg ai/L	> 0.56 mg ai/L
MATC (based on mean measured)		0.89 mg ai/L			

S.D…Standard Deviation, n.a…not applicable, mm…mean measured, nom…nominal
[a] No statistically significant difference between control and solvent control.
[b] Significantly reduced compared to the pooled control, based on the Fisher Exact Test. This treatment level was excluded from further statistical analysis (i.e. reproduction and growth) due to the survival effect observed.

Conclusion: NOEC = 0.56 mg ai/L (immobilisation, reproduction, growth, weight and length)
LOEC > 0.56 mg ai/L (reproduction, growth, weight and length)
EC_{50} = 0.97 mg ai/L (immobilisation)
EC_{50} > 0.56 mg ai/L (reproduction, growth, weight and length)
based on mean measured concentrations

Validity criteria: An immobilisation of daphnids of 10% was observed in the control and solvent control at the end of the test which is in the line with the recommended maximum

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immobilization of 20% according to the OECD guideline. The mean number of living offspring produced per parent animal surviving at the end of the test is ≥ 60 (actual: 135 – 139).

B.9.2.2.5 Chronic toxicity to sediment dwelling organisms (Annex IIA 8.2.7)

Reference:	**S-2200 – Toxicity Test with Sediment-Dwelling Midges (*Chironomus riparius*) Under Static Conditions, Following OECD Guideline 219**
Author(s), year:	Picard, Christian R., 2012
Report/Doc. number:	Report No. ROW-0047, Study No. 13048.6671
Guideline(s):	OECD Guideline 219
GLP:	Yes
Deviations:	The protocol stated that 20 midge larvae will be added to each test vessel. In this study, greater than 20 midge larvae were inadvertently added to one replicate of the control group. A total of 21 midge emerged from the replicate and a percent emergence of 100% was utilised for statistical analyses. Based on the high emergence for all replicates in the control group and all other test groups, this deviation did not have a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:	
Test substance:	S-2200 technical grade, purity: 93.4%, batch: ST-0811G [benzyl-^{14}C] S-2200, radiochemical purity 98.1 – 98.9%, batch: CFQ40467
Reference substance:	MCBX, purity: 96.9%, batch: CTS08015 2-COOH-S-2200, purity: 99.0%, batch: 317-001-47-1 5-COOH-S-2200, purity: 97.6%, batch: 262-005-10-1
Test species:	Midge (*Chironomus riparius*)
Number of organisms:	4 replicates for each treatment level and for the solvent and water controls, 20 midge larvae per replicate
Age:	First instar, 3 days old
Type of test, duration:	Static water/sediment test system (spiked water exposure), 28 d
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.68, 1.5, 3.3, 7.3 and 16 mg ai/L
Initial measured:	(control and solvent control), 0.64, 1.4, 2.7, 4.8 and 8.1 mg ai/L
Solvent:	Acetone (CAS No. 67-64-1)
Test system:	
Water quality:	Laboratory well water, hardness: 56 mg/L as $CaCO_3$, alkalinity: 24 mg/L as $CaCO_3$, pH = 7.0, conductivity: 270 µmhos/cm
Sediment:	Artificial sediment according to OECD 218 and 219 (6.0% sphagnum peat, 20% kaolin clay, 74% fine sand), 1.8% organic carbon content, pH = 7.0, particle size distribution: 77% sand, 4% silt and 19% clay
Size of test vessels:	600 mL glass beakers, 1.5 cm sediment layer
No. of replicates:	4 replicates per treatment level, control and solvent control for biological evaluation and 4 replicates per treatment level, control and solvent control for chemical analysis.
Test condition:	
Temperature:	19 - 20 °C
pH:	5.8 – 8.3

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Hardness: 48 - 64 mg $CaCO_3$/L
Alkalinity: 16 – 46 mg $CaCO_3$/L
Conductivity: 490 – 630 µmhos/cm
Ammonia: 0.15 – 0.60 mg N/L
O_2 content: 6.7 – 9.1 mg O_2/L (> 60 % saturation)
Light regime: 16 hours light / 8 hours darkness, intensity: 520 – 790 lux
Feeding: The midge larvae were fed a suspension of fish food, initially at a daily rate of about 1 mL per vessel until day 10 and thereafter at approximately 2.0 mL per vessel and day until the end of the test.
Test parameters: Midges (behaviour, emergence,…) were examined at test day -1 and daily thereafter, until test termination (day 28). The sex, the time point of emergence and the number of emerged midges were recorded daily from day 10 post-treatment onwards.
For chemical analysis (HPLC-UV/RAM) of S-2200 in the overlaying water column, pore water and sediment samples were taken from fresh at day 0 (test initiation), day 7 and day 28.
Measurements of temperature, pH and dissolved oxygen concentration were made at daily intervals in an alternate replicate vessel of each treatment level and the controls during the 28-day exposure. In addition, measurement of temperature, pH and dissolved oxygen were made on the day the test organisms were added (day -1) and application of the test substance (day 0) and at test termination (day 28) in each exposure vessel.
Total hardness, alkalinity, conductivity, and total ammonia of the test solution were determined on day 0 and at test termination in a composite sample from the highest treatment level and control solution.
Statistics: Comparison of control groups: t-test for homogeneous variances.
Data for all endpoints (midge emergence, male/female combined development rate): Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test.
Emergence and development rate: Equal Variance t Two-Sample Test
EC_{50}, NOEC: empirically estimated to be greater than the highest concentration tested.

Findings:
Analytical data: See Table B.9.2.2.5-1

Table B.9.2.2.5-1: Measured concentrations of S-2200 in the overlaying water, the pore water and the sediment

S-2200 [mg ai/L]	Day 0		Day 7		Day 28	
	Measured concentration	% of nominal	Measured concentration	% of nominal	Measured concentration	% of nominal
	Overlaying water [mg total ^{14}C-residues/L]					
Control	< 0.0050	n.a.	< 0.0051	n.a.	< 0.0050	n.a.
Solvent control	< 0.0050	n.a.	< 0.0050	n.a.	< 0.0050	n.a.
0.68	0.64	94	0.31	45	0.26	39
1.5	1.4	94	0.76	51	0.60	40
3.3	2.7	80	1.6	49	1.3	41
7.3	4.8	66	3.8	52	3.1	42
16	8.1	51	5.6	35	5.4	34
	Pore water [mg total ^{14}C-residues/L]					
Control	< 0.012	n.a.	< 0.012	n.a.	< 0.012	n.a.
Solvent control	< 0.012	n.a.	< 0.012	n.a.	< 0.012	n.a.

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S-2200 [mg ai/L]	Day 0		Day 7		Day 28	
	Measured concentration	% of nominal	Measured concentration	% of nominal	Measured concentration	% of nominal
0.68	0.014	n.a.	0.11	n.a.	0.21	n.a.
1.5	0.095	n.a.	0.22	n.a.	0.51	n.a.
3.3	0.12	n.a.	0.46	n.a.	1.2	n.a.
7.3	0.36	n.a.	1.2	n.a.	2.8	n.a.
16	1.2	n.a.	1.7	n.a.	4.2	n.a.
	Sediment [mg total ^{14}C-residues/kg]					
Control	< 0.0053	n.a.	< 0.0052	n.a.	< 0.0053	n.a.
Solvent control	< 0.0053	n.a.	< 0.052	n.a.	< 0.054	n.a.
0.68	< 0.0053	n.a.	0.69	n.a.	0.62	n.a.
1.5	< 0.12	n.a.	1.6	n.a.	1.2	n.a.
3.3	1.1	n.a.	2.9	n.a.	3.7	n.a.
7.3	4.4	n.a.	9.5	n.a.	8.5	n.a.
16	10	n.a.	13	n.a.	13	n.a.

n.a…not applicable

Effects: Sex ratio: No relationship between treatment and sex ratio was found; therefore number of males and females midges was pooled for further endpoint calculations.
Development rate, midge emergence: No significant differences (Bonferroni's Adjusted t-Test) in mean percent emergence and in combined male/female midge development rate were identified.
For further details see Table B.9.2.2.5-2

Table B.9.2.2.5-2: Effects of S-2200 on midge (*C. riparius*) in a water-spiked test

S-2200 [mg ai/L] Mean measured (overlying water)	Number of emerged midges (out of 80)	Emergence of larvae			Mean development rate [1/d] [a]
		total [%]	male [%]	female [%]	
Control	75	93	52	48	0.0720
Solvent control	77	96	45	55	0.0720
Pooled control	152	95	49	51	0.0720
0.64	74	93	50	50	0.0754
1.4	76	95	43	57	0.0747
2.7	76	95	53	47	0.0773
4.8	79	99	59	41	0.0767
8.1	73	91	52	48	0.0719
NOEC		8.1 mg/L			8.1 mg/L
LOEC		> 8.1 mg/L			> 8.1 mg/L
EC_{50}		> 8.1 mg/L			> 8.1 mg/L

[a] Mean developmental rate is based on combined sex data.

Conclusions: NOEC = 8.1 mg ai/L (total emergence of larvae, mean development rate)
LOEC > 8.1 mg ai/L
EC_{50} > 8.1 mg ai/L
based on mean measured concentrations

Validity criteria: The emergence in the solvent and water controls were between 93 and 96% (required at least 70%) at the end of the test. The emergence to adults from control vessels occurred between 12 and 21 days after their insertion into the vessels (required 12 – 23 days after insertion).
The pH and the dissolved oxygen concentration were measured at test initiation and test termination and were within the recommended range (pH = 6 – 9, oxygen

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concentration at least 60%). The water temperature was between 19 and 20 °C and hence does not differ by more than ± 1.0 °C.

B.9.2.2.6 Microcosm or mesocosm study (Annex IIIA 10.2.2)

No study submitted. Not required because the risk assessment for the intended use (see B.9.2.4) indicates a low risk for aquatic organisms and therefore no further refinement is required.

B.9.2.2.7 Summary of chronic toxicity

Table B.9.2.2.7-1: Summary of chronic toxicity data for aquatic organisms (based on mean measured concentrations)

Test organism	Test condition	Time	Endpoint	NOEC [mg/L]	EC_{50}/LC_{50} [mg/L]	Reference
S-2200						
Pimephales promelas Fathead minnow	flow-through (ELS-test)	32 d	Survival, growth	0.15 ai	> 0.15 ai	Michael, R.L., 2010
Daphnia magna Water flea	flow-through	21 d	Reproduction	0.56 ai	> 0.56 ai	Sayers, L.E., 2010b
Chironomus riparius Midge	static	28 d	Emergence	8.1 ai	> 8.1 ai	Picard, C.R., 2012

B.9.2.3 Bioaccumulation (Annex IIA 8.2.3)

Active substance

Reference:	**Flow-Through Bioconcentration and Metabolism Study of [^{14}C]S-2200 with Bluegill Sunfish (*Lepomis macrochirus*)**
Author(s), year:	[redacted], 2010
Report/Doc. number:	Report No. ROM-0016, Study No. 13048.6627
Guideline(s):	OECD Guideline 305, US EPA FIFRA 165-4, US EPA OPPTS 850.1730, JMAFF 12-Nosan 8147 2-9-17
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	Radiolabeled substance: [Benzyl-^{14}C]S-2200, purity: 98.9 %, batch: CFQ40467 Non-radiolabeled substance: S-2200 PAI, purity: 100 %, batch: 081103G
Reference substances:	S-2200, purity: 100 %, batch:081103G S-2167 (S-2200 *S*-isomer), purity: 100 %, batch: 060020652 S-2354 (S-2200 *R*-isomer), purity: 99.7 %, batch: 060020653 2-COOH-S-2200, purity: 99.8 %, batch: 252-001-60-2 5-COOH-S-2200, purity 99.7 %, batch: 251-011-55-2 2-CH_2OH-S-2200, purity: 98.5 %, batch: CTS08029 5-CH_2OH-S-2200, purity: 99.9 %, batch: CTS08030 4-OH-S-2200, purity: 99.9 %, batch: CTS08026 De-Xy-S-2200, purity: 99.9 %, batch: CTS08001

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	MCBX, purity: 96.9 %, batch: CTS08015 (*R*)-MCBX, purity: 99.8 %, batch: FUJINAMI (*S*)-MCBX, purity: 99.4 %, batch: TM633 2,5-dimethylphenol (CAS No.: 95-87-4), purity: 99.8 %, batch: 01197MJ
Test species:	Bluegill Sunfish (*Lepomis macrochirus*)
Number of organisms:	155 fish per test concentration and 154 fish per solvent control, total biomass per aquarium was 146 g (0.24 g/L of the 24-hour flow-through volume of the aquaria. For the depuration phase the remaining fish (59 fish for the solvent control and the 1.0 µg/L treatment, and 58 fish for the 10 µg/L treatment) were placed in clean water for 7 days. 1 replicate per treatment level and solvent control
Weight, length:	Weight: 1.0 g (0.80 – 1.2 g) n = 465 Length: 37 - 45 mm, n = 30
Type of test, duration:	Flow-through test, 28 d exposure period and 7 d depuration period
Applied concentrations:	
Nominal:	0 (solvent control), 1.0 and 10 µg ai/L
Solvent:	Acetone (CAS No. 67-64-1)
Test conditions:	
Water quality:	Well water, total hardness: 60 - 72 mg/L as $CaCO_3$, total alkalinity: 20 – 23 mg/L as $CaCO_3$
Temperature:	22 - 23 °C
pH:	6.1 – 7.2 (exposure phase), 7.0 – 7.3 (depuration phase)
O_2 content:	Exposure phase: 6.1 – 8.6 mg O_2/L (> 60 % saturation) Depuration phase: 7.1 – 8.6 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding:	Pelleted food: 1 % of biomass daily
Test parameters:	Residues in water: For chemical analysis (LSC, HPLC/RAM) of S-2200 in test solutions samples were taken at -2 and -1 d (pre-exposure phase), 0, 1, 3, 7, 14, 21 and 28 d (exposure phase) and 1, 3, 7 and 14 d (depuration phase). Residues in fish: Samples were taken at day 0, 1, 3, 7, 14, 21, 24 and 28 (exposure phase) and 1, 3 and 7 (depuration phase). Six fish for tissue analysis were removed from each test concentration and control at each sampling time. Concentration of [^{14}C] S-2200 equivalents in fish tissues were determined by LSC-method. Additionally a lipid analysis (by chloroform/methanol extraction) was carried out on fish sampled at day 1, 3, 7, 14, 21, 24 and 28 (exposure phase) and at day 1, 3 and 7 (depuration phase). Daily observations were made of the appearance and behaviour of the fish. Other parameters like temperature, pH and dissolved oxygen concentrations were measured daily in each vessel.
Calculations/statistics:	BCF was calculated as ratio of [^{14}C] S-2200 equivalents concentration in water and [^{14}C] S-2200 equivalents concentration in fish tissues and as ratio of K_d (depuration constant) and K_u (uptake constant), rate constant K was determined by SigmaPlot™.
Findings:	
Analytical data – water:	Throughout the exposure phase, the radioactivity present in the exposure tanks was confirmed to be more than 95% of [Benzyl-^{14}C]S-2200, and the concentrations of measured S-2200 were 0.989 – 1.061 and 10.053 – 10.965 µg/L for low and high concentrations, respectively. Overall, measured exposure concentrations in the nominal 1.0 and 10 µg/L treatments were between 96.9% and 103.3% of nominal.

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Lipid content: During the exposure phase, the percent lipid content based on wet weight for edible, non-edible and whole fish tissue in low and high concentrations ranged from 1.14 – 2.07, 3.43 – 5.51 and 2.45 – 3.63%. During the depuration phase, the percent lipid content based on wet weight for edible, non-edible and whole fish tissue in low and high concentrations ranged from 1.78 – 2.16, 4.64 – 6.23 and 3.16 – 3.94%. Based on these results the lipid content of the whole fish remained relatively consistent over the course of the study and between exposure groups.

Analytical data – fish tissues (LSC): See Table B.9.2.3-1, B.9.2.3-2 and B.9.2.3-3

BCF: See Table B.9.2.3-4

Table B.9.2.3-1: Total radioactive residues (TRR) measured in fish tissue (*Lepomis macrochirus*)

Day	Tissue concentration [µg/g]					
	Edible		Non-edible		Whole fish	
	1.0 µg/L	10 µg/L	1.0 µg/L	10 µg/L	1.0 µg/L	10 µg/L
Exposure phase						
1	0.019	0.227	0.138	1.399	0.078	0.835
3	0.023	0.254	0.214	1.592	0.118	0.927
7	0.026	0.226	0.196	1.757	0.112	1.017
14	0.029	0.267	0.220	2.898	0.125	1.571
21	0.038 [a]	0.291 [a]	0.410 [b]	2.416 [b]	0.222 [c]	1.367 [c]
24	0.030 [a]	0.203 [a]	0.350 [b]	2.678 [b]	0.174 [c]	1.458 [c]
28	0.034 [a]	0.258 [a]	0.300 [b]	3.005 [b]	0.163 [c]	1.651 [c]
Depuration phase						
1	0.011	0.056	0.163	1.413	0.086	0.711
3	0.002	0.031	0.065	0.744	0.033	0.375
7 [d]	0.001	0.011	0.005	0.067	0.003	0.039

[a] The mean steady state concentration in edible fish tissue was determined to be 34 µg/kg (1.0 µg/L) and 250 µg/kg (10 µg/L).
[b] The mean steady state concentration in non-edible fish tissue was determined to be 353 µg/kg (1.0 µg/L) and 2699 µg/kg (10 µg/L).
[c] The mean steady state concentration in whole fish tissue was determined to be 186 µg/kg (1.0 µg/L) and 1492 µg/kg (10 µg/L).
[d] Percent depuration in the low and high exposure fish at day 7 were > 97 %, based on mean steady state whole fish concentration.

Table B.9.2.3-2: Distribution of ^{14}C residues in whole fish samples, low concentration (1.0 µg/L)

Concentration of [^{14}C] residues [µg/g] (%TRR)							
Day	1	3	7	14	21	24	28
Exposure phase							
Whole fish total	0.078 (100)	0.118 (100)	0.112 (100)	0.125 (100)	0.222 (100)	0.174 (100)	0.162 (100)
Extractable	0.076 (98)	0.115 (97)	0.108 (97)	0.119 (96)	0.214 (96)	0.167 (96)	0.156 (96)
Unextractable	0.002 (2)	0.003 (3)	0.004 (3)	0.006 (4)	0.008 (4)	0.007 (4)	0.006 (4)
S-2200	0.018 (23)	0.023 (19)	0.023 (21)	0.021 (17)	0.034 (15)	0.027 (16)	0.019 (12)
10-min	n.d.	n.d.	0.009 (7.7)	0.005 (4)	0.016 (7)	0.014 (8)	0.016 (10)
11-min	0.004 (6)	0.006 (5)	n.d.	0.006 (5)	0.008 (3)	0.007 (4)	0.011 (7)
16-min [a]	0.048 (61)	0.048 (41)	0.050 (44)	0.048 (38)	0.094 (42)	0.050 (29)	0.064 (40)
17-min	n.d.	0.016 (13)	n.d.	n.d.	n.d.	0.005 (3)	0.003 (2)
19-min [a]	0.003 (4)	0.018 (15)	0.017 (15)	0.014 (11)	0.017 (7.8)	0.009 (5)	0.009 (5)
Unknown	0.003 (3)	0.004 (4)	0.010 (9)	0.024 (19)	0.045 (20)	0.054 (31)	0.034 (21)
Acetone extract	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Depuration phase							
Whole fish total	0.086 (100)	0.033 (100)	0.003 (100)	-	-	-	-
Extractable	0.080 (94)	0.030 (89)	0.001 (17)	-	-	-	-
Unextractable	0.005 (6)	0.004 (11)	0.002 (83)	-	-	-	-
S-2200	n.d.	n.d.	n.d.	-	-	-	-
10-min	0.003 (4)	0.003 (9)	n.d.	-	-	-	-
11-min	0.010 (12)	0.003 (9)	n.d.	-	-	-	-

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Concentration of [^{14}C] residues [µg/g] (%TRR)							
Day	1	3	7	14	21	24	28
16-min [a]	0.034 (40)	0.011 (34)	n.d.	-	-	-	-
17-min	0.002 (2)	0.0003 (1)	n.d.	-	-	-	-
19-min [a]	0.011 (12)	0.003 (10)	n.d.	-	-	-	-
Unknown	0.020 (24)	0.009 (26)	0.001 (17)	-	-	-	-
Acetone extract	n.d.	n.d.	n.d.	-	-	-	-

n.d…not detectable
[a] 16-min and 19-min peaks are characterized as the glucuronic acid conjugate and /or sulphate conjugates of hydroxylated S-2200, i.e. 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200.

Table B.9.2.3-3: Distribution of ^{14}C residues in whole fish samples, high concentration (10 µg/L)

Concentration of [^{14}C] residues [µg/g] (%TRR)							
Day	1	3	7	14	21	24	28
Exposure phase							
Whole fish total	0.835 (100)	0.927 (100)	1.017 (100)	1.577 (100)	1.367 (100)	1.458 (100)	1.651 (100)
Extractable	0.820 (98)	0.905 (98)	0.992 (97)	1.533 (97)	1.320 (97)	1.413 (97)	1.598 (97)
Unextractable	0.015 (2)	0.023 (2)	0.026 (3)	0.044 (3)	0.047 (3)	0.045 (3)	0.053 (3)
S-2200	0.191 (23)	0.220 (24)	0.241 (24)	0.246 (16)	0.275 (20)	0.232 (16)	0.255 (15)
10-min	0.036 (4)	0.030 (3)	0.030 (3)	0.047 (3)	0.036 (3)	0.023 (2)	0.050 (3)
11-min	0.054 (6)	0.037 (4)	0.032 (3)	0.045 (3)	0.051 (4)	0.035 (2)	0.045 (3)
16-min [a]	0.305 (37)	0.346 (37)	0.387 (38)	0.687 (44)	0.603 (44)	0.692 (47)	0.731 (44)
17-min	0.034 (4)	0.082 (9)	0.077 (8)	0.012 (1)	n.d.	0.038 (3)	0.036 (2)
19-min [a]	0.130 (16)	0.093 (10)	0.122 (12)	0.230 (15)	0.214 (16)	0.199 (14)	0.139 (8)
Unknown	0.079 (8)	0.097 (10)	0.103 (10)	0.264 (17)	0.141 (10)	0.195 (13)	0342 (21)
Acetone extract	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Depuration phase							
Whole fish total	0.711 (100)	0.375 (100)	0.039 (100)	-	-	-	-
Extractable	0.674 (95)	0.343 (92)	0.022 (57)	-	-	-	-
Unextractable	0.037 (5)	0.031 (8)	0.017 (43)	-	-	-	-
S-2200	n.d.	n.d.	n.d.	-	-	-	-
10-min	0.020 (3)	n.d.	0.0003 (1)	-	-	-	-
11-min	0.008 (1)	0.023 (6)	0.002 (5)	-	-	-	-
16-min [a]	0.358 (50)	0.159 (42)	0.006 (17)	-	-	-	-
17-min	0.033 (5)	0.012 (3)	n.d.	-	-	-	-
10-min [a]	0.105 (15)	0.050 (13)	0.001 (1)	-	-	-	-
Unknown	0.150 (21)	0.099 (26)	0.013 (33)	-	-	-	-
Acetone extract	n.d.	n.d.	n.d.	-	-	-	-

n.d…not detectable
[a] 16-min and 19-min peaks are characterized as the glucuronic acid conjugate and /or sulphate conjugates of hydroxylated S-2200, i.e. 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200.

Table B.9.2.3-4: Summary of bioconcentration factors

	Bioconcentration factors (BCFs)					
	Low concentration (1.0 µg/L)			High concentration (10 µg/L)		
BCF	Edible	Non-edible	Whole body	Edible	Non-edible	Whole body
Total [^{14}C]Residues						
Total ^{14}C water concentration at steady state [µg S-2200 equivalent/L]	1.068	1.068	1.068	10.679	10.679	10.6679
Tissue concentration at steady state [µg S-2200 equivalent/kg]	34	353	186	250	2699	1492
$BCF_{total\ residues}$	32	331	174	23	253	140
Uptake rate constant (K_u)	15.7479	79.5256	49.0911	46.0107	70.0557	44.6165
Depuration rate constant (K_d)	0.5485	0.2776	0.3230	1.9581	0.2776	0.3229

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	Bioconcentration factors (BCFs)					
Kinetic $BCFK_{total\ residues}$	29	286	152	23	252	138
[^{14}C]S-2200						
Measured S-2200 water concentration at steady state [µg S-2200 equivalent/L]	1.035	1.035	1.035	10.345	10.345	10.345
Tissue concentration at steady state [µg S-2200 equivalant/kg]	14	40	27	132	373	254
BCF_{S-2200}	14	39	26	13	36	25
Lipid content (wet weight) at steady state [%]	1.76	4.46	3.04	1.94	4.31	3.14
$BCF_{lipid\ total\ residues}$	18	74	57	12	59	45
$BCF_{lipid\ S-2200}$	8	9	8	7	8	8

Conclusions: S-2200 was stable under the test conditions and reached the steady-state plateau by day 21 of exposure.
The active substance S-2200 accumulated in whole fish with steady-state BCF values in whole fish tissues of 25 and 26. BCF values for the total ^{14}C residues (TRR) were determined to be 140 and 174 for whole fish, and 253 and 331 for non-edible portions.
The uptake and depuration constants based on S-2200 were not obtained as it reached a plateau at an early stage of exposure and was not detected at all during the depuration phase. The modelled uptake rate constants (K_u) for total ^{14}C residues in whole fish tissues ranged from 44.6175 to 49.0911 per day, depuration constants (K_d) for total ^{14}C residues in whole fish tissues ranged from 0.3229 to 0.3230 per day. Greater than 95% of the ^{14}C residues were eliminated during the depuration phase (within 7 d). The depuration half-life (CT_{50}) for total ^{14}C residues was 2.1 d for whole fish.
S-2200 was extensively metabolized in fish. The major residues were the glucuronic acid conjugate and /or sulphate conjugates of hydroxylated S-2200, (i.e. 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200) and the active substance itself. The concentrations of the glucuronic acid conjugate and /or sulphate conjugates of hydroxylated S-2200 were determined to be between 4 and 61% TRR in whole fish during the exposure phase, respectively.

Validity criteria: The temperature is between 22 and 23 °C (recommended variation less than ± 2 °C). The concentration of dissolved oxygen is between 6.1 and 8.6 mg O_2/L (> 60 % air saturation). At the end of the test no mortalities or signs of adverse effects on fish were observed in the control and in the treatment groups (recommended less than 10%).

B.9.2.4 Risk assessment for aquatic organisms

The risk assessment for aquatic organisms is based on the recommendations of the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4 (final), 17 October 2002) and follows a tiered approach.
The standard risk assessment (first tier) is based on the calculation of the toxicity/exposure ratios (TER) taking into consideration the most sensitive organism of each group. TER values will be estimated as the ratio of EC_{50}/LC_{50} or NOEC (relevant endpoints, see Table B.9.2.4-2) to PEC_{sw} (exposure, see Section B.8) calculated with the FOCUS surface water model. This model based on scenarios considering a tiered sequence of exposure assessment steps (Step 1, 2, 3 and 4). The trigger values for the acute risk are TER_A

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> 100 for fish and invertebrates, TER_A > 10 for algae and aquatic macrophytes and for the chronic risk TER_{LT} > 10 for fish and aquatic invertebrates.

The aquatic risk assessment includes S-2200 (as active substance and formulated in the product S-2200 25% SC) and the environmentally relevant metabolites:

- S-2200-OR (max occurrence 20.7% whole system, photolysis study)
- S-2200-ORC (max occurrence 12.5% whole system, photolysis study)
- 5-COOH-S-2200 (max. occurrence 18.3% in soil, groundwater metabolite, PEC_{GW} > 0.1 µg/L)
- 2-COOH-S-2200 (max. occurrence 6.5% in soil, groundwater metabolite, PEC_{GW} > 0.1 µg/L)

Table B.9.2.4-1: Overview of parent compound, metabolites and identified degradation products mentioned in the Section B.8 ("Environmental Fate and Behaviour")

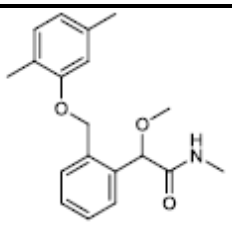
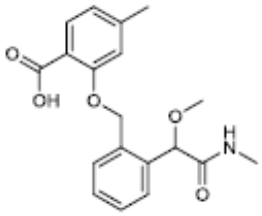
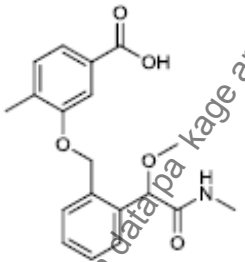
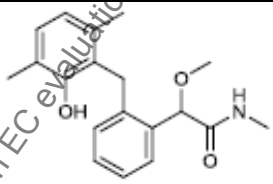
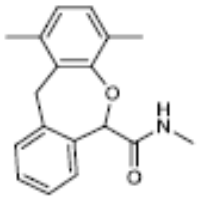
Substance	Structure	Maximum occurrence [% of AR]	Risk assessment required according to Section B.8
Active substance S-2200		-	-
Metabolite 2-COOH-S-2200		Soil: 6.5% Groundwater: > 0.1 µg/L	Risk assessment for aquatic organisms demanded
Metabolite 5-COOH-S-2200		Soil: 18.3% Groundwater: > 0.1 µg/L	Risk assessment for aquatic organisms demanded
Metabolite S-2200-OR		Surface water: 20.7% (photolysis metabolite)	Risk assessment for aquatic organisms demanded
Metabolite S-2200-ORC		Surface water: 12.5% (photolysis metabolite)	Risk assessment for aquatic organisms demanded

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Table B.9.2.4-2: Summary of acute and chronic toxicity data for aquatic organisms which are relevant for risk assessment (most sensitive species of each group)

Group	Test substance	Time-scale (Test type)	Endpoint	Toxicity [mg/L]	Source
Fish					
Oncorhynchus mykiss	S-2200	96 h (static)	Mortality, LC_{50}	0.94	[redacted] 2009a
Pimephales promelas	S-2200	32 d, ELS-test (flow-through)	Survival, growth, NOEC	0.15	[redacted] 2010
Oncorhynchus mykiss	*S*-Isomer (S-2354)	96 h (static)	Mortality, LC_{50}	> 12	[redacted] 2009b
Oncorhynchus mykiss	*R*-Isomer (S-2167)	96 h (static)	Mortality, LC_{50}	0.84	[redacted] 2009c
Oncorhynchus mykiss	2-COOH-S-2200	96 h (static)	Mortality, LC_{50}	> 89	[redacted] 2012a
Oncorhynchus mykiss	5-COOH-S-2200	96 h (static)	Mortality, LC_{50}	> 100	[redacted] 2012b
Oncorhynchus mykiss	S-2200-OR	96 h (static)	Mortality, LC_{50}	> 9.0	[redacted] 2012c
Oncorhynchus mykiss	S-2200-ORC	96 h (static)	Mortality, LC_{50}	4.0	[redacted] 2012d
Oncorhynchus mykiss	S-2200 25% SC	96 h (static)	Mortality, LC_{50}	1.1 ai	[redacted] 2011a
Aquatic invertebrates					
Daphnia magna	S-2200	48 h (static)	Immobility, EC_{50}	1.2	Sayers, L.E., 2010
Daphnia magna	S-2200	21 d (flow-through)	Reproduction, NOEC	0.56	Sayers, L.E., 2010
Daphnia magna	*S*-Isomer (S-2354)	48 h (static)	Immobility, EC_{50}	> 14	[redacted] 2012f
Daphnia magna	*R*-Isomer (S-2167)	48 h (static)	Immobility, EC_{50}	0.92	[redacted] 2012e
Daphnia magna	2-COOH-S-2200	48 h (static)	Immobility, EC_{50}	> 100	[redacted] 2012g
Daphnia magna	5-COOH-S-2200	48 h (static)	Immobility, EC_{50}	> 100	[redacted] 2012h
Daphnia magna	S-2200-OR	48 h (static)	Immobility, EC_{50}	> 14	[redacted] 2012i
Daphnia magna	S-2200-ORC	48 h (static)	Immobility, EC_{50}	9.6	[redacted] 2012j
Daphnia magna	S-2200 25% SC	48 h (static)	Immobility, EC_{50}	0.67 ai	[redacted] 2011b
Sediment dwelling invertebrates					
Chironomus riparius	S-2200	28 d (static)	Emergence, NOEC	8.1	Picard, C.R., 2012
Algae					
P. subcapitata	S-2200	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	0.67 3.4	Softcheck, K.A., 2012a
P. subcapitata	*S*-Isomer (S-2354)	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	12 > 12	Softcheck, K.A., 2012c
P. subcapitata	*R*-Isomer (S-2167)	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	0.38 2.2	Softcheck, K.A., 2012b
P. subcapitata	2-COOH-S-2200	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	58 62	Softcheck, K.A., 2012d
P. subcapitata	5-COOH-S-2200	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	> 54 > 54	Softcheck, K.A., 2012e

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Group	Test substance	Time-scale (Test type)	Endpoint	Toxicity [mg/L]	Source
P. subcapitata	S-2200-OR	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	> 9.9 > 9.9	Softcheck, K.A., 2012f
P. subcapitata	S-2200-ORC	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	> 5.0 > 5.0	Softcheck, K.A., 2012g
P. subcapitata	S-2200 25% SC	72 h (static)	Biomass, E_bC_{50} Growth rate, E_rC_{50}	1.2 ai > 2.8 ai	Softcheck, K.A., 2011

The active substance S-2200 is a racemic mixture (50:50) of two stereoisomers, namely the *R*-isomer (S-2167) and the *S*-isomer (S-2354).
The risk assessment is based on the technical S-2200 as the *S*-isomer and R-isomer were not demonstrated to be of higher toxicity. The results of the laboratory studies show that the *R*-isomer of S-2200 and the active substance S-2200 are of comparable toxicity, whereas the *S*-isomer of S-2200 is significantly less toxic than the *R*-isomer and the active substance.

The risk to aquatic organisms from S-2200, its metabolites and the formulation was assessed for the field application in winter oilseed rape. Global maximum PEC_{sw} values for the intended application scenarios were calculated with the FOCUS surface water model.
For the active substance and the relevant metabolites, S-2200-OR, S-2200-ORC, 5-COOH-S-2200 and 2-COOH-S-2200 the maximum PEC_{SW} values were calculated with FOCUS Step 1 and 2.

Table B.9.2.4-3: Summary of maximum observed PEC_{sw} values of S-2200 and metabolites, FOCUS Step 1 and 2

Substance	Winter oilseed rape, 1 x 0.2 kg ai/ha max. PEC_{sw} [µg/L]	
	FOCUS Step 1	FOCUS Step 2, Southern Europe [a]
S-2200	43.541	5.365
S-2200-OR	1.039	0.381
S-2200-ORC	0.797	0.263
2-COOH-S-2200	4.818	0.458
5-COOH-S-2200	12.323	1.211

[a] FOCUS Step 2 calculations is based on input parameters for Southern Europe (worst-case)

B.9.2.4.1 Acute risk

Table B.9.2.4.1-1: Acute toxicity exposure ratios (TER_A) based on worst case PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Annex VI Trigger
Fish						
Oncorhynchus mykiss	S-2200	0.94	96 h	0.0435	**21.6**	100
Oncorhynchus mykiss	S-2200 25% SC	1.1	96 h	0.0435	**25.3**	100
Oncorhynchus mykiss	S-2200-OR	> 9.0	96 h	0.0010	> 9000	100
Oncorhynchus mykiss	S-2200-ORC	4.0	96 h	0.0008	5000	100
Oncorhynchus mykiss	2-COOH-S-2200	> 89	96 h	0.0048	> 18542	100
Oncorhynchus mykiss	5-COOH-S-2200	> 100	96 h	0.0132	> 7576	100
Aquatic invertebrates						
Daphnia magna	S-2200	1.2	48 h	0.0435	**27.6**	100
Daphnia magna	S-2200 25% SC	0.67	48 h	0.0435	**15.4**	100
Daphnia magna	S-2200-OR	> 14	48 h	0.0010	> 14000	100

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Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Annex VI Trigger
Daphnia magna	S-2200-ORC	9.6	48 h	0.0008	12000	100
Daphnia magna	2-COOH-S-2200	> 100	48 h	0.0048	> 20833	100
Daphnia magna	5-COOH-S-2200	> 100	48 h	0.0132	> 7576	100
Algae						
P. subcapitata	S-2200	0.67	72 h	0.0435	15.4	10
P. subcapitata	S-2200 25% SC	1.2	72 h	0.0435	27.6	10
P. subcapitata	S-2200-OR	> 9.9	72 h	0.0010	> 9900	10
P. subcapitata	S-2200-ORC	> 5.0	72 h	0.0008	> 6250	10
P. subcapitata	2-COOH-S-2200	58	72 h	0.0048	12083	10
P. subcapitata	5-COOH-S-2200	> 54	72 h	0.0132	> 4091	10

Table B.9.2.4.1-2: Acute toxicity exposure ratios (TER_A) based on worst case PEC_{sw} from FOCUS Step 2 – mentioned are only these organisms which failed the trigger at Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Annex VI Trigger
Fish						
Oncorhynchus mykiss	S-2200	0.94	96 h	0.0054	174	100
Oncorhynchus mykiss	S-2200 25% SC	1.1	96 h	0.0054	204	100
Aquatic invertebrates						
Daphnia magna	S-2200	1.2	48 h	0.0054	222	100
Daphnia magna	S-2200 25% SC	0.67	48 h	0.0054	124	100

Acute risk from active substance and formulation:
The active substance S-2200 and the formulation S-2200 25% SC are toxic to standard test species of fish, aquatic invertebrates and algae. All three groups of aquatic organisms, fish, aquatic invertebrates and algae show a comparable toxicity when exposed to the active substance and the formulation. No significant difference in sensitivity and toxicity between the active substance S-2200 and the formulation S-2200 25% SC was observed.

All TER_A values for fish, aquatic invertebrates and algae calculated with global maximum PEC_{sw} from FOCUS Step 2 (Southern Europe) meet the relevant triggers. In conclusion, the acute risk can be considered acceptable for applications in winter oilseed rape.

Acute risk from metabolites:
Aquatic toxicity studies with fish, aquatic invertebrate and algae have been conducted with the metabolites S-2200-OR, S-2200-ORC, 2-COOH-S-2200 and 5-COOH-S-2200.
In conclusion all environmental relevant metabolites are of low acute toxicity to aquatic organisms and all calculated TER_A values for the intended use are well above the relevant trigger values. Thus the risk from metabolites of S-2200 to aquatic organisms is expected to be low.

B.9.2.4.2 Chronic risk

Table B.9.2.4.2-1: Chronic toxicity exposure ratios (TER_{LT}) based on worst case PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Annex VI Trigger
Fish						
Oncorhynchus mykiss	S-2200	0.15	32 d	0.0435	**3.45**	10
Aquatic invertebrates						
Daphnia magna	S-2200	0.56	21 d	0.0435	12.9	10
Chironomus riparius	S-2200	8.1	28 d	0.0435	186	10

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Table B.9.2.4.2-2: Chronic toxicity exposure ratios (TER_{LT}) based on worst case PEC_{sw} from FOCUS Step 2 – mentioned are only these organisms which failed the trigger at Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Annex VI Trigger
Fish						
Oncorhynchus mykiss	S-2200	0.15	32 d	0.0054	27.8	10

Chronic risk from active substance and formulation:
The assessment of the chronic toxicity is based on long-term studies with fish (*O. mykiss*) and aquatic invertebrates (*D. magna* and *C. riparius*) with the active substance.

For fish the safety factor (TER_{LT}) was calculated to be 27.8 based on PEC_{sw} from FOCUS Step 2. For aquatic invertebrates TER_{LT} values of 12.9 and 186 were estimated based on PEC_{sw} from FOCUS Step 1. All TER_{LT} values are above the relevant trigger of 10.

In conclusion for the intended use in winter oilseed rape the chronic risk of S-2200 for aquatic organisms is expected to be low.

B.9.2.4.3 Bioaccumulation

S-2200 has a log P_{OW} of 3.51 and therefore a fish bioconcentration study is triggered. Based on the fish bioaccumulation study (Lentz, N.R., 2010) with *L. macrochirus* a BCF (whole fish) of 25-26 was determined, which indicate a low potential to bioaccumulate in the aquatic food chain.
The active substance was extensively metabolized in fish and the residues were eliminated quickly (CT_{50} = 2.1 d based on total ^{14}C residues).
The major residues were the glucuronic acid conjugate and /or sulphate conjugates of hydroxylated S-2200, (i.e. 2-CH_2OH-S-2200, 4-OH-S-2200 and 5-CH_2OH-S-2200) and the active substance itself. The concentrations of the glucuronic acid conjugate and /or sulphate conjugates of hydroxylated S-2200 were determined to be between 4 and 61 % TRR in whole fish during the exposure phase, respectively.

Metabolites

According to the Guidance Document on Aquatic Ecotoxicology the potential to bioaccumulate has to be addressed for the active substance and its relevant water metabolites if the log P_{OW} is greater than 3.0.
Therefore, a bioconcentration study in fish was conducted for the active substance S-2200 (log P_{OW} = 3.51, 25 °C). The major aquatic metabolites were determined to be 2-COOH-S-2200, 5-COOH-S-2200, S-2200-OR and S-2200-ORC with an estimated (according to KOWWIN, v.1.67 estimate) log P_{OW} of 2.53, 2.88, 3.30 and 4.02, respectively. Hence, the potential of the metabolites to bioaccumulate in the aquatic food chain is considered to be low for the metabolites 2-COOH-S-2200 and 5-COOH-S-2200. However, the potential of the metabolites S-2200-OR and S-2200-ORC to bioaccumulate in the aquatic food chain has to be addressed.

All metabolites making a significant contribution to total radioactive residues in the aqueous exposure medium and in fish tissues were identified and quantified. Additional studies to investigate the bioconcentration potential of metabolites, degradation and reaction products separately are therefore unnecessary and have not been conducted.

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B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

B.9.3.1 Toxicity to mammals

B.9.3.2 Acute oral toxicity

The endpoint from the acute oral study with rats ([redacted] 2010a) for the active substance S-2200 is > 2000 mg a/kg bw. No mortalities occurred at this dose and this endpoint is used for risk assessment.
The acute toxicity of the formulation (S-2200 25SC) was determined to be > 2000 mg prod./kg bw in rats, equivalent to > 499.2 mg ai/kg bw, based on the content of 24.96% active ingredient specified in the study report ([redacted] 2011a).

B.9.3.2.1 Subchronic toxicity

See part B.6 of this DAR.

B.9.3.2.2 Chronic (long-term) toxicity

See part B.6 of this DAR.

B.9.3.2.3 Reproductive toxicity

See part B.6 of this DAR.

Table B.9.3.2.3-1: Summary of reproductive toxicity studies relevant for ecotoxicity

Type of study *reference*	Concentrations tested	NOAEL (mg/kg bw/d)		Adverse effects / target organs at LOAEL
Two-generation reproduction toxicity study (rat) [redacted] *2012*	0, 1000, 3000, 10000 ppm equivalent to: 0, 43 – 163, 132 – 511, 452 – 1688 mg/kg bw/d (rounded)	43 (1000 ppm)	Parents	*Liver:* Increased liver weight and diffuse hepatocellular hypertrophy Brown pigment in bile duct/periportal area Focal periductular inflammatory cell infiltration *Thyroid:* Increased thyroid weight (males)
		56 (1000 ppm)	Offspring	Lower spleen weights at weaning (males)
		559 (10000 ppm)	Repro-duction	No effects at highest dose tested
Developmental toxicity study (rat) [redacted] *2012a*	0, 100, 300, 1000 mg/kg bw/d	1000	Maternal	No treatment-related effects at highest dose tested
Developmental toxicity study (rabbit) [redacted]; 2012b	0, 100, 300, 1000 mg/kg bw/d	1000	Maternal	No treatment-related effects at highest dose tested

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Type of study *reference*	Concentrations tested	NOAEL (mg/kg bw/d)	Adverse effects / target organs at LOAEL
Rat, 90-days (oral) [redacted] *2011a*	0, 800, 4000, 10000, 20000 ppm ♂: 0, 54, 282.6, 742.7, 1544.6 mg/kg bw/day ♀: 0, 61.6, 320.1, 788.5, 1886.5 mg/kg bw/day	800ppm ♂: 54 mg/kg bw/day ♀: 61.6 mg/kg bw/day	↑ abs and rel liver weight (mainly ♂, but effects present also in ♀) Hepatocellular hypertrophy Follicular cell hypertrophy in the thyroid ↓ Bilrubin levels (♂)
Mouse, 90-days (oral) [redacted] 2011b	0, 1750, 3500, 7000 ppm ♂: 0, 204.1, 404.9, 807.3 mg/kg bw/day ♀: 0, 251.8, 529.1, 1111.2 mg/kg bw/day	3500 ppm ♂ 404.9 mg/kg bw/day ♀ 529.1 mg/kg bw/day	↑ abs and rel liver weight

According to the EFSA Guidance Document for the risk assessment for birds and mammals following studies should be taken into account for the selection of the appropriate endpoint for the long-term mammals risk assessment:

OECD 416 – Two generation reproduction toxicity study
OECD 414 – Prenatal developmental toxicity study
OECD 407 – Repeated dose 28-day oral toxicity in rodents
OECD 408 – Subchronic oral toxicity – rodent 90 day study

An oral 28 day study in rodents was not available. For all other study types the summaries of the evaluation as performed for the human toxicology section are given in the table above.

The lowest ecologically relevant NOAELs from the pre-natal developmental toxicity study in rats and rabbits was 1000 mg/kg bw/d (the highest dose tested in both studies).

In the 90 day rat study (IIA 5.3.2/01) the overall NOAEL was considered to be 800 ppm (54 & 61.6 mg/kg/day for males and females, respectively). In the 90 day mouse study the overall NOAEL was considered to be 3500 ppm (404.9 & 529.1 mg/kg/day for males & females, respectively). The NOAEL in the study with rats was based on effects on liver weight, bilirubin levels and hepatocellular hypertrophy and follicular cell hypertrophy in the thyroid. Such effects typically are not considered relevant for the reproductive risk assessment of mammals as they are not considered to have an effect on the reproduction and on the population.

The endpoints of the two-generation rat study for parents, offspring and effects on reproduction were 43, 56 and 559 mg ai/kg bw/d respectively, mainly based on effects on the liver, the spleen and the thyroid (see table above). As mentioned above these types of effects are usually not considered to be of biological significance.
Therefore, overall the NOAEL of 166.3 mg ai/kg bw/d (3000 ppm) from the two-generation rat study based on effects on the pup development is considered to be the worst-case endpoint for use in the risk

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assessment. Effects included suppression of postnatal body weight gain in both sexes of F1 and F2 offspring, reduced food consumption of both F0 and F1 dams and slight delay of sexual maturation at 10000 ppm.

B.9.3.3 Risk assessment for mammals

Mammals may be exposed to S-2200 by eating contaminated vegetation, seeds and fruits, invertebrate prey like arthropods (i.e. insects) or earthworms or vertebrate prey. Another possible route is via drinking water.

The first tier risk assessment was conducted as recommended in the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).
The calculations are based on a single application rate of 200 g ai/ha in oilseed rape. Possible other food items, for which no respective risk assessment is performed, are covered by the calculations presented because residues in these items are expected to be significantly lower.

According to the guidance document an acute and a reproductive risk assessment should be performed starting with a screening step which is based on an "indicator species" in order to identify all those substances that clearly pose a low risk for mammals. For oilseed rape the indicator species is small herbivorous mammal with a shortcut value for the acute assessment of 118.4 and for the reproductive assessment of 48.3. The daily dietary dose for acute exposure is calculated by multiplication of the application rate with the shortcut value. For the reproductive assessment a time weighted average factor (twa) can additionally be applied. If the toxic effect is considered to be caused by long term exposure the twa is 0.53 (based on an averaging interval of 21 days and a default DT_{50} of 10 days). There are no indications that any effects might have been caused by short-term exposure and it is justified to apply a twa of 0.53. A multiple application factor in order to account for the possible build-up of residues was not applied as the intended use only includes one application.

The active ingredient S-2200 is a racemic mixture of two stereoisomers, namely the R-isomer and the S-isomer. A study on the Analytical Method Verification for the Determination of S-2200 TG in Avian Diet (Martin, K.H. and Nixon, W.B. (2009)) indicated that there is no evidence of conversion between the two isomers. An evaluation and summary of the study is included in part B 9.1. No further considerations regarding the risk resulting from the two isomers are considered necessary.

The calculations of TER-values (toxicity exposure ratio) for the acute and the long-term exposure are presented in the tables below.

Table B.9.3.2.3-1: Acute exposure of mammals to S-2200 - calculated toxicity exposure ratios, Screening step

Time scale	Crop	Indicator species	Endpoint [mg ai /kg bw]	Shortcut value	MAF	f_{twa}	DDD [mg/kg bw]	TER
Acute	Oilseed rape 0.2 kg ai/ha	Small herbivorous mammal	> 2000	118.4	-	-	23.68	>84
Acute (product)			> 499.2	118.4	-	-	23.68	>21

MAF…multiple application factor; f_{twa}…time weighted average factor; DDD… daily dietary dose; TER…toxicity exposure ratio

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Table B.9.3.2.3-2: Long-term exposure of mammals to S-2200 - calculated toxicity exposure ratios, Screening step

Time scale	Crop	Indicator species	Endpoint [mg ai/kg bw/d]	Shortcut value	MAF	f_{twa}	DDD [mg/kg bw]	TER
Long-term	Oilseed rape 0.2 kg ai/ha	Small herbivorous mammal	166.3	48.3	-	0.53	5.12	32

MAF…multiple application factor; f_{twa}…time weighted average factor; DDD… daily dietary dose; TER…toxicity exposure ratio

The TER figures for acute and long-term / reproductive exposure exceed the trigger of 10 and 5 as specified in Commission Regulation (EU) No 546/2011 and indicate a low risk to birds after application of S-2200 according to the GAP.

B.9.3.3.1 Assessment of the risk from metabolites formed in potential food items

Plant metabolism studies have shown 4-OH-S-2200 (free and conjugated), 2-CH2OH-S-2200 (free and conjugated) and De-Xy-S-2200 to be major metabolites in crops, as they are found at levels exceeding 10% TRR. However, residue studies using S-2200 25 SC (S-2200, 250 g/L) confirmed that residue levels of 4-OH-S-2200 (free and conjugated), 2-CH2OH-S-2200 (free and conjugated) and De-Xy-S-2200 in oilseed rape seed are non-detectable.

B.9.3.3.2 Risk to mammals from secondary poisoning

As the log P_{ow} of S-2200 is greater than 3 (3.51) the risk to birds and mammals from secondary poisoning was assessed according to Guidance of EFSA (2009).
The major metabolites in soil and water were determined to be 2-COOH-S-2200, 5-COOH-S-2200, S-2200-OR and S-2200-ORC with an estimated (according to KOWWIN, v.1.67 estimate) log P_{OW} of 2.53, 2.88, 3.30 and 4.02, respectively. Hence, the potential of the metabolites to bioaccumulate in the food chain is considered to be low for the soil metabolites 2-COOH-S-2200 and 5-COOH-S-2200. However, the potential of the metabolites S-2200-OR and S-2200-ORC to have an effect on fish eating mammals has to be addressed. As no long-term studies with the metabolites are available, as a conservative approach they are assumed to be 10 times more toxic than the parent compound.

B.9.3.3.2.1 Food chain from earthworm to earthworm-eating mammals

As the log P_{ow} of the metabolites in soil is below the trigger of 3, no assessment for earthworm eating mammals is required. An assessment for birds exposed to the parent compound is presented below in accordance with the Guidance Document (EFSA, 2009).

$$TER = \frac{NOEL_{long-term}}{PEC_{worm} \times 1.28}$$

The factor of 1.28 is used to convert the residues in worms to a daily dose based on a mammal of 10 g eating 12.8 g worms per day, according to Smit. (2005).

$$PEC_{worm} = PEC_{soil} \times BCF$$

$$BCF(C_{worm}/C_{soil}) = \frac{0.84 + 0.012 \times K_{ow}}{f_{oc} \times K_{oc}}$$

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The risk assessment was performed for the single application in oilseed rape.

Table B.9.3.3.2.1-1: Parameters and calculations for the assessment of the long-term risk to earthworm-eating mammals in oilseed rape

Parameter	S-2200
$NOEL_{long\text{-}term}$ [mg ai/kg bw/d]	166.3
K_{oc} (Organic carbon adsorption coefficient)	449
K_{ow} (Octanol water partition coefficient)	3236
f_{oc} (Organic carbon content of soil)	default value: 0.02
PEC_{soil} (max) [mg ai/kg]	0.067
BCF_{worm}	4.42
PEC_{worm} [mg a.s./kg]	0.30
TER	433

The TER-values following use in oilseed rape is above the trigger of 5 for long-term risk, indicating that the use of S-2200 poses a low risk to earthworm-eating mammals.

B.9.3.3.2.2 Food chain from fish to fish-eating mammals

The risk to fish-eating mammals from bioaccumulation of S-2200 is calculated with the following equations in accordance with the Guidance Document (EFSA, 2009). Not only the BCF but also the toxicity for the metabolites is assumed to be 10 times higher than for the parent compound as a worst case approach.

$$TER = \frac{NOEL_{long-term}}{PEC_{fish} \times 0.142}$$

The factor of 0.142 is used to convert the residues in fish to a daily dose based on a mammal of 3000 g eating 425 g per day, according to Smit (2005).

$$PEC_{fish} = PEC_{water} \times BCF$$

The risk assessment is based on the use in oilseed rape. As a worst-case approach the highest PEC_{water} without an ftwa was used.

Table B.9.3.3.2.2-1: Parameters and calculations for the assessment of the long-term risk to fish-eating mammals in oilseed rape

Parameter	S-2200	S-2200-OR	S-2200-ORC
$NOEL_{long\text{-}term}$ [mg ai/kg bw/d]	166.3	16.6[a]	16.6[a]
PEC_{water} (Maximum according FOCUS step 1) [mg ai/L]	0.04354	0.00104	0.0008
BCF_{fish}	26	260[b]	260[b]
PEC_{fish} [mg ai/kg]	1.13	0.27	0.21
TER	1039	433	557

[a] assumed to be 10 times more toxic than the parent compound
[b] BCF for the metabolites is assumed to be 10 times higher than for the metabolites

The TER-values following use in oilseed rape are above the long-term trigger of 5, indicating that the use of S-2200 and its major metabolites in water poses a low risk to fish-eating mammals.

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B.9.3.3.2.3 Biomagnification in terrestrial food chains

ADME studies conducted with rats and a bioaccumulation study in fish demonstrate that S-2200 has a low potential to bioaccumulate and biomagnify.
According to the ADME studies in rats S-2200 is widely distributed throughout the body. After single oral administration there was no major difference in distribution between high and low doses (1000 and 5 mg/kg bw, respectively) or between sexes. The major tissue residues were seen in the gastrointestinal tract, and in liver and kidney, as well as in uterus and ovaries at 168 hours after dosing. There was no evidence of accumulation into tissues. S-2200 was extensively metabolised to numerous metabolites (unchanged parent formed <0.2% of administered dose at low dose). The primary routes of metabolism were by oxidation and subsequent conjugation with glucuronic acid, demethylation with subsequent oxidation, or oxidation with subsequent demethylation.
In addition, metabolism studies in laying hens and lactating goats showed that the metabolic pathways in livestock were similar to that found in the rat. S-2200 is extensively metabolised and readily excreted in the hen and goat. Residue levels were low in eggs, milk, muscle and fat, and higher in liver and kidney. Parent S-2200 was the main component of the residue in eggs, milk fat, muscle (goat) and fat (hen and goat), and the main metabolites were 5-COOH-S-2200 (goat kidney and liver), 4-OH-S-2200 (in hen liver and as the glucuronide in goat kidney) and De-Xy-S-2200 (hen liver). S-2200 has a log Pow of 3.51, however as S-2200 is extensively metabolised, no significant accumulation of residues in tissues, particularly fatty tissues, was seen in the livestock metabolism studies.

B.9.3.3.3 Risk to mammals from consumption of contaminated drinking water

The EFSA guidance document recommends assessing the risk for mammals via the uptake of contaminated drinking water. The relevant scenario is puddles on soil.

Puddle scenario: Mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil.

According to the recommendation given above the puddle scenario is considered to be relevant for the intended use in oilseed rape.
However, due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals (see below), no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (K_{oc} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500 L/kg).
A comparison of the relevant endpoints with the effective application rate for S-2200 is presented below.

Table B.9.3.3.3-1: Risk to mammals exposed via contaminated drinking water

Crop	Exposure scenario	Effective Application Rate [g ai/ha]	K_{oc} [L/kg]	LD_{50} / NOEL [mg ai/kg bw]	Ratio Application Rate:Endpoint
Oilseed rape	Acute	200	449	> 499.2 (product)	< 0.4
	Long-term	200		166	< 1.2

As the ratio for S-2200, a less sorptive substance, is below 50, the risk is indicated to be acceptable. The risk from metabolites formed in the water is likely to be covered by the safety margins for the active ingredient.

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B.9.3.3.4 Overall conclusions

The available data on mammalian toxicity for the active substance S-2200 and the risk assessment indicate an acceptable risk to mammals via dietary exposure. In addition, the risk to mammals from plant metabolites, bioaccumulation or via the drinking water is considered to be low.

B.9.4 Effects on bees (Annex IIA 8.3.1, IIIA 10.4)

B.9.4.1 Acute Toxicity (Annex IIA 8.3.1.1, IIIA 10.4.1)

Active Substance

Reference:	**S-2200 TG – Acute Oral and Contact Toxicity to the Honeybee *Apis mellifera* L. in the Laboratory**
Author(s), year:	Vergé, Emmanuelle, 2009
Report/Doc. number:	Report No.: ROW-0002, Study No.: S09-02321
Guideline(s):	OECD Guideline 213 and 214
GLP:	Yes
Deviations:	Oral toxicity test: Due to technical reasons the temperature was down to 21.5 °C for a total period of approximately 24 hours. The results of the control and reference item treatment groups showed that the deviation had no impact on the validity of the study. Contact toxicity test: Due to technical reasons the temperature was down to 22.5 °C for a total period of approximately 6 hours and the relative humidity was up to 78 % for total period of approximately 20 hours. The results of the control and reference item treatment groups showed that the deviation had no impact on the validity of the study.
Validity:	Acceptable

Material and Methods:

Test substance:	S-2200 technical, purity: 93.4 %, batch: ST-0811G
Reference:	Perfekthion (BAS 152 11 I), dimethoate, purity: 42.2 %, batch: FRE-000627
Solvent:	Acetone
Test species:	*Apis mellifera* L., young worker honey bees
Type of test:	Acute oral and contact limit test
Number of organisms:	Five replicates with 10 bees for both controls, the reference item treatments and the test item treatment groups

Oral toxicity test:

Applied concentrations:	Control: 50% aqueous sucrose solution Solvent control: 1 % acetone aqueous sucrose solution Test item: 100 µg ai/bee (limit test) Reference item: 0.08, 0.11, 0.14 and 0.18 µg dimethoate/bee
Exposure route:	The test item was dissolved in acetone before the 50 % aqueous sucrose solution was added and offered to the bees. The test bees were starved for 2 hours before they were fed with the solutions. After six hours, the feeding troughs were exchanged with clean feeders containing 50 % aqueous sucrose solution and the retrieved containers re-weighed to determine the quantity of feed consumed.
Test conditions:	Temperature: 21.5 – 26.5 °C, relative humidity: 55 - 70 %, no light
Test parameter:	Mortality counts and checks for behavioural abnormalities were made after exposure for 4, 24 and 48 h.

Contact toxicity test:

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Applied concentrations: Control: tap water
Solvent control: acetone
Test item: 100 µg ai/bee (limit test)
Reference item: 0.10, 0.15, 0.23 and 0.34 µg dimethoate/bee

Test parameter: A 2.0 µL droplet of the appropriate solution of S-2200 dissolved in acetone was administered to the thoracic surface of CO_2-anaesthetised bees with a hand-held microapplicator. Solvent control bees were similarly dosed with acetone and control bees were subjected to the anaesthetisation procedure only.
After application the bees were returned to the test cages and feed with a 50 % sucrose aqueous solution *ad libitum*.

Test conditions: Temperature: 22.5 – 25.5 °C, relative humidity: 56 - 78 %, no light

Test parameter: Mortality counts and checks for behavioural abnormalities were made after exposure for 4, 24 and 48 h.

Findings: Oral toxicity test: The actual consumption per bee in the oral test was 110.71 µg ai/bee of technical S-2200. No behavioural differences between the test item treated bees and the control bees were observed during the entire test period.
See Table B.9.4.1-1.

Table B.9.4.1-1: Effects of S-2200 on *Apis mellifera* following 48-h oral exposure in an acute toxicity test (mean cumulative mortality)

Nominal dose [µg ai/bee] (consumed)	Mortality (corrected mortality [a]) [%]	
	24 h	48 h
Control (sugar solution)	0.0 (-)	0.0 (-)
Solvent control (acetone)	0.0 (-)	2.0 (-)
Treatment		
100 (110.71)	2.0 (2.0)	2.0 (0.0)
Reference item		
0.08 (0.09)	20.0 (-)	22.0 (-)
0.11 (0.12)	46.0 (-)	56.0 (-)
0.14 (0.15)	86.0 (-)	90.0 (-)
0.18 (0.20)	96.0 (-)	98.0 (-)
Test substance: 24h / 48h LD_{50} > 110.71 µg ai/bee Reference: 24 h LD_{50} = 0.12 µg dimethoate/bee (95 % C.I. 0.11 – 0.12 µg ai/bee) 48 h LD_{50} = 0.11 µg dimethoate/bee (95 % C.I. 0.10 – 0.12 µg ai/bee)		

[a] Corrected mortality calculated with the mortality of the acetone control group. Corrected mortality was calculated according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Findings: Contact toxicity test: One apathetic bee was observed during the entire 48 h test period of the contact toxicity test. No additional behavioural effects were observed during the entire test period.
See Table B.9.4.1-2.

Table B.9.4.1-2: Effects of S-2200 on *Apis mellifera* following 48-h contact exposure in an acute toxicity test (mean cumulative mortality)

Nominal dose [µg ai/bee]	Mortality (corrected mortality [a]) [%]	
	24 h	48 h
Control (tap water)	2.0 (-)	2.0 (-)
Solvent control (acetone)	0.0 (-)	2.0 (-)
Treatment		
100	2.0 (0.0)	2.0 (0.0)

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Nominal dose [µg ai/bee]	Mortality (corrected mortality [a]) [%]	
	24 h	48 h
Reference item		
0.10	8.0 (6.1)	16.0 (14.3)
0.15	54.0 (53.1)	62.0 (61.2)
0.23	76.0 (75.5)	76.0 (75.5)
0.34	68.0 (67.3)	68.0 (67.3)
Test substance: 24 h / 48 h LD_{50} > 100 µg ai/bee Reference: 24 h LD_{50} = 0.16 µg dimethoate/bee (95 % C.I. 0.15 – 0.18 µg ai/bee) 48 h LD_{50} = 0.15 µg dimethoate/bee (95 % C.I. 0.13 – 0.17 µg ai/bee)		

[a] Corrected mortality calculated with the mortality of the tap water control group. Corrected mortality was calculated according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Conclusions: 48 h LD_{50} > 110.71 µg ai/bee (oral toxicity)
48 h LD_{50} > 100 µg ai/bee (contact toxicity)

Validity criteria: The mean mortality of the controls in the oral and contact toxicity test was about 2 % which is in the line with the recommended maximum mortality of 10 % according to the OECD guidelines.
The 24 h LD_{50} values of the reference item (dimethoate) in the oral (24 h LD_{50} = 0.12 µg ai/bee) and contact (24 h LD_{50} = 0.16 µg ai/bee) toxicity tests were within the recommended range of 0.10 – 0.35 µg ai/bee (oral) and 0.10 – 0.30 µg ai/bee (contact), respectively.
In the contact toxicity test a volume of 2 µL of solution was chosen in deviation to the guideline recommendation of 1 µL, since a higher volume ensures a more reliable dispersion of the test item. Experience of the test facility has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. The RMS agrees with the applicant that under consideration of the results of the study it can be concluded that the use of a higher volume has no impact on the outcome of the acute contact toxicity test.

Formulation

Reference:	**S-2200 25SC - Acute Oral and Contact Toxicity to the Honeybee Apis mellifera L. in the Laboratory**
Author(s), year:	Vergé, Emmanuelle, 2010
Report/Doc. number:	Report No.: ROW-0023, Study No.: S10-02870
Guideline(s):	OECD Guideline 213 and 214
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	S-2200 25 SC, purity: 24.96 %, batch: C09-5F101G
Reference:	Perfekthion (BAS 152 11 I), dimethoate, purity: 414.8 g ai/L, batch: 90924-06
Solvent:	None
Test species:	*Apis mellifera* L., young worker honey bees
Type of test:	Acute oral and contact dose-response test
Number of organisms:	Five replicates with 10 bees for control, the reference item treatments and the test item treatment groups

Oral toxicity test:

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Applied concentrations: Control: tap water
Test item: 6.25, 12.5, 25, 50 and 100 µg ai/bee
Reference item: 0.06, 0.08, 0.11 and 0.15 µg dimethoate/bee

Exposure route: The test item was dissolved in tap water before the 50 % aqueous sucrose solution was added and offered to the bees. The test bees were starved for 2 hours before they were fed with the solutions. After six hours, the feeding troughs were exchanged with clean feeders containing 50 % aqueous sucrose solution and the retrieved containers re-weighed to determine the quantity of feed consumed.

Test conditions: Temperature: 24.5 °C, relative humidity: 52 - 65 %, no light

Test parameter: Mortality counts and checks for behavioural abnormalities were made after exposure for 4, 24 and 48 h.

Contact toxicity test:

Applied concentrations: Control: tap water
Test item: 6.25, 12.5, 25, 50 and 100 µg ai/bee
Reference item: 0.10, 0.15, 0.23 and 0.34 µg dimethoate/bee

Test parameter: A 2.0 µL droplet of the appropriate solution of S-2200 25 SC dissolved in tap water was administered to the thoracic surface of CO_2-anaesthetised bees with a hand-held microapplicator. Control bees were subjected to the anaesthetisation procedure only.
After application the bees were returned to the test cages and feed with a 50 % sucrose aqueous solution *ad libitum*.

Test conditions: Temperature: 25 °C, relative humidity: 64 %, no light

Test parameter: Mortality counts and checks for behavioural abnormalities were made after exposure for 4, 24 and 48 h.
In the contact toxicity test the mortality rose by more than 10% between the 24 and 48 hour assessments and therefore the observation period was prolonged up to 72 hours.

Findings: Oral toxicity test: No mortality in the control or test item treatment groups was observed in the oral toxicity test. In addition, no sublethal effects were observed throughout the 48 hour test period. See Table B.9.4.1-3.

Table B.9.4.1-3: Effects of S-2200 25 SC on *Apis mellifera* following 48-h oral exposure in an acute toxicity test (mean cumulative mortality)

Nominal dose [µg ai/bee] (consumed)	Mortality [%]	
	24 h	48 h
Control (sugar solution)	0.0	0.0
Treatment		
6.25 (7.15)	0.0	0.0
12.5 (14.23)	0.0	0.0
25 (27.78)	0.0	0.0
50 (56.48)	0.0	0.0
100 (109.25)	0.0	0.0
Reference item		
0.06 (0.07)	0.0	0.0
0.08 (0.09)	8.0	10.0
0.11 (0.13)	30.0	42.0
0.15 (0.17)	72.0	88.0
Test substance: 24h / 48h LD_{50} > 109.25 µg ai/bee Reference: 24 h LD_{50} = 0.15 µg dimethoate/bee (95 % C.I. 0.12 – 0.17 µg ai/bee)		

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Nominal dose [µg ai/bee] (consumed)	Mortality [%]	
	24 h	48 h
48 h LD_{50} = 0.14 µg dimethoate/bee (95 % C.I. 0.12 – 0.15 µg ai/bee)		

Findings: Contact toxicity test: In the water control group of the contact toxicity test 2% mortality was observed during the 72 hours observation period. The highest mortality of 14% was observed in the group treated with 25 µg ai/bee 72 hours after application. In the highest dose level of 100 µg ai/bee no mortality was recorded over the 72 hours observation period. This shows that the mortality was not dose related. In addition, no sublethal effects were observed throughout the 72 hour test period.
See Table B.9.4.1-4.

Table B.9.4.1-4: Effects of S-2200 on *Apis mellifera* following 48-h contact exposure in an acute toxicity test (mean cumulative mortality)

Nominal dose [µg ai/bee]	Mortality (corrected mortality [a]) [%]		
	24 h	48 h	72 h
Control (tap water)	0.0 (-)	2.0 (-)	2.0 (-)
Treatment			
6.25	0.0 (-)	6.0 (4.1)	6.0 (4.1)
12.5	0.0 (-)	6.0 (4.1)	6.0 (4.1)
25	0.0 (-)	14.0 (12.2)	14.0 (12.2)
50	2.0 (2.0)	2.0 (0.0)	2.0 (0.0)
100	0.0 (-)	0.0 (- 2.0)	0.0 (- 2.0)
Reference item			
0.10	6.0 (6.0)	18.0 (16.3)	20.0 (18.4)
0.15	52.0 (52.0)	52.0 (51.0)	54.0 (53.1)
0.23	74.0 (74.0)	78.0 (77.6)	84.0 (83.7)
0.34	78.0 (78.0)	78.0 (77.6)	78.0 (77.6)
Test substance: 24 h / 48 / 72 h LD_{50} > 100 µg ai/bee Reference: 24 h LD_{50} = 0.18 µg dimethoate/bee (95 % C.I. 0.16 – 0.20 µg ai/bee) 48 h LD_{50} = 0.16 µg dimethoate/bee (95 % C.I. 0.14 – 0.18 µg ai/bee) 72 h LD_{50} = 0.16 µg dimethoate/bee (95 % C.I. 0.13 – 0.18 µg ai/bee)			

[a] Corrected mortality calculated with the mortality of the tap water control group. Corrected mortality was calculated according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Conclusions: 48 h LD_{50} > 109.25 µg ai/bee (oral toxicity)
72 h LD_{50} > 100 µg ai/bee (contact toxicity)

Validity criteria: The mean mortality of the controls in the oral and contact toxicity test was 0 and 2 % which is in the line with the recommended maximum mortality of 10 % according to the OECD guidelines.
The 24 h LD_{50} values of the reference item (dimethoate) in the oral (24 h LD_{50} = 0.15 µg ai/bee) and contact (24 h LD_{50} = 0.18 µg ai/bee) toxicity tests were within the recommended range of 0.10 – 0.35 µg ai/bee (oral) and 0.10 – 0.30 µg ai/bee (contact), respectively.
In the contact toxicity test a volume of 2 µL of solution was chosen in deviation to the guideline recommendation of 1 µL, since a higher volume ensures a more reliable dispersion of the test item. Experience of the test facility has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. The RMS agrees with the applicant that under consideration

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of the results of the study it can be concluded that the use of a higher volume has no impact on the outcome of the acute contact toxicity test.

B.9.4.2 Bee brood feeding test

Not required.

B.9.4.3 Cage tests (Annex IIIA 10.4.3)

Not required.

B.9.4.4 Field tests

Not required.

B.9.4.5 Summary of effects on bees

Table B.9.4.5-1: Summary of effects of S-2200 and S-2200 25 SC on honey-bees

Test substance	Exposure route	Endpoint	Value	Reference
S-2200	oral contact	48 h LD_{50} 48 h LD_{50}	> 110.71 µg ai/bee > 100 µg ai/bee	Vergé, E., 2009
S-2200 25 SC	oral contact	48 h LD_{50} 72 h LD_{50}	> 109.25 µg ai/bee > 100 µg ai/bee	Vergé, E., 2010

B.9.4.6 Risk assessment for honey bees

Honey-bees may be exposed to formulated S-2200 by direct spraying of the plant protection product while bees are foraging on flowers and weeds present in or adjacent to the crop treated. They may also be exposed through contact with fresh or dry residues or by oral uptake of contaminated pollen, nectar and honey dew. The LD_{50}-values (48 h) for oral and contact toxicity of the active substance and the formulation S-2200 25 SC are above 100 µg/bee, indicating a low toxicity. The toxicity of the technical active substances and the formulation active substance is comparable.
The intended single rate for winter oilseed rape is 0.2 kg ai/ha. In accordance with the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) the acute risk for bees was expressed as a Hazard Quotient (Q_H) calculated by the following formula (single application rate in g/ha, LD_{50} in µg/bee):

Hazard quotient (Q_H) = application rate/LD_{50}

Table B.9.4.6-1: Risk to honey-bees from oral and contact exposure to S-2200 and S-2200 25 SC in winter oilseed rape

Test substance	Exposure route	Application rate [g ai/ha]	Endpoint [µg ai/bee]	Q_H
S-2200	oral	1 x 200	48 h LD_{50} > 110.71	< 1.8
	contact		48 h LD_{50} > 100	< 2.0
S-2200 25 SC	oral	1 x 200	48 h LD_{50} > 109.25	< 1.8
	contact		72 h LD_{50} > 100	< 2.0

The resulting Hazard Quotients are clearly below the trigger of 50 indicating a low risk to honey-bees after the use of S-2200 25 SC according to GAP.

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B.9.5 Effects on other arthropod species (Annex IIA 8.3.2, IIIA 10.5)

B.9.5.1 Acute toxicity (Annex IIA 8.3.2, IIIA 10.5.1)

Reference:	**S-2200 25 SC: Toxicity to the Aphid Parasitoid, *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) in the Laboratory (Rate Response Test)**
Author(s), year:	Klug, Thomas, 2010a
Report/Doc. number:	Report No.: ROW-0021, Study No.: S10-02868
Guideline(s):	Mead-Briggs et al. 2000 (IOBC)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	S-2200 25 SC; batch no.: C09-5F101G, analysed content: 24,96 %
Reference:	Perfekthion (BAS 152 11 I), dimethoate, batch no.: 90924-06, analysed content: 414.8 g/L
Test species:	Adult wasps *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera: Braconidae), < 48 h old
Type of test:	Acute contact laboratory test
Number of organisms:	4 replicates with 10 wasps (5 males and 5 females) for each treatment group. Reproduction: The fertility test was conducted for the control group and for the test item groups treated with 62.5, 125, 500 and 1000 g ai/ha. Up to 17 randomly chosen surviving females were tested in each treatment group.
Treatments:	Control: deionised water Toxic standard: 0.3 mL/ha (40 % Dimethoate, EC) Test item: 62.5, 125, 250, 500, 1000 g ai/ha treatments applied with a calibrated sprayer in 200 L water/ha
Exposure route, duration:	The test units were made up of two glass plates fitted to the top and bottom sections of a steel casing perforated with holes. The holes provided, variously, ventilation (through mesh covers), an entrance aperture for introducing and feeding the wasps. After the sprayed residues had dried on the glass plates the test units were assembled with the treated surfaces of the plates facing inwards and wasps introduced. For the assessment of the parasitic capacity a pot containing 10-15 barley seedling infested with > 100 *Rhopalosiphum padi* was placed on a seed tray. Female parasitoids were introduced individually into each fertility cage. Exposure time: 48 h, parasitisation period: 24 h, post-parasitation period: 11 days.
Feeding:	A honey water solution and a sugar water solution (20 % saccharose) was added *ad libitum*.
Test conditions:	Temperature: 18.0 – 20.5 °C; relative humidity: 63 - 79 %, 16 h light and 8 hours darkness for mortality and reproduction phase, 1000 - 1400 lux for the exposure and the 24 h parasitation phase and 4000 – 7500 lux for the reproduction phase.
Test parameters:	Mortality and behavioural abnormalities were assessed 1, 2, 24 and 48 h after introduction of the wasps to the test units.
Statistics:	Mortality: Fisher's Exact Test (one-tailed, $p \leq 0.05$) was used to detect significant difference between Bonferroni-Holms corrected mortality data of each test item treatment group and the control group.

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Reproduction: Shapiro-Wilk's Test was used to test for normality and homoscedasticity. The Bonferroni U-Test was conducted to detect significant differences between the treatment and control groups. The ER_{50} was based on reproduction data and was calculated by Probit procedure.

Findings: No statistically significant effects of S-2200 25 SC on mortality compared to the control were found at any test item rate.
A statistically significant reduction in reproduction was found between the control group and the test item rates up from and including 250 g ai/ha.
See Table B.9.5.1-1.

Table B.9.5.1-1: Summary of the effects of S-2200 25 SC on *A. rhopalosiphi* following exposure on glass plates for 48 h under laboratory conditions

S-2200 25 SC (nominal) [g ai/ha]	Mortality [a] (out of 40 wasps/treatment)		Parasitation efficiency	
	Mortality [%]	Corrected mortality [%] [b]	Mean number of mummies per female ± SD	Reduction of reproduction rate [%]
Control	0	0	19.9 ± 9.3	-
62.5	2.5	2.5	17.4 ± 15.6	12.6
125	0	0	18.2 ± 7.4	8.5
250	0	0	11.8 ± 8.0 *	40.7
500	10	10	12.9 ± 7.8 *	35.2
1000	10	10	8.7 ± 9.3 *	56.3
Reference item	100 **	100	n.a.	n.a.

[a] Mortality, calculated as mean value of 4 replicates of each treatment, based on the number of dead and moribund wasps.
[b] Corrected mortality according to Schneider-Orelli (1947).
* Statistically significant effects compared to the control (Bonferroni U-Test, one-tailed, $p \leq 0.05$)
** Statistically significant effects compared to the control (Fisher's Exact Test, Bonferroni-Holms corrected, one-tailed, $p \leq 0.05$).
n.a.…not available

Conclusion: 48 h LR_{50} > 1000 g ai/ha
ER_{50} = 757.2 g ai/ha (reduction of reproduction)

Validity criteria: According to the IOBC guideline (Mead-Briggs *et al*., 2000) the study is considered valid because the mean mortality in the control group was ≤ 13 % (actual: 0%), the mean mortality in the reference group was ≥ 50 % (actual: 100 %), the mean control parasitisation rate was > 5 aphid mummies per surviving female (actual: 19.9 mummies/female) and no more than two females of the control group failed to produce mummies (actual: no female of the control group failed to produce mummies).

Reference: **S-2200 25 SC: Toxicity to the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the Laboratory (Rate Response Test)**
Author(s), year: Klug, Thomas, 2010b
Report/Doc. number: Report No.: ROW-0022, Study No.: S10-02869
Guideline(s): Blümel *et al*., 2000
GLP: Yes
Deviations: None
Validity: Acceptable

Material and Methods:
Test substance: S-2200 25 SC; batch no.: C09-5F101G, analysed content: 24,96 %

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Reference:	Perfekthion (BAS 152 11 I), dimethoate, batch no.: 90924-06, analysed content: 414.8 g/L
Test species:	*Typhlodromus pyri* Scheuten protonymphs, < 24 h old
Type of test:	Acute contact laboratory test
Number of organisms:	4 replicates with 20 individuals for each treatment group
Treatments:	Control: deionised water Toxic standard: 12 mL/ha (40 % Dimethoate EC) Test item: 62.5, 125, 250, 500 and 1000 g ai/ha. The test item was sprayed in 200 L/ha on the glasses.
Exposure route, duration:	One exposure unit consisted of two cover slides (glass) which were put together at the longer sides with a narrow gap between the slides. Cover slides were placed on tissue paper which again was placed on top of a wet foam rubber. To avoid the escape of the mites a barrier of sticky material was added. This test arena was transferred to a plastic tray filled with deionised water. The narrow gap between the two cover slides was filled with water by capillary forces to provide the mites with water. Protonymphs were exposed to dried residues for 14 d.
Feeding:	Pollen (birch and bean)
Test conditions:	Temperature: 23.5 - 26°C; relative humidity: 66 - 80 %, 16 h photo period, 4200 lux. The climatic chamber was ventilated during the study.
Test parameter:	Mortality (the number of dead mites, together with any trapped in the glue barrier or missing) was assessed 3 and 7 days after introduction of the protonymphs to the test units. Reproduction: On day 7 of exposure the sex of the test organisms was determined. The number of eggs laid and the number of live and dead juvenile stages per female were counted and removed afterwards at day 9, 11 and 14.
Statistics:	Mortality: Fisher's Exact Test (one-tailed, $p \leq 0.05$) was used to detect significant difference between Bonferroni-Holms corrected mortality data of each test item treatment group and the control group. Reproduction: Shapiro-Wilk's Test was used to test for normality and homoscedasticity. The Dunnett's t-Test was conducted to detect significant differences between the treatment and control groups.
Findings:	No significant effects on mortality and reproduction in the treatment groups were found compared to the control. See Table B.9.5.1-2

Table B.9.5.1-2: Summary of the effects of S-2200 25 SC on *Typhlodromus pyri* following exposure on glass plates for 7 days under laboratory conditions

S-2200 25 SC (nominal) [g ai/ha]	Mortality after 7 days [a] (out of 80 mites/treatment)		Reproduction (days 7 - 14)	
	Mortality [%]	Corrected mortality [%] [b]	Mean no. of eggs per female mite ± SD	Reduction of reproduction [%]
Control	1.3	-	8.3 ± 1.3	-
62.5	3.8	2.5	7.7 ± 1.2	7.2
125	3.8	2.5	7.3 ± 1.3	12.0
250	1.3	0.0	9.1 ± 1.2	- 9.6
500	1.3	0.0	8.3 ± 0.7	0.0
1000	2.5	1.2	7.6 ± 1.8	8.4
Reference item	61.3 *	60.8	n.a.	n.a.

n.a.…not available
A negative value indicates an increase in reproduction compared to the control.

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* Statistically significantly increased compared to the control (Fisher's Exact Test, one-tailed, $p \leq 0.05$)
[a] Mean mortality, based on the number of missing (escaping) and dead mites.
[b] Corrected mortality according to Schneider-Orelli (1947).

Conclusion: 7 d LR_{50} > 1000 g ai/ha
ER_{50} > 1000 g ai/ha

Validity criteria: According to the IOBC guideline (Blümel *et al*., 2000) the study is considered valid because the mean mortality in the control group was ≤ 20 % on day 7 (actual: 1.3 %), the mean mortality (control corrected) in the reference group range between 50 % and 100% (actual: 60.8 %) and the cumulative mean number of eggs per female in the control (from day 7 to day 14) was ≥ 4 eggs per female (actual: 8.3 eggs (female).

B.9.5.2 Semi-field or field tests (Annex IIIA 10.5.1, IIIA 10.5.2)

Not required.

B.9.5.3 Summary

Table B.9.5.3-1: Summary of effects of S-2200 on non-target-arthropods (laboratory studies)

Test species	Exposure	Test item	Rate [g ai/ha]	Type of effect	Effect [%]	Reference
Aphidius rhopalosiphi (adults)	Contact with dried residues on treated glass plates	S-2200 25 SC	62.5 125 250 500 1000	Corrected mortality / Reproduction	2.5 (12.6 0 / 8.5 0 / 40.7 10 / 35.2 10 / 56.3	Klug, T., 2010a
				48 h LR_{50} > 1000 g ai/ha ER_{50} = 757.2 g ai/ha		
Typhlodromus pyri (protonymphs)	Contact with dried residues on treated glass plates	S-2200 25 SC	62.5 125 250 500 1000	Corrected mortality / Reproduction	2.5 / 7.2 2.5 / 12.0 0 / - 9.6 0 / 0 1.2 / 8.4	Klug, T., 2010b
				7 d LR_{50} > 1000 g ai/ha ER_{50} > 1000 g ai/ha		

B.9.5.4 Risk assessment for non-target arthropods

Non-target arthropods may be exposed to formulated S-2200 by direct spraying, contact with fresh or dry residues or by oral uptake of contaminated food.
A risk assessment for non-target arthropods was performed according to the recommendations of ESCORT II. In the first tier hazard quotients were calculated for exposure in in-field and off-field areas according to the following formulas:

$$HQ_{in-field} = \frac{\text{application rate} \times MAF}{LR_{50}}$$

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$$HQ_{off\text{-}field} = \text{correction factor} \times \frac{\text{application rate} \times MAF \times \left(\frac{\text{drift factor}}{\text{vegetation distribution factor}} \right)}{LR_{50}}$$

drift factor = % drift/100 (90 %ile drift according to Ganzelmeier et al. 1995)

The correction factor and the vegetation distribution factor were set to 10. Drift figures were chosen according to crop type in 1 m distance. The intended use for winter oilseed rape is a single rate of 0.2 kg ai/ha, hence no MAF is applied.

Table B.9.5.4-1: HQ calculations for *Aphidius rhopalosiphi* and *Typhlodromus pyri* in winter oilseed rape

Species	Application rate [g ai/ha]	MAF	Drift [%]	LR_{50} [g ai/ha]	$HQ_{in\text{-}field}$	$HQ_{off\text{-}field}$
Aphidius rhopalosiphi	200	1	2.77	> 1000	< 0.2	< 0.006
Typhlodromus pyri		1		> 1000	< 0.2	< 0.006

All HQ-values for both indicator species and the in-field and off-field area are below the trigger of 2 and indicate a low and acceptable risk.

Based on the results of the studies adverse effects on the reproduction of *Aphidius rhopalosiphi* were identified. At test rates up from and including 250 g ai/ha statistically significantly effects on the reproduction rate (number of mummies per female) were observed. However, only at the highest test concentration of 1000 g ai/ha adverse effects of greater than 50% were shown. Hence, the ER_{50} was calculated to be 757.2 g ai/ha.
In the following a risk assessment was conducted taken into account the determined ER_{50} values for *A. rhopalosiphi* and *T. pyri* and the calculated field and drift rates for the intended use in winter oilseed rape.

Table B.9.5.4-2: HQ calculations for *Aphidius rhopalosiphi* and *Typhlodromus pyri* in winter oilseed rape

Species	Application rate [g ai/ha]	ER_{50} [g ai/ha]	Field rate [g ai/ha]	Drift rate [g ai/ha]	In-/off-field risk acceptable?
Aphidius rhopalosiphi	200	757.2	200	5.54	Yes / Yes
Typhlodromus pyri		> 1000			Yes / Yes

Overall conclusion:
Both in- and off-field exposure to formulated S-2200 do not cause unacceptable effects to populations of terrestrial non-target arthropods if S-2200 is applied according to the intended use conditions.

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B.9.6 Effects on earthworms (Annex IIA 8.9, IIIA 10.6.3)

B.9.6.1 Acute toxicity (Annex IIA 8.9.1, IIIA 10.6.1.1)

Active substance

Reference:	**Acute Toxicity of S-2200 TG on Earthworms, *Eisenia fetida* Using an Artificial Soil Test**
Author(s), year:	Stäbler, D. (2009)
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0006
Guideline(s):	OECD 207, ISO 11268-1, EC method C.8. (88/302/EEC)
GLP:	Yes (certified laboratory)
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:

Test substance:	S-2200, purity: 93.4 %, batch: ST-0811G
Test species:	Earthworm (*Eisenia fetida andrei*)
Number of organisms:	4 replicates each with 10 individuals per treatment, control and solvent control
Weight, age:	Average weight: 301 – 450 mg/worm, adults with clitellum, 7 - 9 months
Type of test, duration:	Laboratory acute test, 14 days
Applied concentrations:	100, 178, 316, 562 and 1000 mg test item/kg soil dw corresponding to 93.4, 166, 295, 525 and 934 mg active ingredient/kg soil dw
Control:	Untreated control (deinoised water for moistening) and solvent control (pure acetone and deinonised water for moistening)
Toxic standard:	2-chloroacetamid was routinely tested as toxic reference item in a separate study at concentrations of 8, 13, 22, 36 and 60 mg/kg soil dw.

Test conditions:

Test substrate:	Artificial soil according to OECD 207: 10 % sphagnum peat, 20 % kaolin clay, 69.76 % industrial sand (fine sand dominant with more than 50 % of the particles between 50 and 200 microns), 0.24 % calcium carbonate (the soil pH was adjusted to 6.0 ± 0.5 at the start of the test before adding the test item)
Substrate/test vessel:	750 g wet weight in 1 L glass vessels
Temperature:	18 - 20 °C
Light regime:	Continuous illumination (400 – 600 Lux)
Test parameters:	Assessments of mortality, abnormalities in behaviour and pathological symptoms were performed 7 and 14 days after exposure to the test item by visual examination. Body weight of surviving earthworms was determined individually at the end of the study. pH and the soil water content were measured at the beginning and the end of the study.
Statistics:	The NOEC was determined as the highest concentration with no statistically significant difference in mortality and/or body weight loss to the solvent control. Body weight loss was analysed with Dunnett`s t-Test ($p \leq 0.05$). Mortality data were analysed using Fisher`s Exact Test. The LC_{50} was determined using Probit Procedure (Gompertz distribution based on the goodness of fit values).

Findings:

Moisture:	35.1 – 35.8 % (0 d), 32.6 – 35.1 % (14 d)
pH:	6.17 – 6.28 (0 d), 6.63 – 6.93 (14 d)
Mortality:	No mortalities occurred in the controls. A single mortality was observed in the 93.4 mg ai/kg soil dw group at day 14. 47.5 % mortality were noted in the next higher

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dose group of 166 mg ai/kg soil dw. No worm was found still alive in all other test item groups.
Toxic standard: LC_{50}: 11.7 mg/kg soil dw (lower limit: 11.0, upper limit: 12.4 mg /kg soil dw)

Behavioural effects: Neither abnormalities in behaviour nor pathological symptoms were observed at the 7 day and 14 day assessments.

Body weight: The body weight loss of the test organisms in the test item treatment groups with surviving earthworms was 11.9 % (93.4 mg ai/kg soil dw) and 18.2 % (166 mg ai/kg soil dw) compared to the initial weight. In the untreated control group the average body weight loss was 5.5 % of the initial weight, in the solvent control group the average body weight loss was 7.4 % of initial. Results are summarised in the table below.

Table B.9.6.1-1: Effects of S-2200 on mortality and the mean body weight of *Eisenia fetida*

S-2200 [mg/kg soil dw]	Mean mortality on day 14 [%]	Mean body weight [mg/worm] (SD)		Mean change in body weight, 0 d – 14 d [g]
		Day 0 [g]	Day 14 [g]	
Control [a]	0.0	355.8 (9.8)	336.4 (15.4)	-19.4 (-5.5 %)
Solvent control [b]	0.0	353.2 (4.7)	327.1 (10.2)	-26.1 (-7.4 %)
93.4	2.5	377.7 (8.7)	332.6 (5.3)	-45.1 (-11.9 %)
166	47.5*	384.1 (10.0)	314.2 (11.6)	-69.9 (-18.2 %)**
295	100*	385.3 (19.1)	n.d.	n.d.
525	100*	372.5 (6.6)	n.d.	n.d.
934	100*	359.3 (15.2)	n.d.	n.d.
NOEC (body weight): 93.4 mg ai/kg soil dw NOEC (mortality): 93.4 mg ai/kg soil dw				
LC_{50} = 168 mg ai/kg soil dw (lower limit: 155, upper limit: 193 mg ai/kg soil dw)				

* statistically significantly different from the solvent control (Fisher`s Exact Test, $p \leq 0.05$)
** statistically significantly different from the solvent control (Dunnett`s t-Test, left sided, $p \leq 0.05$)
[a] deionised water only
[b] deionised water and acetone

Conclusion: LC_{50} = 168 mg ai/kg soil dw
$NOEC_{mortality}$ = 93.4 mg ai/kg soil dw
$NOEC_{body\ wheight}$ = 93.4 mg ai/kg soil dw

Validity criteria: The mortality in the solvent control did not exceed 10% at the end of the test. The average loss of biomass of the worms in the solvent control did not exceed 20%.

Metabolites

Reference:	**Acute Toxicity of 2-COOH-S-2200 on Earthworms, *Eisenia fetida* Using an Artificial Soil Test**
Author(s), year:	Gehrig, M. (2011a)
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0029
Guideline(s):	OECD 207, ISO 11268-1, EC method C.8. (88/302/EEC)
GLP:	Yes (certified laboratory)
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:

Test substance:	2-COOH-S-2200, purity: 99.0 %, batch: 317-001-47-1
Test species:	Earthworm (*Eisenia fetida andrei*)
Number of organisms:	4 replicates each with 10 individuals per treatment, control and solvent control

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Weight, age:	Average weight: 300 – 450 mg/worm, adults with clitellum, at least 2 months in age, but less than 1 year
Type of test, duration:	Laboratory acute test, 14 days
Applied concentrations:	100, 178, 316, 562 and 1000 mg active ingredient/kg soil dw
Control:	Untreated control (untreated quartz sand) and solvent control (quartz sand and acetone)
Toxic standard:	2-chloroacetamid was tested as toxic reference item in a separate study at concentrations of 5, 9, 16, 27 and 45 mg/kg soil dw.
Test conditions:	
Test substrate:	10 % sphagnum peat, 20 % kaolin clay, 69.76 % industrial sand (fine sand dominant with more than 50 % of the particles between 50 and 200 microns), 0.12 % calcium carbonate (the soil pH was adjusted to 6.0 ± 0.5 at the start of the test before adding the test item)
Substrate/test vessel:	750 g wet weight in 1 L glass vessels
Temperature:	18.5 – 21.5 °C
Light regime:	Continuous illumination (550 – 750 Lux)
Test parameters:	Assessments of mortality, abnormalities in behaviour and pathological symptoms were performed 7 and 14 days after exposure to the test item by visual examination. Body weight of surviving earthworms was determined individually at the end of the study. pH and the soil water content were measured at the beginning and the end of the study.
Statistics:	The NOEC was determined as the highest concentration with no statistically significant difference in mortality and/or body weight loss to the control. Body weight loss was analysed with Dunnett`s t-Test ($p \leq 0.05$). As no mortalities occurred no respective statistical analyses was required.
Findings:	
Moisture:	35.9 – 38.4 % (0 d), 33.1 – 35.5 % (14 d)
pH:	6.25 – 6.43 (0 d), 6.75 – 7.11 (14 d)
Mortality:	After 7 and 14 days of exposure to the test item no mortality was observed in the controls and in any of the test item treatment groups. Toxic standard: LC_{50}: 15.7 mg/kg soil dw (lower limit: 14.4, upper limit: 17.1 mg /kg soil dw)
Behavioural effects:	Neither abnormalities in behaviour nor pathological symptoms were observed at the 7 day and 14 day assessments.
Body weight:	The body weight change of the test organisms in the test item treatment groups was between +1.5 % (1000 mg/kg soil dw) and -2.9 % (316 mg/kg soil dw) compared to the initial weight. In the control groups the average body weight loss was 3.9 % (solvent control) and 6.2 % (control) of the initial weight. Results are summarised in the table below.

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Table B.9.6.1-2: Effects of 2-COOH-S-2200 on mortality and the mean body weight of *Eisenia fetida*

2-COOH-S-2200 [mg/kg soil dw]	Mean mortality on day 14 [%]	Mean body weight [mg/worm] (SD)		Mean change in body weight, 0 d – 14 d [g]
		Day 0 [g]	Day 14 [g]	
Control [a]	0.0	356.3 (10.1)	342.5 (8.7)	-13.8 (-3.9)
Solvent control [b]	0.0	351.7 (11.0)	329.9 (20.0)	-21.8 (-6.2)
100	0.0	342.5 (4.7)	338.2 (4.8)	-4.3 (-1.3)
178	0.0	349.6 (11.6)	344.5 (24.4)	-5.1 (-1.5)
316	0.0	359.7 (10.3)	349.4 (3.2)	-10.3 (-2.9)
562	0.0	342.1 (9.9)	342.3 (19.4)	-0.2 (0.1)
1000	0.0	349.5 (9.9)	354.8 (10.3)	-5.3 (1.5)
NOEC (body weight): 1000 mg/kg soil dw NOEC (mortality): 1000 mg/kg soil dw				
LC_{50} >1000 mg/kg soil dw				

[a] quartz sand
[b] quartz sand and acetone

Conclusion: LC_{50} >1000 mg/kg soil dw
$NOEC_{mortality}$ = 1000 mg/kg soil dw
$NOEC_{body\ wheight}$ = 1000 mg/kg soil dw

Validity criteria: The mortality in the solvent control did not exceed 10% at the end of the test. The average loss of biomass of the worms in the solvent control did not exceed 20%.

Reference:	**Acute Toxicity of 5-COOH-S-2200 on Earthworms, *Eisenia fetida* Using an Artificial Soil Test**
Author(s), year:	Gehrig, M. (2011b)
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0030
Guideline(s):	OECD 207, ISO 11268-1, EC method C.8. (88/302/EEC)
GLP:	Yes (certified laboratory)
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:

Test substance:	5-COOH-S-2200, purity: 97.6 %, batch: 262-005-10-1
Test species:	Earthworm (*Eisenia fetida andrei*)
Number of organisms:	4 replicates each with 10 individuals per treatment, control and solvent control
Weight, age:	Average weight: 300 – 450 mg/worm, adults with clitellum, at least 2 months in age, but less than 1 year
Type of test, duration:	Laboratory acute test, 14 days
Applied concentrations:	100, 178, 316, 562 and 1000 mg active ingredient/kg soil dw
Control:	Untreated control (untreated quartz sand) and solvent control (quartz sand and acetone)
Toxic standard:	2-chloroacetamid was tested as toxic reference item in a separate study at concentrations of 5, 9, 16, 27 and 45 mg/kg soil dw.

Test conditions:

Test substrate:	10 % sphagnum peat, 20 % kaolin clay, 69.76 % industrial sand (fine sand dominant with more than 50 % of the particles between 50 and 200 microns), 0.12 % calcium carbonate (the soil pH was adjusted to 6.0 ± 0.5 at the start of the test

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	before adding the test item)
Substrate/test vessel:	750 g wet weight in 1 L glass vessels
Temperature:	18.5 – 21.5 °C
Light regime:	Continuous illumination (550 – 750 Lux)
Test parameters:	Assessments of mortality, abnormalities in behaviour and pathological symptoms were performed 7 and 14 days after exposure to the test item by visual examination. Body weight of surviving earthworms was determined individually at the end of the study. pH and the soil water content were measured at the beginning and the end of the study.
Statistics:	The NOEC was determined as the highest concentration with no statistically significant difference in mortality and/or body weight loss to the control. Body weight loss was analysed with Williams test ($p \leq 0.05$). The LC_{50} value could not be calculated.
Findings:	
Moisture:	35.9 – 37.9 % (0 d), 33.0 – 37.3 % (14 d)
pH:	6.25 – 6.50 (0 d), 6.75 – 7.16 (14 d)
Mortality:	After 7 and 14 days only one single mortality was observed in the 100 mg/kg soil dw treatment group. No further mortalities were noted in any of the other groups. Toxic standard: LC_{50}: 15.7 mg/kg soil dw (lower limit: 14.4, upper limit: 17.1 mg /kg soil dw)
Behavioural effects:	Neither abnormalities in behaviour nor pathological symptoms were observed at the 7 day and 14 day assessments.
Body weight:	The body weight change of the test organisms in the test item treatment groups was between +0.6 % (100 mg/kg soil dw) and -11.2 % (178 mg/kg soil dw) compared to the initial weight. In the control groups the average body weight loss was 3.9 % (solvent control) and 6.2 % (control) of the initial weight. Results are summarised in the table below.

Table B.9.6.1-3: Effects of 5-COOH-S-2200 on mortality and the mean body weight of *Eisenia fetida*

5-COOH-S-2200 [mg/kg soil dw]	Mean mortality on day 14 [%]	Mean body weight [mg/worm] (SD)		Mean change in body weight, 0 d – 14 d [g]
		Day 0 [g]	Day 14 [g]	
Control [a]	0.0	356.3 (10.1)	342.5 (8.7)	-13.8 (-3.9)
Solvent control [b]	0.0	351.7 (11.0)	329.9 (20.0)	-21.8 (-6.2)
100	0.0	334.1 (4.3)	336.2 (12.9)	2.1 (0.6)
178	0.0	354.2 (9.0)	314.5 (21.9)	-39.7 (-11.2)
316	0.0	357.7 (10.4)	345.5 (8.5)	-12.2 (-3.4)
562	0.0	363.5 (9.3)	337.4 (15.5)	-26.1 (-7.2)
1000	0.0	345.8 (10.9)	330.3 (14.3)	-15.5 (-4.5)
NOEC (body weight): 1000 mg/kg soil dw NOEC (mortality): 1000 mg/kg soil dw				
LC_{50} >1000 mg/kg soil dw				

[a] quartz sand
[b] quartz sand and acetone

Conclusion:	LC_{50} >1000 mg/kg soil dw $NOEC_{mortality}$ = 1000 mg/kg soil dw $NOEC_{body\ wheight}$ = 1000 mg/kg soil dw
Validity criteria:	The mortality in the solvent control did not exceed 10% at the end of the test. The average loss of biomass of the worms in the solvent control did not exceed 20%.
Comment RMS:	It is noted that the same controls were used for the acute earthworm studies with the metabolites 2-COOH-S-2200 and 5-COOH-S-2200. Assuming that the studies were

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performed in parallel this procedure is not considered to invalidate the studies.

B.9.6.2 Sublethal effects (Annex IIA 8.9.2, IIIA 10.6.4)

Active substance

Reference:	**Sublethal Toxicity of S-2200 TG to the Earthworms *Eisenia fetida* in Artificial Soil**
Author(s), year:	Ganssmann, M. (2011)
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0027
Guideline(s):	OECD 222, ISO 11268-2
GLP:	Yes (certified laboratory)
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:	
Test substance:	S-2200 TG, purity: 93.4 %, batch: ST-0811G
Test species:	Earthworm (*Eisenia andrei*)
Number of organisms:	4 replicates per treatment and 8 replicates per control, solvent control and positive control, each with 10 individuals.
Weight, age:	Mean: 390 - 546 mg/worm, 9-10 months old with a clitellum
Type of test, duration:	Laboratory sublethal test, 8 weeks
Applied concentrations:	Nominal: 0 (control, solvent control), 3.8, 7.5, 15, 30, 60 mg ai/kg soil dw
Solvent/vehicle:	Acetone, quartz sand
Toxic standard:	Carbendazim (formulated as Nociolex) was tested in the testing facility in a separate toxic reference item study at 0 and 0.653 mg ai/kg soil dw.
Test conditions:	
Test substrate:	Artificial soil, 10 % sphagnum peat, 21 % kaolin clay, 69 - 70 % industrial sand (fine sand dominant with more than 50 % of the particles between 50 and 200 microns), approx. 1 % calcium carbonate – precipitated extra pure (the soil pH was adjusted to 6.0 ± 0.5 at the start of the test before adding the test item), 10 g air dried finely ground cow manure per 1 kg substrate, Water content was adjusted to 55 % WHCmax.
Substrate/test vessel:	590 g dry substrate + 10 g quartz sand/test container (1000cm^3)
Temperature:	19. – 20.5 °C
Light regime:	16 hours light (400 – 650 Lux) / 8 hours dark
Feeding:	One day after application earthworms were fed with 4 g finely ground cow manure per vessel on the soil surface. On day 7, 14 and 21 food was added to the soil depending on the feeding activity. After 28 days 4 g food was added for the reproduction test and units were moistened with 4 g deionised water.
Test parameters:	Mortality, behavioural abnormalities and pathological symptoms were recorded 28 days after application. Body weight was recorded individually at test initial and after 28 days of exposure. The number of offspring was determined after 56 days. pH and soil water content were determined at the beginning of the test and 56 days after application.
Statistics:	Reference parameter for all evaluations was the solvent control. Normality of data was tested using the Shapiro-Wilks test. Homoscedasticity was tested using Levene`s test for body weight change and reproduction, followed by William`s test (two-tailed) for the body weight change and reproduction capacity (p

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≤ 0.05).

Findings:

Water content: Day 0: 28.4 – 31.4 %
Day 56: 27.1 – 31.0 %

pH: 6.25 – 6.36 (day 0), 6.93 – 6.99 (day 56)

Toxic reference item: Toxic reference item: A statistically significant reduction in reproduction and mean body weight change was observed at 0.653 mg ai/kg soil dw.

Mortality: After 28 days, no mortality of adult earthworms was observed in any of the test item treatment groups or the control groups.

Body weight: In the solvent control, the mean body weight change was +38.2 % and in the control the mean body weight change was 30.9 % in relation to the initial weight. Statistically significant differences as compared to the solvent control occurred at the highest test item treatment group.

Reproduction capacity: The mean number of juveniles in the solvent control and in the control was 193.6 and 211.3, respectively. A statistically significant effect on reproduction in comparison to the solvent control was noted at 15, 30 and 60 mg ai/kg soil dw.

Table B.9.6.2-1: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

Test item	Solvent control	control	S-2200				
Exposure [mg ai/kg soil dw]	-	-	3.8	7.5	15	30	60
Mortality of adult earthworms [%] after 28 d	0	0	0	0	0	0	0
Mean weight change [mg/worm]	169.8	137.0	144.5	143.5	173.6	170.5	129.3
Mean weight change [%]	38.2	30.9	32.2	32.6	39.9	38.1	29.3*
Mean number of Juveniles/replicate (±SD)	193.6 (10.6)	211.3 (9.1)	214.5 (15.9)	217.3 (12.1)	155.5 (10.4)	187.0 (5.8)	153.5 (11.6)
Reproduction [%] (deviation from control)	--	+9.1	+10.8	+12.2	-19.7*	-3.4*	-20.7*
EC_{50} > 60 mg ai/kg soil dw $NOEC_{body\ weight}$ = 30 mg ai/kg soil dw $NOEC_{reproduction}$ = 7.5 mg ai/kg soil dw $NOEC_{overall}$ = 7.5 mg ai/kg soil dw							

* Statistically significantly different to the solvent control (William`s Test, descending order, two sided, p ≤ 0.05)

Conclusion:
EC_{50} > 60 mg ai/kg soil dw
$NOEC_{body\ weight}$ = 30 mg ai/kg soil dw
$NOEC_{reproduction}$ = 7.5 mg ai/kg soil dw
$NOEC_{overall}$ = 7.5 mg ai/kg soil dw

Validity criteria: The average mortality of adults in both controls was ≤ 10 %. The number of juveniles per vessel in both controls was ≥ 30. The coefficient of variance of reproduction in both controls was ≤ 30 % (solvent control: 10.6 %; control: 9.1 %).

B.9.6.3 Field studies

Not required, since the risk assessment (see Risk assessment for earthworms (Annex IIIA 10.6.1)) indicates an acceptable acute and long-term risk for earthworms regarding the intended uses. Therefore, no higher tier risk assessment is required.

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B.9.6.4 Summary of effects on earthworms

Table B.9.6.4-1: Summary of effects of the active substance S-2200 on earthworms

Substance	Species	Endpoint	Time	LC_{50}/EC_{50} [mg/kg soil dw]	NOEC [mg/kg soil dw]	Reference
S-2200	*Eisenia fetida*	Mortality	14 d	**LC_{50} =168**	93.4	Stäbler D., 2009
	Eisenia fetida	Reproduction Body weight	56 d	EC_{50} > 60*	**7.5**	Ganssmann M., 2011
Metabolite 2-COOH-S-2200	*Eisenia fetida*	Mortality	14 d	LC_{50} >1000	1000	Gehrig M., 2011a
Metabolite 5-COOH-S-2200	*Eisenia fetida*	Mortality	14 d	**LC_{50} >1000**	1000	Gehrig M., 2011b

Bold written values were used for the risk assessment.
* The EC_{50} for reproduction and body weight change could not be calculated, but it is assumed to be greater than 60 mg ai/kg soil dw.

B.9.6.5 Risk assessment for earthworms (Annex IIIA 10.6.1)

TER values for earthworms were calculated as the ratio between acute effect concentrations (LC_{50}) or sublethal no observed effect concentrations (NOEC), respectively, and the maximum initial PEC_{soil}. The PEC_{soil} used for the 1st tier risk assessment is based on the maximum plateau (minimum plateau plus single application) and was calculated to be 0.067 mg/kg (see fate section). No plateau was calculated for the metabolite 5-COOH-S-2200. The relevant PEC_{soil} is 0.011 mg/kg.

Acute effects

Table B.9.6.5-1: TER acute for earthworms

GAP use	Test substance	14 d LC_{50} [mg ai/kg soil dw]	max PEC_{soil} [mg/kg soil dw]	TER_A	Trigger
Oilseed rape, 200 g ai/ha	S-2200	84 *	0.067	1254	10
	5-COOH-S-2200	> 500 *	0.011	> 45455	10

* corrected by a factor of 2 due to the log P_{OW} of S-2200 > 2 (3.51 for S-2200, 2.88 for 5-COOH-S-2200, estimated with KOWIN)

Long term effects

Table B.9.6.5-2: TER long-term for earthworms

GAP use	Test substance	NOEC [mg ai/kg soil dw]	max PEC_{soil} [mg/kg soil dw]	TER_{LT}	Trigger
Oilseed rape, 200 g ai/ha	S-2200	3.75 *	0.067	56	5

* corrected by a factor of 2 due to the log P_{OW} of S-2200 > 2 (3.51 for S-2200)

Active substance and formulation

The calculated TER_A value for the active substance S-2200 is well above the trigger value of 10, thus the acute risk to earthworms after the application according to GAP use in oilseed rape can be considered as low.

Furthermore, the long-term risk for earthworms from exposure to the active substance was considered to be acceptable. The TER_{LT} value for the long-term exposure was calculated to be 54. Hence, S-2200 is expected not to pose an acute and long-term risk for earthworms when applied according to GAP.

The risk assessment for earthworms is based on toxicity studies with the technical active substance S-2200. Neither an acute toxicity study nor a sub-lethal reproduction study with the lead formulation S-2200 25 SC was submitted by the applicant.

According to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) studies like earthworm reproduction studies should be conducted with the formulated product instead of the active

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substance. However, considering the low acute and reproductive toxicity of the active substance to earthworms the risk for earthworms can be assumed to be acceptable considering exposure to the lead formulation.

Metabolites

5-COOH-S-2200 was found to be the only potentially relevant metabolite in soil and a respective acute risk assessment was performed. The second metabolite 2-COOH-S-2200 does not occur at amounts >10% and is therefore not considered to be relevant for the risk assessment. A long-term risk assessment based on a study addressing effects on reproduction should be performed for substances with a DT_{90f} between 100 and 365 and/or the number of application is between 3 and 6 on a case by case basis. The worst-case DT_{90} for 5-COOH-S-2200 in the laboratory is 454.4 days, the geometric mean is 93.04 days. As the GAP only includes one application and the TER values for the acute and the long-term risk for the parent and for the acute risk for 5-COOH-S-2200 are quite high, no further chronic study is considered necessary.

B.9.7 Effects on soil non-target macro-organisms (Annex IIA 8.14, IIIA 10.6.6)

B.9.7.1 Effects on collembola or gamasid mites

According to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) studies on other soil macro-organisms (collembolan, gamasid mites) are only required for persistent active substances or metabolites with a DT_{90f} between 100 and 365 days and a standard HQ for arthropods (*A. rhopalosiphi* and *T. pyri*) of greater than 2.
The worst-case DT_{90f} of the active substance S-2200 is 225 days and the standard HQ values for arthropods are below the trigger of 2. Additionally, the acute and long-term earthworm toxicity studies indicate a low risk for earthworms from exposure to the active substance S-2200.

No DT_{90f} is available for the metabolite 5-COOH-S-2200. The worst-case DT_{90lab} is 454.4 days, the geometric mean is 93.04 days. The long DT_{90} was mainly due to slow degradation of the parent compound in this study. As the degradation of the parent compound in the field was demonstrated to be more rapid, also the DT_{90f} for 5-COOH-S-2200 can be expected to be substantially shorter.

Based on the available information the risk for other soil macro-organisms can be assumed to be low. Therefore, neither a collembolan reproduction study nor a test on gamasid mites is required for the active substance S-2200 and its metabolite 5-COOH-S-2200.

B.9.7.2 Effects on the degradation in litter bags (Annex IIIA 10.6.7)

According to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) litter bag tests are only required for persistent active substances or metabolites with a DT_{90f} of greater than 365 days, effects on soil micro-organisms greater than 25% after 100 days and a TER_{LT} for earthworms of less than 5.
The worst-case DT_{90f} of the active substance S-2200 is 225 days and therefore below the trigger value of 365 days. In addition, the effects on soil micro-organisms were observed to be below 25% after 28 days of exposure and the TER_{LT} for earthworms was above the trigger of 5.

The worst-case DT_{90lab} for the metabolite 5-COOH-S-2200 is above 365 days (454.4 days), but as explained above the DT_{90f} is likely to be considerably shorter.

Based on the available information the risk regarding soil degradation can be assumed to be low. Therefore, no litter bag study is required for the active substance S-2200.

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B.9.7.3 Risk assessment of other soil non-target macro-organisms

Based on the available information for the persistence of the active substance S-2200 and the metabolite 5-COOH-S-2200 in soil and the low toxicity towards soil organisms like earthworms, soil micro-organisms and non-target arthropods the risk for soil macro-organism can be assumed to be low. Therefore, neither studies with other soil macro-organisms (collembolan, gamasid mites) nor litter bag tests are required.

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.10, IIIA 10.7)

B.9.8.1 Nitrogen and carbon mineralisation

Active Substance

Reference:	**Effect of S-2200 TG on the Activity of the Soil Microflora**
Author(s), year:	Gehrig M., 2011c
Report/Doc. number:	Sumitomo Chemical Co. Ltd Report No.: ROW-0028
Guideline(s):	OECD 216 and 217
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:	
Test substance:	S-2200 TG, purity (analysed): 93.4 %, Batch No.: ST-0811G
Test species:	Soil microflora
Type of test, duration:	Nitrogen and carbon transformation test, 28 days
Applied concentrations:	Untreated and solvent control, 0.3 mg ai/kg soil dw, 1.5 mg ai/kg soil dw
Solvent/vehicle:	Acetone / quartz sand
Toxic standard:	Sodium chloride, 20 g ai/kg soil dw
Test conditions:	
Test substrate:	Common agricultural soil (characterized as medium loamy sand), collected on January 28th, 2011 at a depth of 0 - 20 cm, from a field located in Offenbach, Germany: dry weight [%]: 95.0; pH: 6.8; total organic carbon [%] based on soil dw: 1.03; microbial biomass [mg C/100 g soil dw] calculated from respiration activity: 17.3; microbial biomass [% of the TOC] calculated from respiration activity: 1.68; NO_3^- N [mg /kg soil dw]: 12.3; total nitrogen [% N]: 0.09; WHC_{max} [%]: 35.45 Texture (DIN 4220): 8,3 % clay, 27,7 % silt, 64,0 % sand, For the nitrogen turnover the soil and finely ground Lucerne meal were mixed before application to achieve a final concentration of 0.5 % of soil dw. For the short-term respiration a concentration of 300 mg glucose/100 g soil wet weight was used.
Test design:	3 replicates/treatment group (soil subsamples of approx. 1100 g)
Incubation:	20 ± 2 °C in the dark, about 45 % WHC_{max}
Test parameters:	Soil dry weight and pH were determined 0, 7, 14 and 28 days after application. Nitrogen mineralisation: Soil nitrogen content was determined 0, 7, 14 and 28 days after application of the test item. Soil nitrification was determined by measuring the NO_3^- contents of aqueous soil extracts by means of calibrated ion sensitive electrode and Orion expandable Ionanalyser.

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Carbon mineralisation: Short-term respiration was measured in soil samples take 0, 7, 14 and 28 day after test initiation by means of the OxiTop® method. The principle of the OxiTop® is the measurement of the decrease of pressure in the test bottles as a result of the oxygen consumption by microbial activity.
The rate of oxygen consumption during the measurement period was determined within the first 12 hours.

Statistics: The results of the nitrification and short-term respiration measurements were tested for normality using Shapiro-Wilk's test and residual analysis. Homogeneity of variances was tested using Levene's test or Bartlett's test. For the nitrogen content at day 28, the nitrogen turnover (nitrate-N formation rate of the last sampling interval (14 day sampling to 28 day sampling), for the nitrate-N formation rate of the whole study period (0 day sampling to 28 day sampling) and for the short-term respiration the data comply with the requirements for a multiple-t-test. Hence, the Dunnett's t-Test was used to analyse the data for significance.

Findings:

pH: Nitrogen turnover: 7.19 – 7.40 (start), 7.12 – 7.14 (end)
Short-term respiration: 6.61 – 6.84 (start), 6.84 – 6.87 (end)

Effects test item: Nitrogen turnover: Regarding the nitrate content at day 28, the nitrate formation rate for the interval between the 0 day sampling and the 28 day sampling as well as for the interval between the 14 day sampling and the 28 day sampling no statistically significant differences were detected between the control and soil treated with 0.30 mg ai/kg soil dry weight and 1.5 mg ai/kg soil dw.
After 28 days of incubation the mean deviations to the solvent control of the amount of nitrate-N and the nitrate formation rates were below the threshold value of 25 %, except for the value for the nitrate formation (day 0 - day 28) at 0.3 mg ai/kg soil dw (26.2 % deviation to the solvent control).
Short-term respiration: The short-term respiration rate in soil treated with the test item at 0.30 and 1.5 mg ai/kg soil dry weight did not differ significantly from the solvent control at study termination (28 day incubation). Deviations from the solvent control at test end were -1.47 % at 0.30 mg ai/kg soil dw and -2.01 % at 1.5 mg ai/kg soil dw. Tabulated results are presented in the table below.

Table B.9.8.1-1: Effects of S-2200 on nitrogen transformation

Test item mg ai/kg soil dw	NO_3^--N Levels (Day 28)		NO_3^--N formation rate (Day 0 – 7)		NO_3^--N formation rate (Day 0 – 14)		NO_3^--N formation rate (Day 0 – 28)	
	mg/kg soil dw	% of solvent control[a]	mg/kg soil dw/day	% of solvent control[a]	mg/kg soil dw/day	% of solvent control[a]	mg/kg soil dw/day	% of solvent control[a]
Solvent control	25.2	-	-0.746	-	0.164		0.461	-
Control	28.1	11.5	-0.707	-5.23	0.329	101	0.546	18.4
0.30	29.4	16.7	-0.760	1.88	0.650	113	0.582	26.2[b]
1.5	28.1	11.5	-0.810	8.58	0.443	170	0.536	16.3

[a] negative value indicates inhibition, positive value indicates stimulation
[b] regarded as not treatment related

Table B.9.8.1-2: Effects of S-2200 on glucose induced short-term respiration

Test item mg ai/kg soil dw	Respiration rate (Day 0)		Respiration rate (Day 7)		Respiration rate (Day 14)		Respiration rate (Day 28)	
	mg CO_2/h/kg soil dw	% of solvent control[a]	mg CO_2/h/kg soil dw	% of solvent control[a]	mg CO_2/h/kg soil dw	% of solvent control[a]	mg CO_2/h/kg soil dw	% of solvent control[a]

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Test item mg ai/kg soil dw	Respiration rate (Day 0)		Respiration rate (Day 7)		Respiration rate (Day 14)		Respiration rate (Day 28)	
Solvent control	9.91	-	9.55	-	9.03	--	7.48	-
Control	10.5	5.95	9.64	0.942	9.24	2.33	7.43	0.668
0.30	10.3	3.94	9.49	-0.628	8.89	-4.55	7.37	-1.47
1.5	9.63	-2.83	8.93	-6.49	8.63	-4.43	7.33	-2.01

[a] negative value indicates inhibition, positive value indicates stimulation

Conclusion: After 28 days of incubation the deviations between control soil and soil treated with S-2200 (applied with dose rates of 0.3 and 1.5 mg ai/kg soil dw) was < 25 %, thus S-2200 had no adverse effects on nitrogen- and carbon-transformation of soil micro-organisms.

Validity criteria: Coefficient of variation in the control ≤ 15 %

Comment RMS: The deviation the nitrate formation (day 0 - day 28) at 0.3 mg ai/kg soil dw was 26.2 % compared to the solvent control at the end of study, but the test was not prolonged. According to the study author this deviation was not considered treatment related as no effect >25% were observed at the higher treatment rate. The argumentation of the study author can be followed and the study is considered valid and useful for the risk assessment.

B.9.8.2 Summary of effects on non-target micro-organisms

Table B.9.8.2-1: Summary of effects n non-target micro-organisms

Test substance	Test parameter	Test concentration	Time	Effects (deviation from control)	Reference
S-2200	Nitrogen mineralisation	0.3 mg ai/kg soil dw	28 days	26.2 % [a] *	Gehrig M., 2011c
		1.5 mg ai/kg soil dw		16.3 % [a]	
S-2200	Carbon mineralisation	0.3 mg ai/kg soil dw	28 days	-1.47 % [b]	
		1.5 mg ai/kg soil dw		-2.01 % [b]	

[a] NO_3^- - N formation rate (Day 0 – 28)
[b] Respiration rate (Day 28)
* regarded as not treatment related

B.9.8.3 Risk assessment for non-target micro-organisms

S-2200 did not significantly affect the activity of the soil micro-flora respiration and soil nitrogen transformation under test conditions at application rates up to 1.5 mg ai/kg soil. The relevant PEC_{soil} was the maximum plateau of 0.067 mg ai/kg (minimum plateau plus single application).

Thus the exposure concentrations used in the tests was approximately 4 and 22 times higher than the maximal expected PEC_{soil} when applied according to the GAP.

No study with the lead formulation S-2200 25 SC was submitted by the notifier. According to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) studies like soil micro-flora studies should be conducted with the formulated product instead of the active substance. However, as a study with the technical active substance is available, the information on formulation toxicity is dispensable with regard to the risk assessment.

The relevant metabolite 5-COOH-S-2200 occurs at an amount of 18.3 % after 60 days and it can therefore not be expected to be covered by the available test with the parent compound. However, the acute study with earthworms and also further data with aquatic organisms indicate that the metabolite is less toxic than the parent compound and no further studies are considered necessary.

Regarding the risk of the different isomers it can be assumed that the behaviour in different soils is comparable and therefore no further information is considered necessary for the assessment of S-2200.

According to the results of the data provided for the active substance S-2200 it can be assumed that the risk for soil micro-organisms is low when applied according to the GAP.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be a risk (Annex IIA 8.6)

B.9.9.1 Effects on terrestrial non-target plants

Reference:	**S-2200 25 SC: Toxicity Effects on the Seedling Emergence of six Species of Plants**
Author(s), year:	Sindermann, Anne B., Porch, John R., Krueger, Henry O., Martin, Kathy H., 2012a
Report/Doc. number:	Report No.: ROW-0045; Study No. 166-202
Guideline(s):	OECD 208
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	S-2200 25 SC; batch no.: C09-5F101G, purity: 24,96%, average density: 1.054 g/mL
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.4%, batch: AS 2263a S-2167 (S-2200 *R*-isomer), purity: 99.8%, batch: AS 2262a
Test species:	Monocotyledonae: Wheat (*Triticum aestivum*, Poaceae), Corn (*Zea mays*, Poaceae) Dicotyledonae: Cucumber (*Cucumis sativa,* Cucurbitaceae), Soybean (*Glycine max*, Fabaceae), Tomato (*Solanum lycopersicum,* Solanaceae), Lettuce (*Lactuca sativa*, Asteraceae)
Test soil:	The test soil was characterised as a loamy sand soil (85% sand, 6% silt, and 9% clay), and was a mixture of kaolinite clay, industrial quartz sand, and peat, with limestone assed to buffer the pH. Organic carbon content: 0.71%, pH: 6.2
Type of test:	Greenhouse seedling emergence test
Number of organisms:	Ten seeds per replicate (= pot); four replicates for each treatment group
Applied concentrations:	Deionised water control; test item: 200 g ai/ha
Test duration:	21 days from application onwards
Test units:	Plastic pots
Test procedure:	The application was performed on the day of sowing of the seeds (pre-emergence) in an application volume of 200 L/ha water.
Test conditions:	Temperature: 17.7-32.8°C; relative humidity: 13.1-86.7 %, light: 16L:8D
Test parameters:	Observations for seedling emergence were conducted on 7, 14 and 21 days after application. On test termination, height measurements and seedling conditional assessments were made. Plant fresh weight was assessed at test termination.
Statistics:	Statistical analyses were performed using SAS Proprietary Software Version 8.
Findings:	Since S-2200 is a mixture of two isomers, S-2354 and S-2167, the mean recovery of S-2200 was calculated by the addition of the results of the analysis for S-2354 and S-2167 from aqueous solutions. The mean measured concentrations were in the range from 101-103% of nominal. There were no adverse effects on the emergence, survival, height, dry weight, and plant condition of the species tested. No treatment group mean was significantly

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different (p > 0.05) from the respective control mean.

Table B.9.3.3.4-1: Effects of S-2200 25 SC on seedling emergence

Test species		Effects on seedling emergence (Reduction compared to control)			
		% Emergence (% Reduction)	% Survival (% Reduction)	Dry weight [mg] (% Reduction)	Height [cm] (% Reduction)
Trticum aestivum	Control	92.5	100	266	44.9
	200 g ai/ha	97.5 (- 6%)	100 (0%)	244 (8%)	44.7 (0%)
Zea mays	Control	97.5	100	622	56.2
	200 g ai/ha	100 (- 3%)	100 (0%) [b]	646 (- 4%)	55.2 (2%)
Cucumis sativa	Control	97.5	100	454	14.3
	200 g ai/ha	97.5 (0%)	100 (0%)	428 (6%)	14.0 (2%)
Glycine max	Control	97.5	97.5 [c]	530	21.4
	200 g ai/ha	97.5 (0%)	100 (- 3%)	554 (- 5%)	22.1 (- 3%)
Lactuca sativa	Control	75.0	90.2 [b, c]	86.7	11.8
	200 g ai/ha	90.0 (- 20%)	91.7 (- 2%) [c]	70.3 (19%) [a]	12.3 (- 4%)
Solanum lycopersicum	Control	90.0	100	203	13.1
	200 g ai/ha	95.0 (- 6%)	100 (0%)	203 (0%)	14.7 (- 12%)

Negative value indicates an increase of emergence, survival, weight and height of the seedlings.
[a] Since the treatment group mean was not statistically significantly different from the control group mean this reduction (19%) was not considered to be an adverse effect.
[b] One seedling was noted with signs of visual phytotoxicity.
[c] One to three seedlings died.

Conclusion: No adverse effects on seedling emergence and shoot biomass were observed in the seedling emergence test after application of 200 g ai/ha. The ER_{50} is therefore considered to be > 200 g ai/ha for six species of plants and 200 g ai/ha can also be considered as NOER.

The mean emergence in the control was at least 70% (actual: 75-97.5%). The mean control survival was at least 90% (actual: 90.2-100%). The control seedling did not exhibit visible effects, and the environmental conditions and growing medium were the same for all test groups of a species.

The validity criteria were met with one exception: One *L. sativa* seedling in the control group was observed to be necrotic. Since occasional signs of phytotoxicity are observable in natural conditions the one necrotic seedling was not considered to be abnormal. Control seedlings of all species were considered to be acceptable for treatment group comparison.

Reference:	**S-2200 25 SC: Toxicity Effects on the Vegetative Vigour of six Species of Plants**
Author(s), year:	Sindermann, Anne B., Porch, John R., Krueger, Henry O., Martin, Kathy H., 2012b
Report/Doc. number:	Report No.: ROW-0046; Study No. 166-203
Guideline(s):	OECD 227
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	S-2200 25 SC; batch no.: C09-5F101G, purity: 24,96%, average density: 1.054 g/mL
Reference substance:	S-2354 (S-2200 *S*-isomer), purity: 99.4%, batch: AS 2263a S-2167 (S-2200 *R*-isomer), purity: 99.8%, batch: AS 2262a

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Test species:	Monocotyledonae: Wheat (*Triticum aestivum*, Poaceae), Corn (*Zea mays*, Poaceae) Dicotyledonae: Cucumber (*Cucumis sativa,* Cucurbitaceae), Soybean (*Glycine max*, Fabaceae), Tomato (*Solanum lycopersicum,* Solanaceae), Lettuce (*Lactuca sativa*, Asteraceae)
Type of test:	Greenhouse vegetative vigour test
Number of organisms:	Five plants per replicate (= pot); six replicates for each treatment group
Applied concentrations:	Deionised water control; test item: 200 g ai/ha; Items were applied at a nominal spray volume of 200 L/ha.
Test duration:	21 days from application onwards
Test units:	plastic pots, bottom watering
Test procedure:	The application was performed at the 1 to 4 leaf stage of the plants, depending on the plant species.
Test conditions:	Temperature: 18.3 – 32.0°C; relative humidity: 24.0 – 89.8%, light: 16L:8D
Test parameters:	Observations for plant condition were made on days 0 (prior to application), 7, 14 and 21 (days after application). Observations of height were made on days 0 (prior to application), 7 and 21 (days after application). After the final observations were made, plant shoots were collected, dried, and weighed.
Statistics:	Statistical analyses were performed using SAS Proprietary Software Version 8.
Findings:	Since S-2200 is a mixture of two isomers, S-2354 and S-2167, the mean recovery of S-2200 was calculated by the addition of the results of the analysis for S-2354 and S-2167 from aqueous solutions. The mean measured concentrations were in the range from 97-103% of nominal. There were no adverse effects on the survival, height, dry weight, and plant condition of the species tested. No treatment group mean was significantly difference ($p > 0.05$) from the respective control mean.

Table B.9.3.3.4-2: Effects of S-2200 25 SC on vegetative vigour

Test species		Height [cm] (% Reduction)		% Survival (% Reduction)	Dry weight [mg] (% Reduction)
		Day 7	Day 21		
Trticum aestivum	Control	40.2	58.5	100	1.25
	200 g ai/ha	42.2 (- 5%)	57.8 (1%)	100 (0%)	1.31 (- 5%)
Zea mays	Control	45.8	90.9	100	3.00
	200 g ai/ha	46.7 (- 2%)	92.7 (- 2%)	100 (0%)	3.26 (- 9%)
Cucumis sativa	Control	10.6	28.9	100	3.04
	200 g ai/ha	11.0 (- 3%)	29.7 (- 3%)	100 (0%)	2.84 (6%)
Glycine max	Control	25.6	99.4	100 [a]	4.10
	200 g ai/ha	25.9 (- 1%)	100.5 (- 1%)	100 (0%) [b]	3.78 (8%)
Lactuca sativa	Control	13.6	22.9	100	2.48
	200 g ai/ha	14.1 (- 4%)	22.3 (3%)	100 (0%)	2.72 (- 10%)
Solanum lycopersicum	Control	18.2	43.3	100	3.89
	200 g ai/ha	18.2 (0%)	41.1 (5%)	100 (0%) [c]	3.56 (8%)

Negative values indicates an increase of emergence, survival, weight and height of the seedlings.
[a] In the control group the plants showed abnormal appearance, which was inadvertently not receiving water at test termination. This replicate was excluded from day 21 control group height and dry weight means and condition assessment.
[b] Two plants exhibited possible signs of phytotoxicity on day 21, both showed necrosis and one was also chlorotic and the plants were somewhat reduced in height in comparison to other plants in the group.
[c] Two plants exhibited signs of phytotoxicity at day 14, however the plants were observed to be normal on day 21.

Conclusion:	No adverse effects on height, survival, dry weight and plant condition were observed in the vegetative vigour test after application of 200 g ai/ha. The ER_{50} is therefore considered to be > 200 g ai/ha for six species of plants and 200 g ai/ha

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can also be considered as NOER.

The germination of seeds used in the test was at least 70%. Survival in the control group was at least 90% for plant species at test termination (actual: 100%). The control seedling did not exhibit visible effects, and the environmental conditions and growing medium were the same for all test groups of a species.
The study is considered valid as all validity criteria according to the OECD guideline were met.

Reference:	**Evaluation of Herbicidal Activity of De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and S-2200**
Author(s), year:	Takaishi, Masanao, 2010a
Report/Doc. number:	Report No.: ROG-0002, Study No.: 000-002
Guideline(s):	None cited, in-house methods
GLP:	No
Deviations:	Not applicable
Validity:	Test considered as additional information.

Material and Methods:

Test substance:	S-2200; batch no.: 07112F, purity: 99% De-Xy-S-2200 (metabolite), batch no.: HS2070313, purity: 100% 2-COOH-S-2200 (metabolite), batch no.: CTS07017, purity: 99.93% 5-COOH-S-2200 (metabolite), batch no.: CTS07018, purity: 99.98%
Test species:	Blackgrass (*Alopecurus myosuroides*), Italien ryegrass (*Lolium multiflorum*), Barnyardgrass (*Echinochloa grus-galli*), Giant foxtail (*Setaria faberi*), Cleavers/Catchweed bedstraw (*Galium aparine*), Velvetleaf (*Abutilon theophrasti*), Wheat (*Triticum aestivum*)
Type of test:	Phytotoxic activity screening test
Number of organisms:	Numbers of crop and weed seeds were 5 and 20 per plot, respectively. Crops and weeds were sown in a separate pot. Two replicates for each treatment group
Applied concentrations:	De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and S-2200 were formulated as 8% w/w formulation using N,N-diemethylformamide containing 2% w/w Tween 20. The dose rate of 400 g ai/ha (500 L/ha) was applied to the test plants in post-emergence application.
Test duration:	21 days from application onwards
Test procedure:	The test plants were sown in a plastic pot (8.8 cm in diameter) and cultivated in greenhouse at 25°C (day) and 20°C (night). Soil type was sandy clay (organic matter content: 1.7%, pH: 5.2).
Test parameters:	The herbicidal activity of each chemical was assessed 21 days after application. Criteria of assessment were stunting, yellowing of plants and inhibition of germination.
Statistics:	None
Findings:	None of the chemicals showed herbicidal activity at an application rate of 400 g ai/ha. None of them showed crop injury on wheat.
Conclusion:	The effects of post-emergence exposure of weeds and crops to S-2200 and the metabolites De-Xy-S-2200, 2-COOH-S-2200 and 5-COOH-S-2200 applied at rates of 1 x 400 g ai/ha did not have an adverse impact (i.e. phytotoxic symptoms).

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B.9.9.2 Risk assessment for terrestrial non-target plants

The effects of S-2200 25 SC on non-target plants were assessed in two greenhouse trials. The results are summarised in Table B.9.9.2-1

Table B.9.9.2-1: Summary of effects of S-2200 25 SC on non-target plants

Greenhouse vegetative vigour and seedling emergence tests			
Test species	**Test system**	**Toxicity [g ai/ha]**	**Reference**
		ER_{50} vegetative vigour and seedling emergence	
Trticum aestivum	seedling emergence/ vegetative vigour	> 200	Sindermann, A. B., Porch, J. R., Krueger, H. O., Martin, K. H., 2012a,b
Zea mays		> 200	
Cucumis sativa		> 200	
Glycine max		> 200	
Lactuca sativa		> 200	
Solanum lycopersicum		> 200	

B.9.9.2.1 Risk to other non-target organisms

A quantitative risk assessment is performed which follows a deterministic TER approach. As specified in the Guidance Document on Terrestrial Ecotoxicology. Under Council Directive 91/414/EEC the risk for non-target plants is considered acceptable if the TER for the most sensitive species is greater than 5.
Spray drift is considered to be the relevant exposure route for terrestrial non-target plants. Consequently the PECs can be calculated based on the drift models produced by the BBA (Ganzelmeier et al., 1995; updated by Rautmann et al., 2001).
The intended field use is in winter oilseed rape with a single rate of 0.2 kg ai/ha. The overall EC_{50} is > 200 g ai/ha.

Table B.9.9.2.1-1: PEC off-field and TER resulting from spray-drift after application of S-2200

Crop	No. of applications	Application rate [g ai/ha]	Drift rate [%] (distance to field edge)	PEC [g ai/ha]	TER
Winter oilseed rape	1	200	2.77 (1 m)	5.54	< 36.1

The resulting TER value is above the trigger indicating a low risk to terrestrial non-target plants if S-2200 25 SC is applied according to GAP.

B.9.9.2.2 Overall conclusions

The risk to non-target plants due to application of S-2200 25 SC under the intended representative use conditions is considered acceptable at the regular (1 m) distance to the field edge. No risk mitigation is required.

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B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

Active substance

Reference:	**S-2200 Technical Grade: Toxicity to Activated Sludge in a Respiration Inhibition Test**
Author(s), year:	Eisner, G., 2010
Report/Doc. number:	Report No. ROW-0018, Study No. C91005
Guideline(s):	OECD 209 (1984), EC Guideline Annex V - Method C.11
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	S-2200, batch: ST-0811G, purity: 93.4 %
Reference substance:	3,5-Dichlorophenol, batch: A0201703, purity: 98.8%
Test species:	Activated sludge Source: domestic sewage treatment plant, ARA Ergolz II, Füllinsdorf, Switzerland
Type of test, duration:	Laboratory aerobic activated sludge inhibition test, 3 hours
Applied concentrations:	
Nominal:	11, 34, 197, 343 and 1071 mg/L corrected for the purity of the test item (equivalent to 10, 32, 100, 320 and 1000 mg ai/L tap water) Toxic standard: 5, 16 and 50 mg/L
Test conditions:	
Substrate/test vessel:	Activated sludge and synthetic sewage (according OECD guideline) treated with active substance, reference substance and inoculum control
Incubation:	Aerated for 3 hours at 20°C
Test procedure:	Aerobic activated sludge was incubated for three hours in the presence of various concentrations of the test item under defined conditions. At the start of the test, synthetic sewage feed and activated sludge inoculum were added. The inoculum had a sludge concentration of 2.3 g/L dry weight (corresponding to about 0.9 g dry material per litre test medium). During the incubation period of 3 hours all test media and the controls were continuously aerated by intense stirring on magnetic stirrers to avoid possible foaming and/or stripping of the test item.
Test parameters:	Inhibition of respiration rate (rate of oxygen uptake). pH-value, temperature and dissolved oxygen concentration were determined at the start and at the end of the test.
Statistics:	EC_{50} of the reference substance was calculated by Probit analyses, NOEC and EC_{50} of the test substances directly from the raw data.
Findings/conclusions:	
Oxygen consumption	No significant effects (< 15%) on oxygen consumption were observed in the control and all treatment groups up to a test concentration of 1071 mg ai/L. At the highest test concentration of 1071 mg ai/L, the inhibitory effect increased to 8.8%. See Tabke B.9.10-1.

Table B.9.10-1: Effect of S-2200 on the respiration rate of activated sludge

Test substance	Nominal concentration [mg/L]	pH		Oxygen consumption rate [mg O_2/L/min]	Oxygen concentration [mg O_2/L]		Inhibition [%] [a]
		Start	End		Start	End	
Control [b]	-	7.3	8.3	0.998	8.3	8.8	-
Control [b]	-	7.5	8.2	1.056	8.3	8.0	-

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Test substance	Nominal concentration [mg/L]	pH		Oxygen consumption rate [mg O_2/L/min]	Oxygen concentration [mg O_2/L]		Inhibition [%] [a]
		Start	End		Start	End	
Toxic standard	5	7.6	8.2	0.77	8.5	8.8	24.3
Toxic standard	16	7.5	8.2	0.268	8.4	8.6	73.9
Toxic standard	50	7.5	8.4	0.100	8.4	8.7	90.3
S-2200	11	7.6	8.1	0.995	8.3	8.4	3.1
S-2200	34	7.5	8.0	1.042	8.4	8.3	- 1.5
S-2200	107	7.5	8.1	1.018	8.5	8.5	0.9
S-2200	343	7.5	8.2	1.022	8.3	8.5	0.5
S-2200	1071	7.6	8.2	0.937	8.3	8.4	8.8
3 h EC_{50} (nominal)		> 1071 mg ai/L					
3 h NOEC (nominal)		1071 mg/L					

[a] Negative values indicate an increase of the oxygen concentration
[b] Mean control oxygen consumption rate = 1.027 mg O_2/L/min

Conclusions: NOEC = 1071 mg ai/L
EC_{50} > 1071 mg ai/L
based on nominal concentrations.

Validity criteria: The test is considered valid because the two control respiration rates are within 15% of each other (actual: 6%) and the 3h EC_{50} of the toxic standard (3,5-dichlorophenol) is in the accepted range of 5 to 30 mg/L (actual: EC_{50} = 9 mg/L).

B.9.10.1 Risk assessment for biological methods of sewage treatment

For the effects of S-2200 on the inhibition of the respiration rate of aerobic wastewater micro-organisms an EC_{50} > 1071 mg/L was determined. Therefore it can be assumed that adverse effects on methods of sewage treatment are unlikely, when S-2200 is applied according to GAP.

B.9.11 Other/special studies (Annex IIA 8.16)

Additional studies on the insecticidal and fungicidal activity of the active substance S-2200 and its metabolites De-Xy-S-2200, 2-COOH-S-2200 and 5-COOH-S-2200 were submitted.

Reference:	**Evaluation of Insecticidal Activity of De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and S-2200**
Author(s), year:	Takaishi, Masanao, 2010b
Report/Doc. number:	Report No.: ROG-0003, Study No.: 000-003
Guideline(s):	None cited, in-house methods
GLP:	No
Deviations:	Not applicable
Validity:	Test considered as additional information.

Material and Methods:

Test substance: S-2200; batch no.: 07112F, purity: 99%
De-Xy-S-2200 (metabolite), batch no.: HS2070313, purity: 100%
2-COOH-S-2200 (metabolite), batch no.: CTS07017, purity: 99.93%
5-COOH-S-2200 (metabolite), batch no.: CTS07018, purity: 99.98%

Test species: Diamondback moth (*Plutella xylostella*), Cotton aphid (*Aphis gossypii*), Tobacco whitefly (*Bemisia tabaci*), Brown planthopper (*Nilaparvata lugens*), Two-spotted spider mite (*Tetranychus urticae*)

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Type of test:	Insecticidal activity screening test
Applied concentrations:	12 mg of crystal of each chemical (De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and S-2200) along with 7 mg of solid spreader adjuvant (polyoxyethylene alkyl ether fulfate ammonium salt) was crashed by a homogenizer. This procedure was done with adding 60 mL of water in order to avoid generation of heat (200 ppm as ai).
Test procedure:	Diamondback moth (*Plutella xylostella*): The test chemicals were sprayed to a cabbage seedling in 5 leaf stage. Ten 3rd-instar larvae of diamondback moth were released on the seedling one hour after the spraying. Mortality of the larvae was determined 4 day after releasing of the larvae. Cotton aphid (*Aphis gossypii*): The test chemicals were sprayed to a cucumber seedling in 1 leaf stage, which was infested with ca. 30 aphids. Mortality was determined 6 days after treatment. Tobacco whitefly (*Bemisia tabaci*): The test chemicals were sprayed to a tomato seedling in 2-3 leaf stage, which was infested with ca. 100 nymphae. Efficacy was determined 7 days after treatment. Brown planthopper (*Nilaparvata lugens*): The test chemicals were sprayed to rice seedling (2 leaf stage) grown in a plastic cup. Twenty nymphae of brown planthopper were released onto the seedlings. Mortality was determined 6 days after releasing of nymphae. Two-spotted spider mite (*Tetranychus urticae*): The test chemicals were sprayed to a seedling of kidney bean, which was with a pair of primary leaf and infested with 20-30 individuals of *T. urticae*. The efficacy was determined 7 days after spraying based on the damage in leaves.
Statistics:	None
Findings:	None of the chemicals showed insecticidal activity at an application rate of 200 ppm compared to the untreated control. None of them showed crop injury on wheat.
Conclusion:	The effects of post-emergence exposure of weeds to S-2200 and the metabolites De-Xy-S-2200, 2-COOH-S-2200 and 5-COOH-S-2200 applied at rates of 1 x 200 ppm did not have an adverse impact on the survival of the tested insects.

Reference:	**Comparison of Fungicidal Activity of 2-COOH-S-2200, 5-COOH-S-2200 with S-2200**
Author(s), year:	Kiguchi, So, 2011
Report/Doc. number:	Report No.: ROG-0004, Study No.: 000-004
Guideline(s):	None cited, in-house methods
GLP:	No
Deviations:	Not applicable
Validity:	Test considered as additional information.

Material and Methods:

Test substance:	S-2200; batch no.: NO060912-1, purity: 99.7% 2-COOH-S-2200 (metabolite), batch no.: 317-001-47-1, purity: 99% 5-COOH-S-2200 (metabolite), batch no.: 262-005-10-1, purity: 97.6%
Test species:	White mould (*Sclerotinia sclerotiorum*), stain JPU (susceptible to S-2200)
Type of test:	Fungicidal activity screening test
Applied concentrations:	0.08, 0.04, 0.2, 1 and 5 ppm of 2-COOH-S-2200, 5-COOH-S-2200 and S-2200

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Test procedure:	The mycelia of the test strain, *Sclerotinia sclerotiorum*, was inoculated onto potato dextrose agar contain both 100 ppm salicylhydroxamic acid (SHAM) and the predetermined concentration of the test chemicals. The radius of the mycelial growth was measured after incubation for three days at 23 °C.
Statistics:	None
Findings:	The metabolite 2-COOH-S-2200 and 5-COOH-S-2200 show no fungicidal activity as no growth inhibition was observed at any of the tested concentrations. Hence, the EC_{50} was determined to be greater than 5 ppm. For the active substance S-2200 an EC_{50} of 0.032 ppm was determined. At the test concentrations of 0.008, 0.04, 0.2, 1 and 5 ppm growth inhibition was shown of 32.5, 47.5, 75, 100 and 100%, respectively.
Conclusion:	The results of the screening test indicate that both 2-COOH-S-2200 and 5-COOH-S-2200 did not show any remarkable fungicidal activity to the test strains. This result indicates that both metabolites are not relevant metabolites of S-2200 with regard to their fungicidal activity.

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B.9.12 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Annex II Data and Information					
IIA, 8.1.1 / 01	[redacted]	2009	S-2200 TG: An Acute Oral Toxicity Study with the Northern Bobwhite [redacted] (Sumitomo ROW-0001) GLP, Unpublished	Y	SUM
IIA, 8.1.2 / 01	[redacted]	2009a	S-2200 TG: A Dietary LC50 Study with the Northern Bobwhite [redacted] (Sumitomo ROW-0004) GLP, Unpublished	Y	SUM
IIA, 8.1.2 / 02	Martin, K.H. & Nixon, W.B.	2009	Analytical Method Verification for the Determination of S-2200 TG In Avian Diet Wildlife International, Ltd., Report No. 166C-111 (Sumitomo ROW-0003) GLP, Unpublished	Y	SUM
IIA, 8.1.3 / 01	[redacted]	2009b	S-2200 TG: A Dietary LC50 Study with the Mallard [redacted] (Sumitomo ROW-0005) GLP, Unpublished	Y	SUM
IIA, 8.1.4 / 01	[redacted]	2011a	S-2200: A Reproduction Study with the Northern Bobwhite [redacted] (Sumitomo ROW-0031) GLP, Unpublished	Y	SUM
IIA, 8.1.4 / 02	[redacted]	2011b	S-2200: A Reproduction Study with the Mallard [redacted] (Sumitomo ROW-0032) GLP, Unpublished	Y	SUM
IIA, 8.2.1.1 / 01	[redacted]	2009a	S-2200 Technical Grade - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203, EC Guideline L383A, Method C.1 and OPPTS Draft Guideline 850.1075 [redacted] (Sumitomo ROW-0007) GLP, Unpublished	Y	SUM
IIA, 8.2.1.1 / 02	[redacted]	2009b	S-2354 (S-Isomer of S-2200) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203, EC Guideline L383A, Method C.1 and OPPTS Draft Guideline 850.1075 [redacted] (Sumitomo ROW-0011) GLP, Unpublished	Y	SUM

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IIA, 8.2.1.1 / 03	[redacted]	2009c	S-2167 (R-Isomer of S-2200) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203, EC Guideline L383A, Method C.1 and OPPTS Draft Guideline 850.1075 [redacted] (Sumitomo ROW-0010) GLP, Unpublished	Y	SUM
IIA, 8.2.1.2 / 01	[redacted]	2009d	S-2200 Technical Grade - Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Static Conditions, Following OECD Guideline #203, EC Guideline L383A, Method C.1 and OPPTS Draft Guideline 850.1075 [redacted] (Sumitomo ROW-0008) GLP, Unpublished	Y	SUM
IIA, 8.2.1.2 / 02	[redacted]	2009e	S-2200 Technical Grade - Acute Toxicity to Fathead Minnow (*Pimephales promelas*) Under Static Conditions, Following OECD Guideline #203, EC Guideline L383A, Method C.1 and OPPTS Draft Guideline 850.1075 [redacted] (Sumitomo ROW-0009) GLP, Unpublished	Y	SUM
IIA, 8.2.1.3 / 01	[redacted]	2012a	2-COOH-S-2200 - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203 and The Official Journal of the European Communities L 142/446 Method C.1 [redacted] (Sumitomo ROW-0033) GLP, Unpublished	Y	SUM
IIA, 8.2.1.3 / 02	[redacted]	2012b	5-COOH-S-2200 - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203 and The Official Journal of the European Communities L 142/446 Method C.1 [redacted] (Sumitomo ROW-0034) GLP, Unpublished	Y	SUM
IIA, 8.2.1.3 / 03	[redacted]	2012c	S-2200-OR - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203 and The Official Journal of the European Communities L 142/446 Method C.1 [redacted] (Sumitomo ROW-0035) GLP, Unpublished	Y	SUM
IIA, 8.2.1.3 / 04	[redacted]	2012d	S-2200-ORC - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203 and The Official Journal of the European Communities L 142/446 Method C.1 [redacted] (Sumitomo ROW-0036) GLP, Unpublished	Y	SUM

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Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.2.4 / 01	[redacted]	2010	S-2200 Technical Grade – Early Life-Stage Toxicity Test with Fathead Minnow, *Pimephales promelas,* Following OECD Guideline #210 and OPPTS Draft Guideline 850.1400 [redacted] (Sumitomo ROW-0019) GLP, Unpublished	Y	SUM
IIA, 8.2.6.1 / 01	[redacted]	2010	Flow-Through Bioconcentration and Metabolism Study of [^{14}C]S-2200 with Bluegill Sunfish (*Lepomis macrochirus*) [redacted] GLP, Unpublished	Y	SUM
IIA, 8.3.1.1 / 01	Sayers, L.E.	2010a	S-2200 Technical Grade - Acute Toxicity To Water Fleas (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202, OPPTS Draft Guideline 850.1010, The Official Journal of the European Communities L383A, Method C.2 and JMAFF 12 Nohsan, No. 8147 Daphnia Acute Immobilization Test (2-7-2-1) and JMAFF 13 SeiSan No. 3986 Springborn Smithers Laboratories Study No. 13048.6638 (Sumitomo ROW-0013) GLP, Unpublished	Y	SUM
IIA, 8.3.1.1 / 02	[redacted]	2012e	S-2167 (R-Isomer of S-2200) - Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of the European Communities L 142/456, Method C.2 [redacted] (Sumitomo ROW-0048) GLP, Unpublished	Y	SUM
IIA, 8.3.1.1 / 03	[redacted]	2012f	S-2354 (S-Isomer of S-2200) - Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of the European Communities L 142/456, Method C.2 [redacted] (Sumitomo ROW-0049) GLP, Unpublished	Y	SUM
IIA, 8.3.1.1 / 04	[redacted]	2012g	2-COOH-S-2200 - Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of the European Communities L 142/456, Method C.2 [redacted] (Sumitomo ROW-0037) GLP, Unpublished	Y	SUM
IIA, 8.3.1.1 / 05	[redacted]	2012h	5-COOH-S-2200 - Acute Toxicity To Water Fleas (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of the European Communities L 142/456, Method C.2 [redacted] (Sumitomo ROW-0038) GLP, Unpublished	Y	SUM

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Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.3.1.1 / 06	[redacted]	2012i	S-2200-OR - Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of the European Communities L 142/456, Method C.2 [redacted] (Sumitomo ROW-0039) GLP, Unpublished	Y	SUM
IIA, 8.3.1.1 / 07	[redacted]	2012j	S-2200-ORC - Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202 and The Official Journal of the European Communities L 142/456, Method C.2 [redacted] (Sumitomo ROW-0040) GLP, Unpublished	Y	SUM
IIA, 8.3.2.1 / 01	Sayers, L.E.	2010b	S-2200 Technical Grade - Full Life Cycle Toxicity Test with Water Fleas (*Daphnia magna*) Under Static Renewal Conditions, Following OPPTS Draft Guideline 850.1300, OECD Guideline #211, The Official Journal of the European Communities L225, Method C.20 JMAFF 12 NohSan, No. 8147 Daphnia spp. Reproduction Toxicity Studies (2-7-2-3) and JMAFF 13 SeiSan No. 3986 Springborn Smithers Laboratories Study No. 13048.6639 (Sumitomo ROW-0020) GLP, Unpublished	Y	SUM
IIA, 8.4 / 01	Softcheck, K.A.	2012a	S-2200 Technical Grade - 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata,* Following the Official Journal of the European Communities L383A, Method C.3 and JMAFF 12 Nohsan, No. 8147 Alga, Growth Inhibition Test 2-7-7 Smithers Viscient Study No. 13048.6640 (Sumitomo ROW-0015) GLP, Unpublished	Y	SUM
IIA, 8.4 / 02	Softcheck, K.A.	2012b	S-2167 (R-Isomer of S-2200) - 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata,* Following the Official Journal of the European Communities L383A, Method C.3 Smithers Viscient Study No. 13048.6703 (Sumitomo ROW-0050) GLP, Unpublished	Y	SUM
IIA, 8.4 / 03	Softcheck, K.A.	2012c	S-2354 (S-Isomer of S-2200) - 72-Hour Acute Toxicity Test with Freshwater Green Alga, *Pseudokirchneriella subcapitata,* Following the Official Journal of the European Communities L383A, Method C.3 Smithers Viscient Study No. 13048.6705 (Sumitomo ROW-0051) GLP, Unpublished	Y	SUM

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IIA, 8.4 / 04	Softcheck, K.A.	2012d	2-COOH-S-2200 - 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata,* Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga, Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986 Smithers Viscient Study No. 13048.6683 (Sumitomo ROW-0041) GLP, Unpublished	Y	SUM
IIA, 8.4 / 05	Softcheck, K.A.	2012e	5-COOH-S-2200 - 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata,* Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga, Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986 Smithers Viscient Study No. 13048.6687 (Sumitomo ROW-0042) GLP, Unpublished	Y	SUM
IIA, 8.4 / 06	Softcheck, K.A.	2012f	S-2200-OR - 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata,* Following The Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga, Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986 Smithers Viscient Study No. 13048.6691 (Sumitomo ROW-0043) GLP, Unpublished	Y	SUM
IIA, 8.4 / 07	Softcheck, K.A.	2012g	S-2200-ORC - 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata,* Following the Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga, Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986 Smithers Viscient Study No. 13048.6695 (Sumitomo ROW-0044) GLP, Unpublished	Y	SUM
IIA, 8.5.2 / 01	Picard, C.R.	2012	S-2200 – Toxicity Test with Sediment-Dwelling Midges (*Chironomus riparius*) Under Static Conditions, Following OECD Guideline 219 Smithers Viscient Study No. 13048.6671 (Sumitomo ROW-0047) GLP, Unpublished	Y	SUM
IIA, 8.7.1 / 01	Vergé, E.	2009	S-2200 TG - Acute Oral and Contact Toxicity to the Honeybee *Apis mellifera L.* in the Laboratory. Report amendment 1 Eurofins GAB GmbH Study Code S09-02321 (Sumitomo ROW-0002) GLP, Unpublished	Y	SUM
IIA, 8.7.2 / 01	Vergé, E.	2009	S-2200 TG - Acute Oral and Contact Toxicity to the Honeybee *Apis mellifera L.* in the Laboratory. Report amendment 1 Eurofins GAB GmbH Study Code S09-02321 (Sumitomo ROW-0002) GLP, Unpublished	Y	SUM

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IIA, 8.8.1.1 / 01	Klug, T.	2010a	S-2200 25SC: Toxicity to the Aphid Parasitoid, *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) in the Laboratory (Rate Response test) Eurofins Agroscience Services GmbH Study Code S10-02868 (Sumitomo ROW-0021) GLP, Unpublished	Y	SUM
IIA, 8.8.1.2 / 01	Klug, T.	2010b	S-2200 25SC: Toxicity to the Predatory Mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the Laboratory (Rate Response Test) Eurofins Agroscience Services GmbH Study Code S10-02869 (Sumitomo ROW-0022) GLP, Unpublished	Y	SUM
IIA, 8.9.1 / 01	Stäbler, D.	2009	Acute Toxicity of S-2200 TG on Earthworms *Eisenia fetida* Using an Artificial Soil Test Eurofins GAB GmbH Study Code S09-02322 (Sumitomo ROW-0006) GLP, Unpublished	Y	SUM
IIA, 8.9.1 / 02	Gehrig, M.	2011a	Acute Toxicity of 2-COOH-S-2200 on Earthworms *Eisenia fetida* Using an Artificial Soil Test Eurofins Agroscience Services GmbH Study Code S11-00145 (Sumitomo ROW-0029) GLP, Unpublished	Y	SUM
IIA, 8.9.1 / 03	Gehrig, M.	2011b	Acute Toxicity of 5-COOH-S-2200 on Earthworms *Eisenia fetida* Using an Artificial Soil Test Eurofins Agroscience Services GmbH Study Code S11-00146 (Sumitomo ROW-0030) GLP, Unpublished	Y	SUM
IIA, 8.9.2 / 01	Ganssmann, M.	2011	Sublethal Toxicity of S-2200 TG to the earthworm *Eisenia fetida* in Artificial Soil Eurofins Agroscience Services GmbH Study Code S10-03686 (Sumitomo ROW-0027) GLP, Unpublished	Y	SUM
IIA 8.10.1/01 & 8.10.2/01	Gehrig, M.	2011c	Effects of S-2200 TG on the Activity of the Soil Microflora Eurofins Agroscience Services GmbH Study Code S11-00170 (Sumitomo ROW-0028) GLP, Unpublished	Y	SUM
IIA, 8.12 / 01	Sinderman, A.B., Porch, J.R., Krueger, H.O. & Martin, K.H.	2012a	S-2200 25SC: Toxicity Effects on the Seedling Emergence of Six Species Of Plants Wildlife International, Ltd. Report No. 166-202 (Sumitomo ROW-0045) GLP, Unpublished	Y	SUM
IIA, 8.12 / 02	Sinderman, A.B., Porch, J.R., Krueger, H.O. & Martin, K.H.	2012b	S-2200 25SC: Toxicity Effects on the Vegetative Vigour Of Six Species Of Plants Wildlife International, Ltd. Report No. 166-203 (Sumitomo ROW-0046) GLP, Unpublished	Y	SUM

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IIA, 8.14 / 01	Takaishi, M.	2010a	Evaluation of Herbicidal Activity of De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, and S-2200 Agricultural Chemicals Research Laboratory, Sumitomo Chemical Co. Ltd. Report No. 000-002 (Sumitomo ROG-0002) Non-GLP, Unpublished	Y	SUM
IIA, 8.14 / 02	Takaishi, M.	2010b	Evaluation of Insecticidal Activity of De-Xy-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, and S-2200 Agricultural Chemicals Research Laboratory, Sumitomo Chemical Co. Ltd. Report No. 000-003 (Sumitomo ROG-0003) Non-GLP, Unpublished	Y	SUM
IIA, 8.14 / 03	Kiguchi, S	2011	Comparison of Fungicidal Activity of 2-COOH-S-2200 and 5-COOH-S-2200 with S-2200 Health & Crop Sciences Research Laboratory, Sumitomo Chemical Co. Ltd. (Sumitomo ROG-0004) Non-GLP, Unpublished	Y	SUM
IIA, 8.15 / 01	Eisner, G.	2010	S-2200 Technical Grade: Toxicity to Activated Sludge in a Respiration Inhibition Test Harlan Laboratories Study C91005 (Sumitomo ROW-0018) GLP, Unpublished	Y	SUM
Annex III Data and Information					
IIIA, 10.2.2.1 / 01	[redacted]	2011a	S-2200 25% SC - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Following OECD Guideline #203, EC Guideline L383A, Method C.1, OPPTS Draft Guideline 850.1075, JMAFF 12 NohSan, No. 8147 Fish Acute Toxicity Test (2-7-1-1) and JMAFF 13 SeiSan No. 3986 [redacted] [redacted] (Sumitomo ROW-0024) GLP, Unpublished	Y	SUM
IIIA, 10.2.2.2 / 01	[redacted]	2011b	S-2200 25% SC - Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Static Conditions, Following OECD Guideline #202, OPPTS Draft Guideline 850.1010, The Official Journal of the European Communities L383A, Method C.2 and JMAFF 12 NohSan, No. 8147 Daphnia Acute Immobilization Test (2-7-2-1) and JMAFF 13 SeiSan No. 3986 [redacted] (Sumitomo ROW-0025) GLP, Unpublished	Y	SUM
IIIA, 10.2.2.3/01	Softcheck, K.A.	2011	S-2200 25% SC - 72-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Following The Official Journal of the European Communities L383A, Method C.3, JMAFF 12 Nohsan, No. 8147 Alga, Growth Inhibition Test 2-7-7 and JMAFF 13 Seisan No. 3986 Smithers Viscient Study No. 13048.6676 (Sumitomo ROW-0026) GLP, Unpublished	Y	SUM

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IIIA, 10.4.2.1 / 01	Vergé, E.	2010	S-2200 25SC - Acute Oral and Contact Toxicity to the Honeybee *Apis mellifera* L. in the Laboratory. Eurofins Agroscience Services GmbH Study Code S10-02870 (Sumitomo ROW-0023) GLP, Unpublished	Y	SUM
IIIA, 10.4.2.2 / 01	Vergé, E.	2010	S-2200 25SC - Acute Oral and Contact Toxicity to the Honeybee *Apis mellifera* L. in the Laboratory. Eurofins Agroscience Services GmbH Study Code S10-02870 (Sumitomo ROW-0023) GLP, Unpublished	Y	SUM

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