Annex II Ecotoxicology (AS) to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

Captan (ISO) 1,2,3,6-tetrahydro-N-(trichloromethylthio)phthalimide

EC Number: 205-087-0
CAS Number: 133-06-2
Index Number: 613-044-00-6

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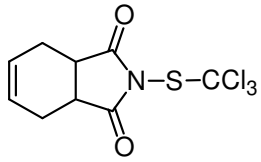
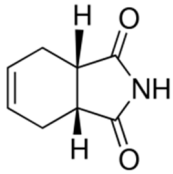
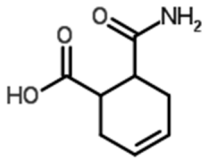
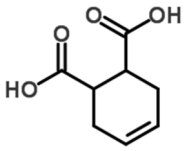
B.9. ECOTOXICOLOGY DATA

Table B.9-1: Substances and metabolites of environmental relevance (structure, synonyms and codes)

Codes and synonyms	Description (IUPAC)	Compound found in	Structure
Captan	N-(trichloromethylthio)cyclohex-4-ene-(1,2 14C)-1,2-dicarboxyimide	All matrices	
THPI	cis-4-cyclohexane-1.2-dicarboximide	Environment (soil, groundwater, surface water, sediment), plant, rat	
THPAM	3-cyclohexene-1-carboxylic acid-6-(aminocarbonyl)	Environment (soil, groundwater, surface water), rat	
THPAI	4-cyclohexene-1,2-dicarboxylic acid	Environment (sediment)	

Metabolites occurring in plants, the rat and livestock are listed and discussed in the risk assessment for birds and mammals.

B.9.2. EFFECT ON AQUATIC ORGANISMS

In order to complete the aquatic risk assessment and to address the new data requirements for active substances (Regulation 283/2013) and plant protection products (Regulation 284/2013) according to Regulation (EC) No. 1107/2009, additional studies were submitted by the notifiers Adama/Arysta.
Studies submitted during the first EU peer-review of the active substance (greyed out reference field) were evaluated according to the currently valid test guidelines and are summarised below.

B.9.2.1. Acute toxicity to fish

The acute toxicity of captan and its metabolites has been investigated in toxicity studies with several fish species. The active substance captan is unstable in the aquatic system due to rapid hydrolyses. The hydrolysis DT_{50} of captan is between 3.6 and 8.3 minutes at pH 9, and between 11.0 and 18.8 hours at pH 5. At a neutral pH value, the DT_{50} is in a range of 2.6 to 4.9 hours.

The majority of the studies were conducted using a static test design to reflect realistic conditions. However, these studies were considered not valid by the RMS as they do not meet the validity criteria according to the OECD test guideline. Taking into account the validity criteria, the test concentration of the test substance has to be satisfactorily maintained throughout the study duration. In case of the static fish toxicity tests conducted with the active substance captan an exposure over 96 hours could not be maintained. The measured concentrations of captan were below the LOQ or LOD at the first sampling time, 24 hours after test start.

In addition, a lack of analytical verification of the test substance was identified. In some of these studies (Jenkins 2002a, 2002b, 2002c, 2002d, 2002e, 2002f) concentrations were measured in the stock solutions and were not measured in the test media. This issue was already discussed during the first EU peer review. The applicant submitted two further studies for the AIR process using a design and methodology (Jenkins, 2004a and 2004b) comparable to the 2002 studies, but this time with measurements of captan concentrations in the test media. In addition, the other four studies out of the original six (Jenkins 2002b, 2002c, 2002e, 2002f) were repeated once more under static conditions with analysis of test media to verify initial exposure concentrations.
The applicant argued that "*…these studies gave initial measured concentrations close to nominal and that the biological results were also similar to the corresponding 2002 studies (Jenkins 2002a and 2002d). Hence, the conclusion by EFSA and MS's during the previous review was that, taken as a whole, the package of studies on the six species was valid for deriving the risk assessment endpoint for fish. For the current EU review, the endpoints derived from the set of studies can be regarded as acceptable.*"
The RMS does not agree on the applicant's statement considering the lack of analytical verification in the test medium and the insufficient duration of exposure during the acute toxicity tests.
According to the EFSA Technical Report (EFSA supporting publication 2015:EN-924) the validity of a study should be questioned when the test concentrations were not maintained and significant residues were not present at the end of the exposure period.

However, taking into account that fish are the most sensitive group of aquatic organisms and trigger the aquatic risk assessment as well as due to animal welfare reasons the RMS is of the opinion that the results of the studies might be of use in a higher tier approach even though the studies are formally not valid.

It is also stated in the EFSA Technical Report that *"…if peak or initial measured concentrations are used to express the endpoint, and concentrations were bit maintained then such endpoint should only be considered in the context of a higher tier risk assessment, ensuring that the exposure in the study is sufficiently representative of the predicted exposure profile*".

For information considering the aquatic risk assessment, refer to Volume 3, B.9 (PPP).

Active substance:

Reference:	**Captan: Acute toxicity to rainbow trout (*Salmo gairdneri*)**
Author(s), year:	1991
Report/Doc. number:	BL3995/B, FT76/90
Guideline(s):	OECD test guideline 203 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:	
Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : RS 309/H, purity : 90,0% w/w
Test species:	Rainbow trout (*Salmo gairdneri*, known as *Oncorhynchus mykiss*)
Number of organisms:	10 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	The weight and length of fish in the dilution water control was measured at the end of the exposure period. Length: 30 – 38 mm (mean: 34 mm) Weight: 0.30 – 0.67 g (mean: 0.41 g)
Loading	0.09 g/L fish loading per test vessel
Type of test:	Flow-through, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 10, 18, 32, 56, 100 and 180 µg ai/L
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 32.3 – 40.7 mg /L as $CaCO_3$, alkalinity: 23.2 – 23.4 mg/L as $CaCO_3$ Free and combined residual chlorine: < 4 µg/L
Conductivity:	123 - 128 µS/cm
Temperature:	15.0 - 15.2 °C
pH:	7.54 - 7.65

O_2 content:	8.6 - 10.0 mg dissolved oxygen/L (> 60% air saturation)
Light regime:	Light/dark cycle of 16/8, with 10 min transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria of ca. 54 litres capacity with and a working volume of 45 litres. The test solutions were renewed at a nominal rate of 250 mL/minutes. At the initiation of the study ten fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness, conductivity and the free and residual chlorine content was determined daily.
Analytical measurements:	The active substance captan hydrolysed rapidly in aqueous conditions. Therefore, only samples of the stock solution were analysed to confirm that the correct nominal concentrations had been made. Samples of the stock solutions were taken for analysis at 0 hours, 48 hours and 96 hours. The analysis of the test solutions were conducted by high performance liquid chromatography.
Statistics:	The LC_{50} was calculated at various time intervals using Stephan's method. The results of the moving average angle method were selected and the dose response curve was plotted with a Hewlett-Packard plotter.

Findings:

Analytical data:	The mean concentrations of captan in the stock solutions ranged from 88 to 100% of nominal values.

Table B. 9.2.1-1: Analytical measurements in the stock solutions

Nominal concentration of captan techn. in the stock solutions [mg ai/L]	Measured concentration of captan in the stock solutions [mg/L]					Mean measured concentration	
	0 h (off)	0 h (on)	48 h (off)	48 h (on)	96 h (off)	[mg/L]	% of nominal
Solvent control	< 7	< 7	< 7	< 7	< 5	< 7	-
100	74	88	89	95	95	88	88
180	140 140 140	170	170 150 150	180	150	160	89
320	230	350	320	350	310	310	97
560	530	570	590	500	-	550	98
1000	750	1000	930	TD	TD	890	89
1800	1700	1800	2000	TD	TD	1800	100

TD…Terminated

Biological results:	The lowest concentration that resulted in 100% mortality within the period of the test was 100 µg ai/L.

No mortalities occurred at the three lowest test concentrations (10, 18 and 32 µg ai/L) over the 96 hour exposure period although partial effects on the behaviour of fish (11 – 30% of fish) were observed.

Table B. 9.2.1-2: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Mortality [%]			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
Solvent control	0	0	0	0
10	0	0	0	0
18	0	0	0	0
32	0	0	0	0 [a]
56	0 [abe]	50 [ab]	70 [ab]	80 [abd]
100	50 [abcdef]	100	100	100
180	100	100	100	100

[a] Quiescent, [b] Surfacing, [c] Weak, [d] Loss of balance, [e] Dark discoloured, [f] Rapid respiration

Conclusion: Based on these results a LC_{50} of 50 µg ai/L (95% C.I. = 37 – 71 µg ai/L) based on nominal concentrations was determined. The NOEC of 18 µg ai/L is based on behavioural effects, i.e. quiescent behaviour.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

All validity criteria are met, except for the analytical measurements of the active substance. The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The fish were not fed for 48 hours before the test start. According to the guideline, the fish should not be fed 24 hours before test start.

- The measured length of fish (measured at the end of exposure only) was below the recommended total length of fish (50 mm ± 10 mm).

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the low recovery of the test item.

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to mirror carp (*Cyprinus carpio*)**
Author(s), year:	1991
Report/Doc. number:	BL4153/B, FT77/90, TMN-0004
Guideline(s):	OECD test guideline 203 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : RS 309/H, purity : 90,0% w/w
Test species:	Mirror carp (*Cyprinus carpio*)
Number of organisms:	10 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	The weight and length of fish in the dilution water control was measured at the end of the exposure period. Length: 32 - 43 mm (mean: 37 mm) Weight: 0.94 – 1.70 g (mean: 1.19 g)
Loading	0.26 g/L fish loading per test vessel
Type of test:	Flow-through, 96 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 18, 32, 56, 100, 180 and 320 µg ai/L
Solvent:	DMF (0.1 mL/L)

Test conditions:

Water quality:	Dilution water, hardness: 39.0 - 41.0 mg /L as $CaCO_3$, alkalinity: 23.2 mg/L as $CaCO_3$ Free and combined residual chlorine: < 4 µg/L
Conductivity:	176.1 - 193.4 µS/cm
Temperature:	21.7 - 21.9 °C
pH:	7.55 - 7.85
O_2 content:	7.6 - 8.4 mg dissolved oxygen/L (> 60% air saturation)

Light regime:	Light/dark cycle of 16/8, with 10 min transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria of ca. 54 litres capacity with and a working volume of 45 litres. The test solutions were renewed at a nominal rate of 250 mL/minutes. At the initiation of the study ten fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness, conductivity and the free and residual chlorine content was determined daily.
Analytical measurements:	The active substance captan hydrolysed rapidly in aqueous conditions. Therefore, only samples of the stock solution were analysed to confirm that the correct nominal concentrations had been made. Samples of the stock solutions were taken for analysis at 0 hours, 48 hours and 96 hours. The analysis of the test solutions were conducted by high performance liquid chromatography.
Statistics:	The LC_{50} was calculated at various time intervals using Stephan's method. The results of the moving average angle method were selected and the dose response curve was plotted with a Hewlett-Packard plotter.

Findings:

Analytical data:	The mean captan concentration in the stock solutions ranged from 94 to 100% of nominal values.

Table B. 9.2.1-3: Analytical measurements in the stock solutions

Nominal concentration of captan techn. in the stock solutions [mg ai/L]	Measured concentration of captan in the stock solutions [mg/L]					Mean measured concentration	
	0 h (off)	0 h (on)	48 h (off)	48 h (on)	96 h (off)	[mg/L]	% of nominal
Solvent control	< 7	< 7	< 7	< 7	< 5	< 7	-
180	170	180	160	170	180	170	94
320	300	320 320 320	290 290 290	310	300	300	94
560	530	550	510	540	540	530	95
1000	990	1100	880	980	900	970	97
1800	1800	1900	1700	1900	1800	1800	100
3200	3300	3200	3100	TD	TD	3200	100

TD…Terminated

Biological : At the highest test concentration 100% mortality was observed. There were no mortalities at the lower test concentrations (18-180 µg ai/L).

Table B. 9.2.1-4: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Mortality [%]			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
Solvent control	0	0	0	0
18	0	0	0	0
32	0	0	0	0
56	0	0	0	0
100	0	0	0	0
180	0	0	0 [a]	0
320	100	100	100	100

[a] Loss of balance

Conclusion: Based on these results a LC_{50} of 240 µg ai/L (95% C.I. = 200-310 µg ai/L) based on nominal concentrations was determined. The NOEC of 180 µg ai/L was determined based on effects on the mortality at the highest test concentration.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

All validity criteria are met, except for the analytical measurements of the active substance. The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The fish were not fed for 72 hours before the test start. According to the test guideline, fish should not be fed for 24 hours before the test is started.
- The measured length of fish (measured at the end of exposure only) was below the recommended total length of fish (50 mm ± 10 mm).

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.
Endpoints: No reliable endpoint could be determined based on the lack of analytical verification.
Conclusion of the RMS: Taking into account the validity criteria according to the OECD test guideline the acute fish toxicity test is not considered valid due lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to rainbow trout**
Author(s), year:	2002a
Report/Doc. number:	MAK660/013072, R-12335, TMN-0020A
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:	
Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 01330243, purity : 95.2%
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 53 mm
	Weight: mean 1.9 g
Loading	0.37 g/L fish loading per test vessel
Type of test:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 30.1, 66.1, 145, 320 and 704 µg ai/L
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 178 - 180 mg /L as $CaCO_3$
Temperature:	12.4 - 13.5 °C
pH:	7.8 - 8.5
O_2 content:	88 - 106% air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with 10 min transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 35 litres to a depth of 20 cm. At the initiation of the study 7 fish were allocated at random to each test vessel.

Test parameters: All test vessels were monitored for mortality and sub-lethal effects after 15 minutes, 2, 4, 24, 48, 72 and 96 hours.
Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.

Analytical measurements: Samples of the stock solutions were taken for analysis prior to the start (0 hours). No analytical measurements were conducted at the end of the test (96 hours).
The analysis of the test solutions were conducted by high performance liquid chromatography (HPLC).

Statistics: LC_{10} and LC_{50} values were estimated by Probit regression versus log 10 dose using SAS 6.12. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986)

Findings:

Analytical data: The mean captan concentration in the stock solutions ranged from 91 to 102% of nominal values.

Table B. 9.2.1-5: Analytical measurements in the stock solutions

Nominal concentrations [mg/mL]		Measured concentrations [mg/mL]	
Captan techn.	**Captan**	**Captan**	**% of nominal**
0.301	0.287	0.265	92
0.661	0.629	0.593	94
1.45	1.38	1.26	91
3.2	3.05	3.12	102
7.04	6.70	6.60	99

Biological effects: The highest nominal concentration resulting in 0% mortality was 138 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 305 µg ai/L.
One fish in each of the controls (control and solvent control) exhibited aggressive behaviour towards the other fish in the vessel; these aggressive fish might have been responsible for the darkened pigmentation exhibited by some of the control fish. After the aggressive fish were isolated behind a screen placed in each vessel, the other fish recovered and appeared normal.
Sub-lethal effects were observed at 66.1 µg ai/L and higher concentration.

Table B. 9.2.1-6: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0.25 h	2 h	4 h	24 h	48 h	72 h	96 h	%
Control	0	0 [a]	0 [a]	0 [a]	0	0	0	-
Solvent control	0	0 [a]	0 [a]	0 [a]	0 [a]	0 [a]	0	-
30.1	0	0	0	0	0	0	0	-
66.1	0	0 [b]	0 [b]	0 [abc]	0 [abc]	0 [ab]	0 [ab]	-
145	0	0 [b]	0 [b]	0 [abc]	0 [abc]	0 [abc]	0 [abc]	-
320	0 [b]	0 [ab]	2 [abc]	7	7	7	7	100
704	0 [b]	4 [abc]	7	7	7	7	7	100

[a] Darkened pigmentation, [b] Hyperventilation, [c] Lethargy

Conclusion: Based on these results a LC_{50} of 215 µg ai/L (95% C.I. = 145 - 320 µg ai/L) based on nominal concentrations was determined. The LC_{10} was 203 µg ai/L (95% C.I. = 125 - 320 µg ai/L). The NOEC based on behavioural effects was 30.1 µg ai/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the low recovery of the test item.

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to brown trout**
Author(s), year:	2002b
Report/Doc. number:	MAK661/013073, R-12336, TMN-0020B
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:	
Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 01330243, purity : 95.2%
Test species:	Brown trout (*Salmo trutta*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 60 mm Weight: mean 3.16 g
Loading	0.63 g/L fish loading per test vessel
Type of test:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 13.7, 30.1, 66.1, 145 and 320 µg ai/L
Measured (mean):	No analytical measurements conducted
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 176 - 182 mg /L as $CaCO_3$
Temperature:	13.1 - 14.4 °C
pH:	7.8 - 8.5
O_2 content:	83 - 106% air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with 10 min transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 35 litres to a depth of 20 cm. At the initiation of the study 7 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 15 minutes, 2, 4, 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.
Analytical measurements:	Samples of the stock solutions were taken for analysis prior to the start (0 hours). No analytical measurements were conducted at the end of the test (96 hours). The analysis of the test solutions were conducted by high performance liquid chromatography (HPLC).

Statistics: LC_{10} and LC_{50} values were estimated by probit regression versus log 10 dose using SAS 6.12. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986)

Findings:

Analytical data: The mean captan concentration in the stock solutions ranged from 91 to 102% of nominal values.

Table B. 9.2.1-7: Analytical measurements in the stock solutions

Nominal concentrations [mg/mL]		Measured concentrations [mg/mL] at test start	
Captan techn.	**Captan**	**Captan**	**% of nominal**
0.137	0.130	0.118	91
0.301	0.287	0.265	92
0.661	0.629	0.593	94
1.45	1.38	1.26	91
3.2	3.05	3.12	102
7.04 (stock)	6.70	6.60	99

Biological : The highest nominal concentration resulting in 0% mortality was 66.1 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 145 µg ai/L.
Sub-lethal effects were observed at 13.7 µg ai/L and higher concentration. At 13.7 µg ai/L the fish were only affected during the initial hours of exposure, at the end of the test they appeared to have recovered.

Table B. 9.2.1-8: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0.25 h	**2 h**	**4 h**	**24 h**	**48 h**	**72 h**	**96 h**	**%**
Control	0	0	0	0	0	0	0	-
Solvent control	0	0	0	0	0	0	0	-
13.7	0	0	0	0 [a]	0 [a]	0 [a]	0	-
30.1	0	0	0	0 [a]	0 [a]	0 [a]	0 [a]	-
66.1	0	0 [a]	0 [a]	0 [a]	0 [a]	0 [abc]	0 [ac]	-
145	0 [a]	0 [ab]	0 [abc]	7	7	7	7	100
320	0 [a]	0 [abc]	7	7	7	7	7	100

[a] Hyperventilation, [b] Darkened pigmentation, [c] Lethargy

Conclusion: Based on these results a LC_{50} of 98 µg ai/L (95% C.I. = 66 - 145 µg ai/L) based on nominal concentrations was determined. The LC_{10} was 92 µg ai/L (95% C.I. : 57 - 145 µg ai/L). The NOEC based on behavioural effects was < 13.7 µg ai/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).
- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the low recovery of the test item.

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to brown trout**
Author(s), year:	2016a
Report/Doc. number:	ENV-16-013, R-37526 (Report Amendment, 13. September 2016)
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable (acceptable to be considered in a Tier 2C risk assessment)

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 94138737, purity : 95.6%
Test species:	Brown trout (*Salmo trutta*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Llength, weight:	Length: 40 - 60 mm
	Weight: 0.6 - 1.3 g
Loading	0.3-0.36 g/L fish loading
Type of test:	Static, 96 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 13.7, 30.1, 66.1, 145 and 320 µg ai/L
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 210 mg /L as $CaCO_3$
Temperature:	13.0 - 14.1 °C
pH:	7.71 - 8.03
O_2 content:	≥ 97.7% air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with 10 min transition periods
Feeding	The fish were not fed within the 24 h period before the study start and during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 20 litres. At test start, seven fish were added at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 2, 4, 6.3 (only mortality), 10, 24, 48, 72 and 96 hours. Measurements of dissolved oxygen, pH and temperature were conducted al test every 24 hours. In addition, temperature was recorded continuously in the control tank. In addition, water hardness was determined.
Analytical measurements:	All treatment rates were analysed at the 0 h and 4 h time point. In addition, the 66.1 µg/L treatment rate was analysed at the 0.5, 1, 10 and 24 h time points. No analytical measurements were conducted at the end of the test (96 hours). The analysis of the test solutions were conducted by gas chromatography with mass spectrometric detection (GC-MS).
Statistics:	The 50% lethal concentration (LC_{50}) was calculated using a binominal graphical method (Spearman Käber).
Findings:	
Analytical data:	After 4 h of exposure, the mean measured concentrations were between 43 and 81% of the nominal concentrations.

Table B. 9.2.1-9: Analytical measurements for the test media at 0 h and 4 h

Nominal concentrations [µg test item/L]	Measured concentrations at 0 h		Measured concentrations at 4 h	
	Captan [µg ai/L]	% of nominal	Captan [µg ai/L]	% of nominal
Control	1.61 *	-	-	-
Solvent control	1.09 *	-	-	-
13.7	15.7	120	10.6	81
33.1	36.8	116	24.6	78
66.1	66.9	106	38.7	61
145	129	93	60.1	43
320	278	91	163	53

* These values are just above the LOQ of 1 µg ai/L.

Table B. 9.2.1-10: Analytical measurements for the treatment rate 66.1 µg ai/L

Nominal concentrations [µg test item/L]	Measured concentrations of captan [µg ai/L]				
	0.5 h	1 h	4 h	10 h	24 h
66.1	78.8	74.8	38.7	12.0	2.55

After 24 h the measured concentrations of captan were 3.9% of the nominal test concentrations.

Biological : The highest nominal concentration resulting in 0% mortality was 66.1 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 145 µg ai/L. Sub-lethal effects were observed at 66.1 µg ai/L and higher concentration. At 66.1 µg ai/L the fish were only affected during the initial hours of exposure, at the end of the test they appeared to have recovered.

Table B. 9.2.1-11: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)									
	0 h	2 h	4 h	6.3 h	10 h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0	0	0	-
Solvent control	0	0	0	0	0	0	0	0	0	-
13.7	0	0	0	0	0	0	0	0	0	-
30.1	0	0	0	0	0	0	0	0	0	-
66.1	0	0 [ab]	0 [b]	0	0 [bc]	0	0	0	0	-
145	0	0 [abc]	0 [abc]	2	7	7	7	7	7	100
320	0	0 [abc]	7	7	7	7	7	7	7	100

[a] Sedentary, [b] Hyperventilation, [c] Darkened pigmentation

Conclusion: Based on these results a LC_{50} of 97.9 µg ai/L (95% C.I. = 66.1-145 µg test item/L) based on nominal concentrations was determined. The NOEC based on behavioural effects was 30.1 µg ai/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.

- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured at the start of the test and after 4 hours. No further analytical measurements were conducted. Due to the missing analytical verification it could not be demonstrated that the test substance was satisfactory maintained throughout the study. Hence, the study is not considered valid.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The measured concentrations of captan in the control and solvent control were slightly above the LOQ. However, no adverse effects were observed in the controls; hence, an exposure of control fish to captan can be considered negligible.

Acceptability of the analytical methods used in the test: The study is not considered valid according to the OECD test guideline. However, taking into account that fish species are the most sensitive group of aquatic organisms, a validation of the analytical measurements was conducted since the endpoint might be of relevance for a higher tier risk assessment.

The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ of 1 µg ai/L.

Endpoints: No reliable endpoint based on mean measured concentrations could be calculated considering the static exposure system used in the test and the fast degradation of the active substance in the water sediment system. However, the results of the study might be used in a Tier 2 risk assessment; hence, the RMS conducted a statistical re-evaluation of the results based on initial measured concentrations.

A 96 h LC_{50} of 91.265 µg ai/L (95% C.I. 75.759-111.719 µg ai/L) was determined based on Probit analysis using linear max. likelihood regression. The LC_{10} was calculated to be 67.834 µg ai/L (95% C.I. 49.831-81.001 µg ai/L) and the NOEC was determined to be 36.8 µg ai/L based on behavioural effects (hyperventilation).

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to lack of analytical verification of the test substance in the test medium.

However, the endpoints might be reliable to be considered in a Tier 2C risk assessment. Regarding the assessment of the reliability of the endpoints in connection with the intended uses and the corresponding FOCUS exposure profiles please refer to section B.9.4.

The endpoints calculated by the RMS are comparable to those endpoints determined by the study author. However, the study author used the nominal concentrations instead of the initial measured concentrations. For the higher tier risk assessment both endpoints, based on the nominal and the initial measured concentrations are presented.

Reference:	**Captan: Acute toxicity to common carp**
Author(s), year:	2002c
Report/Doc. number:	MAK662/013074, R-12337, TMN-0004B
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:	
Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 01330243, purity : 95.2%
Test species:	Common carp (*Cyprinus carpio*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 40 mm Weight: mean 1.7 g
Loading	0.48 g/L fish loading per test vessel
Type of test:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 51.2, 113, 248, 545 and 1200 µg ai/L
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 172 - 174 mg /L as $CaCO_3$
Temperature:	20.6 - 21.0 °C
pH:	8.1 - 8.5
O_2 content:	87 - 100% air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with 10 min transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 25 litres to a depth of 14 cm. At the initiation of the study 7 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 15 minutes, 2, 4, 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.
Analytical measurements:	Samples of the stock solutions were taken for analysis prior to the start (0 hours). No analytical measurements were conducted at the end of the test (96 hours). The analysis of the test solutions were conducted by high performance liquid chromatography (HPLC).

Statistics: LC_{10} and LC_{50} values were estimated by probit regression versus log 10 dose using SAS 6.12. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986)

Findings:

Analytical data: The mean captan concentration in the stock solutions ranged from 95 to 98 % of nominal values.

Table B. 9.2.1-12: Analytical measurements in the stock solutions

Nominal concentrations [mg/mL]		Measured concentrations [mg/mL] at test start	
Captan techn.	**Captan**	**Captan**	**% of nominal**
0.512	0.487	0.475	98
1.13	1.08	1.03	95
2.48	2.36	2.25	95
5.45	5.19	5.0	96
12.0	11.4	11.1	97

Biological : The highest nominal concentration resulting in 0% mortality was 2481 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 1200 µg ai/L. Sub-lethal effects were observed at 113 µg ai/L and higher concentration. At 113 and 248 µg ai/L the fish were only affected during the initial hours of exposure, at the end of the test they appeared to have recovered.

Table B. 9.2.1-13: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0.25 h	**2 h**	**4 h**	**24 h**	**48 h**	**72 h**	**96 h**	**%**
Control	0	0	0	0	0	0	0	-
Solvent control	0	0	0 [a]	0	0	0	0	-
51.2	0	0	0	0	0	0	0	-
113	0 [a]	0 [a]	0	0 [a]	0 [d]	0	0	-
248	0	0 [c]	0	0 [ab]	0 [abc]	0 [ab]	0	-
545	0 [a]	0 [a]	0 [a]	3 [abe]	6 [ab]	6 [ab]	6 [abc]	86
1200	0 [a]	1 [bef]	6 [f]	7	7	7	7	100

[a] Hyperventilation, [b] Darkened pigmentation, [c] Lethargy, [d] Nervous behaviour, [e] Loss of co-ordination, [f] Moribund

Conclusion: Based on these results a LC_{50} of 492 µg ai/L (95% C.I. = 326 - 545 µg ai/L) based on nominal concentrations was determined. The LC_{10} was 434 µg ai/L (95% C.I. := 193 - 545 µg ai/L). The NOEC of 113. µg ai/L based on behavioural effects.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the lack of analytical verification.

Conclusion of the RMS: Taking into account the validity criteria according to the OECD test guideline the acute fish toxicity test is not considered valid due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to common carp**
Author(s), year:	2016b
Report/Doc. number:	ENV-16-021, R-37527
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 94138737, purity : 95.8%
Test species:	Common carp (*Cyprinus carpio*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 51.2 - 52.1 mm
	Weight: mean 1-78 - 1.99 g
Loading	0.52 - 0.74 g/L fish loading per test vessel
Type of test:	Static, 96 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 51.2, 113, 248, 545 and 1200 µg ai/L
Solvent:	DMF (0.1 mL/L)

Test conditions:

Water quality:	Dilution water, hardness: 196 mg /L as $CaCO_3$
Temperature:	20.0 - 20-2 °C
pH:	7.64 - 8.00
O_2 content:	≥ 99% air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8
Feeding	The fish were not fed within the 24 h period before the study start and during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 20 litres. At test start, seven fish were added at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 2, 4, 24, 48, 72 and 96 hours. Measurements of dissolved oxygen, pH and temperature were conducted al test every 24 hours. In addition, temperature was recorded continuously in the control tank. In addition, water hardness was determined.
Analytical measurements:	All treatment rates were analysed at the 0 h and 4 h time point. No analytical measurements were conducted at the end of the test (96 hours). The analysis of the test solutions were conducted by gas chromatography with mass spectrometric detection (GC-MS).
Statistics:	The 50% lethal concentration (LC_{50}) was calculated using a binominal graphical method (Spearman Käber).

Findings:

Analytical data:	After 4 h of exposure, the mean measured concentrations were between 43 and 81% of the nominal concentrations.

Table B. 9.2.1-14: Analytical measurements for the test media at 0 h and 4 h

Nominal concentrations [µg test item/L]	Measured concentrations at 0 h		Measured concentrations at 4 h	
	Captan [µg ai/L]	% of nominal	Captan [µg ai/L]	% of nominal
Control	nd	-	-	-
Solvent control	nd	-	-	-
51.2	57.2	117	10.4	21
113	126	116	24.4	22
248	248	104	39.5	16
545	519	99	87.6	16
1200	1147	100	175	15

nd…not determined

Biological :	The highest nominal concentration resulting in 0% mortality was 248 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 545 µg ai/L.

Sub-lethal effects were observed at 248 µg ai/L and higher concentration. At 248 the fish were only affected during the initial hours of exposure, at the end of the test they appeared to have recovered.

Table B. 9.2.1-15: Mortality and sub-lethal effects

Test concentration [µg test item/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0 h	2 h	4 h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0	-
Solvent control	0	0	0	0	0	0	0	-
51.2	0	0	0	0	0	0	0	-
113	0	0	0	0	0	0	0	-
248	0	0	0 [ab]	0	0	0	0	-
545	0	0 [b]	1 [abcde]	7	7	7	7	100
1200	0	0 [abc]	7	7	7	7	7	100

[a] Sedentary, [b] Loss of equilibrium, [c] Hyperventilation, [d] Mucus secretion, [e] Darkened pigmentation

Conclusion: Based on these results a LC_{50} of 368 µg test item/L (95% C.I. = 248 - 545 µg ai/L) based on nominal concentrations was determined. The NOEC of 113 µg ai/L based on behavioural effects.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured at the start of the test and after 4 hours. No further analytical measurements were conducted. Due to the missing analytical verification it could not be demonstrated that the test substance was satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: The study is not considered valid according to the OECD test guideline. However, taking into account that fish species are the most sensitive group of aquatic organisms, a validation of the analytical measurements was conducted since the endpoint might be of relevance for a higher tier risk assessment.

The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ of 1 µg ai/L.

Endpoints: No reliable endpoint based on mean measured concentrations could be calculated considering the static exposure system used in the test and the fast degradation of the active substance in the water sediment system. Considering the lack of appropriate analytical verification of the test substance in the test system no exposure profile could be determined. Hence, the use of the laboratory static study as a refined exposure study (Tier 2C) is not considered acceptable.

The RMS conducted a statistical re-evaluation of the results based on initial measured concentrations.

A 96 h LC_{50} of 351.8 µg ai/L (95% C.I. 286.1-439.6 µg ai/L) was determined based on Probit analysis using linear max. likelihood regression. The LC_{10} was calculated to be 255.3 µg ai/L (95% C.I. 181.3-310.5 µg ai/L) and NOEC was determined to be 126 µg a.s./L based on behavioural effects (hyperventilation,…).

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to the low recovery of the test item.

Reference:	**Captan: Acute toxicity to 3-spined stickleback**
Author(s), year:	2002d
Report/Doc. number:	MAK663/013075, R-12338, TMN-0014B
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 01330243, purity : 95.2%
Test species:	3-spined stickleback (*Gasterosteus aculeatus*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 34.6 mm Weight: mean 0.60 g
Loading	0.42 g/L fish loading per test vessel
Type of test:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 23.3, 51.2, 113, 248, 545 and 1200 µg ai/L
Measured (mean):	No analytical measurements conducted
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 174 - 190 mg /L as $CaCO_3$
Temperature:	12.0 - 13.6 °C
pH:	7.9 - 8.4
O_2 content:	90 - 104 % air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with 10 min transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 10 litres to a depth of 13 cm. At the initiation of the study 7 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 15 minutes, 2, 4, 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.
Analytical measurements:	Samples of the stock solutions were taken for analysis prior to the start (0 hours). No analytical measurements were conducted at the end of the test (96 hours).

The analysis of the test solutions were conducted by high performance liquid chromatography (HPLC).

Statistics: LC_{10} and LC_{50} values were estimated by probit regression versus log 10 dose using SAS 6.12. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986)

Findings:

Analytical data: The mean captan concentration in the stock solutions ranged from 86 to 98 % of nominal values.

Table B. 9.2.1-16: Analytical measurements in the stock solutions

Nominal concentrations [mg/mL]		Measured concentrations [mg/mL] at test start	
Captan techn.	**Captan**	**Captan**	**% of nominal**
0.233	0.222	0.192	86
0.512	0.487	0.475	98
1.13	1.08	1.03	95
2.48	2.36	2.25	95
5.45	5.19	5.0	96
12.0	11.4	11.1	97

Biological : The highest nominal concentration resulting in 0% mortality was 113 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 545 µg ai/L.
Sub-lethal effects were observed at 23.3 µg ai/L and higher concentration. At 23.3 µg ai/L and 51.2 µg ai/L the fish were only affected during the initial hours of exposure, at the end of the test they appeared to have recovered.

Table B. 9.2.1-17: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0.25 h	**2 h**	**4 h**	**24 h**	**48 h**	**72 h**	**96 h**	**%**
Control	0	0	0	0	0	0	0	-
Solvent control	0 [ac]	0	0	0	0	0	0	-
23.3	0	0 [a]	0	0	0 [c]	0	0	-
51.2	0	0 [a]	0 [a]	0	0	0	0	-
113	0 [ac]	0 [ac]	0 [ac]	0 [a]	0 [a]	0 [ac]	0 [ac]	
248	0	0 [ac]	0 [ac]	0 [abc]	1 [ab]	1 [bc]	1 [ac]	14
545	0 [ac]	0 [abc]	1 [abc]	7	7	7	7	100
1200	0 [ac]	0 [abcd]	6 [abc]	7	7	7	7	100

[a] Hyperventilation, [b] Darkened pigmentation, [c] Lethargy, [d] Spiral swimming

Conclusion: Based on these results a LC_{50} of 275 µg ai/L (95% C.I. = 48 - 415 µg ai/L) based on nominal concentrations was determined. The LC_{10} was 243 µg ai/L (95% C.I. : 152 - 301 µg ai/L). The NOEC of 23.3 µg ai/L is based on behavioural effects.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the low recovery of the test item.

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to bream**
Author(s), year:	2002e
Report/Doc. number:	MAK665/013077, R-12340, TMN-0014C
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 01330243, purity : 95.2%
Test species:	Bream (*Abramis brama*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 47 mm
	Weight: mean 1.88 g
Loading	0.55 g/L fish loading per test vessel

Type of test:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 19.2, 42.3, 93.2, 205 and 450 µg ai/L
Measured (mean):	No analytical measurements conducted
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 172 - 184 mg /L as $CaCO_3$
Temperature:	12.7 - 13.3 °C
pH:	7.9 - 8.5
O_2 content:	89 - 102 % air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 24 litres to a depth of 24 cm. At the initiation of the study 7 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 15 minutes, 2, 4, 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.
Analytical measurements:	Samples of the stock solutions were taken for analysis prior to the start (0 hours). No analytical measurements were conducted at the end of the test (96 hours). The analysis of the test solutions were conducted by high performance liquid chromatography (HPLC).
Statistics:	LC_{10} and LC_{50} values were estimated by Probit regression versus log 10 dose using SAS 6.12. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986)
Findings:	
Analytical data:	The mean captan concentration in the stock solutions ranged from 87 to 94 % of nominal values.

Table B. 9.2.1-18: Analytical measurements in the stock solutions

Nominal concentrations [mg/mL]		Measured concentrations [mg/mL] at test start	
Captan techn.	**Captan**	**Captan**	**% of nominal**
0.192	0.183	0.165	90
0.423	0.403	0.363	90
0.932	0.887	0.794	90
2.05	1.95	1.70	87
4.5	4.28	2.03	94

Biological : The highest nominal concentration resulting in 0% mortality was 93.2 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 450 µg ai/L. Sub-lethal effects were observed at 42.3 µg ai/L and higher concentration.

Table B. 9.2.1-19: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0.25 h	2 h	4 h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0	-
Solvent control	0	0	0	0	0	0	0	-
19.2	0	0	0	0	0	0	0	-
42.3	0 [a]	0	0	0	1	1	1	14
93.2	0	0	0	0 [bd]	1 [ab]	1	1	14
205	0	0	0 [ad]	1 [ab]	5 [abd]	6 [a]	6	86
450	0 [a]	0 [ab]	0 [abcde]	7	7	7	7	100

[a] Hyperventilation, [b] Darkened pigmentation, [c] Lethargy, [d] Loss of co-ordination, [e] Slow respiration

Conclusion: Based on these results a LC_{50} of 119 µg ai/L (95% C.I. = 80 - 184 µg ai/L) based on nominal concentrations was determined. The LC_{10} was 53 µg ai/L (95% C.I. : 24 - 84 µg ai/L). The NOEC of 42.3 µg ai/L is based on behavioural effects.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the low recovery of the test item.

Aside from the discussion of the validity of the study, the RMS does not agree with the assessment of the study director considering the mortalities observed in the test concentrations of 42.3 and 93.2 µg ai/L. There are no indications that the observed mortalities of one fish per test concentrations were not treatment-related. Even though the observed sub-lethal effects (hyperventilation, darkened pigmentation and loss of co-ordination) were observed at only some observation periods does not mean that the observed toxic symptoms had no effect on the survival of test fish. Hence, the RMS is of the opinion that the highest nominal concentration resulting in 0% mortality was 19.2 µg ai/L, resulting in NOEC of 19.2 µg ai/L.

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to bream**
Author(s), year:	2017a
Report/Doc. number:	ENV-16-022, R-37528
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 94138737, purity : 95.8%
Test species:	Bream (*Abramis brama*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: 53.18 - 62.93 mm
	Weight: mean 971.9 - 1707.7 mg
Loading	0.43 - 0.50 g fish/L
Type of test:	Static, 96 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 19.2, 42.3, 93.2, 205 and 450 µg ai/L
Solvent:	DMF (0.1 mL/L)

Test conditions:

Water quality:	Dilution water, hardness: 207 mg /L as $CaCO_3$
Temperature:	10.7 °C (range: 10.3 - 11.3°C)
pH:	7.71 - 8.04
O_2 content:	≥ 98 % air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with transition periods
Feeding	The fish were not fed during the 96 hours study period.

Methods: The test was carried out in glass aquaria with a working volume of 20 litres. Tanks were filled with 20 L test medium. At test start, 7 fish were allocated at random to each test vessel. Each tank was gently aerated throughout the study.

Test parameters: All test vessels were monitored for mortality and sub-lethal effects after 2, 4, 24, 48, 72 and 96 hours.

Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.

Analytical measurements: All treatment rates were analysed at the 0 h and 4 h time point

The analysis of the test solutions were conducted by gas chromatography with mass spectrometric detection (GC-MS).

Statistics: The 50% lethal concentration (LC_{50}) was calculated using a binominal graphical method (Spearman Käber).

Findings:

Analytical data: After 4 h of exposure, the mean measured concentrations were between 44 and 67% of the nominal concentrations.

Table B. 9.2.1-20: Analytical measurements for the test media at 0 h and 4 h

Nominal concentrations [µg test item/L]	Measured concentrations at 0 h		Measured concentrations at 4 h	
	Captan [µg ai/L]	% of nominal	Captan [µg ai/L]	% of nominal
Control	nd	-	-	-
Solvent control	nd	-	-	-
19.2	21.3	116	8.4	46
42.3	42.1	104	25.2	62
93.2	97.5	109	59.4	67
205	212	108	107	54
450	399	93	191	44

nd…not determined

Biological effects: The highest nominal concentration resulting in 0% mortality was 205 µg ai/L.

In the highest test concentration, a maximal mortality of 86% was observed.

Sub-lethal effects were observed at 93.2 µg ai/L and higher concentration. At 93.2 µg ai/L (and higher) the fish were only affected during the initial hours of exposure, at the end of the test they appeared to have recovered.

Table B. 9.2.1-21: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0 h	2 h	4 h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0	-
Solvent control	0	0	0	0	0	0	0	-
19.2	0	0	0	0	0	0	0	-

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0 h	2 h	4 h	24 h	48 h	72 h	96 h	%
42.3	0	0	0	0	0	0	0	-
93.2	0	0 [ab]	0 [c]	0	0	0	0	-
205	0	0 [ade]	0 [acd]	0 [a]	0	0	0	-
450	0	0 [acde]	0 [acd]	6 [fg]	6	6	6	86

[a] Lethargy, [b] Fish swim at the bottom of the tank, [c] Larger mouth movements, [d] "Hanging" in water column, [e] Slower movements than in control, [f] Mucus secretion, [g] Sedentary

Conclusion: Based on these results a LC_{50} of 310.7 µg ai/L (95% C.I. = 269.7-357.9 µg ai/L) based on nominal concentrations was determined. The NOEC of 42.3 µg ai/L based on behavioural effects.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured at the start of the test and after 4 hours. No further analytical measurements were conducted. Due to the missing analytical verification it could not be demonstrated that the test substance was satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: The study is not considered valid according to the OECD test guideline. However, taking into account that fish species are the most sensitive group of aquatic organisms, a validation of the analytical measurements was conducted since the endpoint might of relevance for a higher tier risk assessment.

The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ of 1 µg ai/L.

Endpoints: No reliable endpoint based on mean measured concentrations could be calculated considering the static exposure system used in the test and the fast degradation of the active substance in the water sediment system. Considering the lack of appropriate analytical verification of the test substance in the test system no exposure profile could be determined. Hence, the use of the laboratory static study as a refined exposure study (Tier 2C) is not considered acceptable.

The RMS conducted a statistical re-evaluation of the results based on initial measured concentrations.
A 96 h LC_{50} of 305.5 µg ai/L (95% C.I. 236.5-453.0 µg ai/L) was determined based on Probit analysis using linear max. likelihood regression. The LC_{10} was calculated to be 183.5 µg ai/L (95% C.I. 113.4-237.1 µg ai/L) and NOEC was determined to be 42.1 µg a.s./L based on behavioural effects (hyperventilation,…).
Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to roach**
Author(s), year:	2002f
Report/Doc. number:	MAK664/013076, R-12339, TMN-0014A
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 01330243, purity : 95.2% w/w
Test species:	Roach (*Rutilus rutilus*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 40.4 mm Weight: mean 1.1 g
Loading	0.51 g/L fish loading per test vessel
Type of test:	Static, 96 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 19.2, 42.3, 93.2, 205 and 450 µg ai/L
Solvent:	DMF (0.1 mL/L)

Test conditions:

Water quality:	Dilution water, hardness: 172 - 184 mg /L as $CaCO_3$
Temperature:	12.9 - 13.9 °C
pH:	7.9 - 8.6
O_2 content:	95 - 101 % air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 15 litres to a depth of 20.5 cm. At the initiation of the study 7 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 15 minutes, 2, 4, 24, 48, 72 and 96 hours.

Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.

Analytical measurements: Samples of the stock solutions were taken for analysis prior to the start (0 hours). No analytical measurements were conducted at the end of the test (96 hours).
The analysis of the test solutions were conducted by high performance liquid chromatography (HPLC).

Statistics: LC_{10} and LC_{50} values were estimated by probit regression versus log 10 dose using SAS 6.12. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986)

Findings:

Analytical data: The mean captan concentration in the stock solutions ranged from 87 to 94 % of nominal values.

Table B. 9.2.1-22: Analytical measurements in the stock solutions

Nominal concentrations [mg/mL]		Measured concentrations [mg/mL] at test start	
Captan techn.	**Captan**	**Captan**	**% of nominal**
0.192	0.183	0.165	90
0.423	0.403	0.363	90
0.932	0.887	0.794	90
2.05	1.95	1.70	87
4.5	4.28	2.03	94

Biological : The highest nominal concentration resulting in 0% mortality was 42.3 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 450 µg ai/L.
Sub-lethal effects were observed at 42.3 µg ai/L and higher concentration.

Table B. 9.2.1-23: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0.25 h	**2 h**	**4 h**	**24 h**	**48 h**	**72 h**	**96 h**	**%**
Control	0	0	0	0	0	0	0	-
Solvent control	0	0	0	0	0	0	0	-
19.2	0	0	0	0	0	0	0	-
42.3	0 [d]	0	0	0	0 [a]	0	0	-
93.2	0	0	0	1 [b]	1 [ad]	1 [a]	1 [a]	14
205	0	0 [a]	0 [a]	3 [ab]	5 [abd]	5 [c]	5 [ac]	71
450	0	0 [a]	0 [a]	7	7	7	7	100

[a] Hyperventilation, [b] Darkened pigmentation, [c] Lethargy, [d] Nervous behaviour

Conclusion: Based on these results a LC_{50} of 154 µg ai/L (95% C.I. = 109 - 219 µg ai/L) based on nominal concentrations was determined. The LC_{10} was 88 µg ai/L (95% C.I. : 44 - 128 µg ai/L). The NOEC based on behavioural effects was 42.3 µg ai/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the low recovery of the test item.

Aside from the discussion of the validity of the study, the RMS does not agree with the assessment of the study director considering the determination of the NOEC. Even though the observed sub-lethal effects (hyperventilation and nervous behaviour) were observed at only some observation periods does not mean that the observed toxic symptoms had no effect on the survival of test fish. Hence, the RMS is of the opinion that the NOEC of 19.2 µg ai/L should be considered.

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to roach**
Author(s), year:	2017b
Report/Doc. number:	ENV-16-023, R-37529
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None of relevance
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 94138737, purity : 95.8% w/w

Test species:	Roach (*Rutilus rutilus*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: 91.64 - 118.33 mm
	Weight: 5423.3 - 14127.5 mg
Loading	1.03 – 128 g fish/L
Type of test:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 19.2, 42.3, 93.0, 205 and 450 µg ai/L
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 211 mg /L as $CaCO_3$
Temperature:	10.7 - 11.3 °C
pH:	7.72 - 8.00
O_2 content:	≥ 96% air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 50 litres. Tanks were filled with 50 L test medium. At test start, 7 fish were allocated at random to each test vessel. Each tank was gently aerated throughout the study.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 2, 4, 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.
Analytical measurements:	All treatment rates were analysed at the 0 h and 4 h time point The analysis of the test solutions were conducted by gas chromatography with mass spectrometric detection (GC-MS).
Statistics:	The 50% lethal concentration (LC_{50}) was calculated using a binominal graphical method (Spearman Käber).
Findings:	
Analytical data:	The mean captan concentration in the stock solutions ranged from 55 to 76 % of nominal values. The active substance captan was detected in the analysis for the two control tanks in the 0 h sampling. There was no captan detected in any of the other control water samples used in the method of validation which indicated that the presence of captan in the 0 h samples is unusual and the cause for its presence remains unknown. The levels were more than 10-fold lower than the lowest test dose and there were no effects seen in the fish in the control tanks.

Table B. 9.2.1-24: Analytical measurements for the test media at 0 h and 4 h

Nominal concentrations [µg test item/L]	Measured concentrations at 0 h		Measured concentrations at 4 h	
	Captan [µg ai/L]	% of nominal	Captan [µg ai/L]	% of nominal
Control	1.20 *	-	-	-
Solvent control	1.42 *	-	-	-
19.2	21.1	115	13.6	74
42.3	40.7	100	27.4	68
93.0	81.7	92	49.1	55
205	203	103	156	76
450	466	108	282	63

* These values are just above the LOQ of 1 µg ai/L

Biological : The highest nominal concentration resulting in 0% mortality was 42.3 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 450 µg ai/L. Sub-lethal effects were observed at 42.3 µg ai/L and higher concentration. However, fish were only affected during the initial hours of exposure, at the end of the test they appeared to have recovered.

Table B. 9.2.1-25: Mortality and sub-lethal effects

Test concentration [µg ai/L]	Cumulative mortality (initial number = 7 fish/concentration)							
	0 h	2 h	4 h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0	-
Solvent control	0	0	0	0	0	0	0	-
19.2	0	0	0	0	0	0	0	-
42.3	0	0	0	0	0	0	0	-
93.0	0	0 [a]	0 [a]	0 [bc]	0 [b]	0	0	-
205	0	0 [adef]	0 [ab]	0 [bcg]	0 [b]	0	0	-
450	0	0 [adefg]	0 [abcdefhi]	7	7	7	7	100

[a] Larger mouth movements, [b] Less active than control, [c] Mucus secretion, [d] Hyperventilation, [e] Gasping, [f] "Hanging" in water column, [g] Dorsal fin less erect, [h] Sedentary, [i] Loss of equilibrium

Conclusion: Based on these results a LC_{50} of 290.9 µg ai/L (95% C.I. = 196.4 - 431.1 µg ai/L) based on nominal concentrations was determined. The NOEC of 42.3 µg ai/L based on behavioural effects.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.

- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured at the start of the test and after 4 hours. No further analytical measurements were conducted. Due to the missing analytical verification it could not be demonstrated that the test substance was satisfactorily maintained throughout the study. Hence, the study is not considered valid.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The loading rates are higher than the recommended rate of 1.0 g fish/L according to the OECD test guideline. However, there were no signs of any loading-related distress in any fish. All other water quality criteria parameters were met so there is no impact on the integrity of the study.
- The measured concentrations of captan in the control and solvent control were slightly above the LOQ. However, no adverse effects were observed in the controls; hence, an exposure of control fish to captan can be considered negligible.
- The study report states that fish used will be 9 ± 2 cm in total length. Two fish which were over 11 cm at 11.2 and 11.8 cm. There was no apparent size effect on toxicity; therefore, this deviation had no effect on the validity of the study.

Acceptability of the analytical methods used in the test: The study is not considered valid according to the OECD test guideline. However, taking into account that fish species are the most sensitive group of aquatic organisms, a validation of the analytical measurements was conducted since the endpoint might be of relevance for a higher tier risk assessment.

The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ of 1 µg ai/L.

Endpoints: No reliable endpoint based on mean measured concentrations could be calculated considering the static exposure system used in the test and the fast degradation of the active substance in the water sediment system. Considering the lack of appropriate analytical verification of the test substance in the test system no exposure profile could be determined. Hence, the use of the laboratory static study as a refined exposure study (Tier 2C) is not considered acceptable.

The RMS conducted a statistical re-evaluation of the results based on initial measured concentrations.

A 96 h LC_{50} of 289.7 µg ai/L (95% C.I. 223.7-395.1 µg ai/L) was determined based on Probit analysis using linear max. likelihood regression. The LC_{10} was calculated to be 182.7 µg ai/L (95% C.I. 118.0-235.4 µg ai/L) and NOEC was determined to be 40.7 µg a.s./L based on behavioural effects (large mouth movements, lethargy,…).

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to due to lack of analytical verification of the test substance in the test medium.

Reference:	**Captan: Acute toxicity to rainbow trout**
Author(s), year:	2004a
Report/Doc. number:	MAK836/043023, R-17596
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None of relevance
Validity:	Not acceptable

Material and methods:	
Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 70138427, purity : 95.4% w/w
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 53 mm Weight: mean 1.9 g
Loading	0.37 g/L fish loading per test vessel
Type of test:	Static, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 30.1, 66.1, 145, 320 and 704 µg ai/L
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 140 - 152 mg /L as $CaCO_3$
Temperature:	13.5 - 14.7 °C
pH:	7.96 - 8.36
O_2 content:	81 - 102 % air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 35 litres to a depth of 20 cm. At the initiation of the study, 7 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 2, 4, 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.
Analytical measurements:	Samples of the test medium were taken from each control or test vessel at the start of the test (0 hours, fresh media) and after approximately 48 hours; no samples were taken at 96 hours because no test substance was found in the samples removed at 48 hours.

The analysis of the test solutions were measured using a gas chromatographic method of chemical analyses.

Statistics: LC_{10} and LC_{50} values were estimated by non-linear interpolation using angular transformed % mortality versus log 10 dose using SAS 8.2. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986), based on the Probit regression model.

Findings:

Analytical data: The mean captan concentration in the test media (at 0 hours) ranged from 79 to 81 % of nominal values.

Table B. 9.2.1-26: Analytical measurements in the test medium

Nominal concentrations [µg/L]		Measured concentrations [µg/L], 0 hours (% of nominal concentration)			Measured concentrations [µg/L], 48 hours	
Captan techn.	**Captan**	**Captan**		**Mean**	**Captan**	
Control	-	nd	nd	-	nd	nd
Solvent control	-	nd	nd	-	nd	nd
30.1	28.7	22.7 (79)	23.4 (82)	23.1	nd	nd
66.1	63.1	57.7 (91)	53.3 (84)	55.5	nd	nd
145	139	117.5 (85)	119.3 (86)	118	nd	nd
320	305	275.6 (90)	257.0 (84)	266	nd	nd
704	672	579.6 (86)	609.4 (91)	595	nd	nd

nd…not determined

Biological : The highest nominal concentration resulting in 0% mortality was 124 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 279 µg ai/L.

Sub-lethal effects were observed at all test concentrations. Sub-lethal effects (hyperventilation) were observed in the control group, but not in the solvent control group. The observations of hyperventilation in the water-only control were (out of 7 fish): 2 h = 0; 4 h = 2; 24 h = 0; 48 h = 2; 72 h = 1; 96 h = 0.

Hence, the hyperventilation was transient. The fish in the solvent control (from same batch as the water-control) did not show hyperventilation at any observation timepoint. At the end of the test no adverse effects could be observed.

The symptoms were attributed to the presence of an aggressive fish which when isolated behind a screen placed in the vessel; the other fish recovered and appeared normal.

Table B. 9.2.1-27: Mortality and sub-lethal effects

Test concentration [µg ai/L], initial measured		Cumulative mortality (initial number = 7 fish/concentration)						
Captan techn. *	**Captan**	**2 h**	**4 h**	**24 h**	**48 h**	**72 h**	**96 h**	**%**
Control	-	0	0 [a]	0	0 [a]	0 [a]	0	-
Solvent control	-	0	0	0	0	0	0	-
24.2	23.1	0 [b]	0 [b]	0 [b]	0 [b]	0 [b]	0 [b]	-

Test concentration [µg ai/L], initial measured		Cumulative mortality (initial number = 7 fish/concentration)						
Captan techn. *	Captan	2 h	4 h	24 h	48 h	72 h	96 h	%
58.2	55.5	0 [a]	0 [a]	0	0	0	0	-
124	118	0 [a]	0 [a]	0 [ab]	0 [b]	0 [ab]	0 [abc]	-
279	266	0 [ab]	1 [abd]	7	7	7	7	100
623	595	1 [ae]	7	7	7	7	7	100

[a] Hyperventilation, [b] Darkened pigmentation, [c] Lethargy, [d] Bleeding (fin), [e] Loss of co-ordination
* Concentration of captan technical was calculated from the initial mean measured level of captan and purity of 95.4%

Conclusion: Based on these results a LC_{50} of 186 µg ai/L (95% C.I. = 124 - 279 µg ai/L) based on mean measured initial concentrations was determined. The LC_{10} was 142 µg ai/L (95% C.I. = 106 - 279 µg ai/L).

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured at the start of the test and after 48 hours. After 48 hours the measured concentrations were below the LOD. No further analytical measurements were conducted. Due to the missing analytical verification it could not be demonstrated that the test substance was satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint based on mean measured concentrations could be calculated considering the static exposure system used in the test and the fast degradation of the active substance in the water sediment system. Considering the lack of appropriate analytical verification of the test substance in the test system no exposure profile could be determined. Hence, the use of the laboratory static study as a refined exposure study (Tier 2C) is not considered acceptable.

The RMS conducted a statistical re-evaluation of the results based on initial measured concentrations.

A 96 h LC_{50} of 173.6 µg a.s./L (95% C.I. 138.065-221.528 µg a.s/L) was calculated based on Probit analyses using max. likelihood regression. The LC_{10} was determined to be 121.9 µg a.s./L (95% C.I. 83.544-151.348 µg

a.s./L). No reliable NOEC could be determined taking into account the observed behavioural effects at the lowest test concentration.
Further, a 96 h LC_{50} of 211.33 (95% C.I 168.82-267.799 µg ai/L) based on nominal concentrations was calculated (Probit analysis using max. likelihood regression). The 96 h LC_{10} was determined to be 149.626 µg ai/L (95% C.I. 102-922-184-875 µg ai/L).
The differences in the endpoints derived by the RMS and the study author are because of the different statistical methods used. The RMS considers the Probit analysis to be the more robust method than the non-linear interpolation used by the study author.
Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to the low recovery of the test item.

Reference:	**Captan: Acute toxicity to three-spine stickleback**
Author(s), year:	2004b
Report/Doc. number:	MAK837/043024, R-17597
Guideline(s):	OECD test guideline 203 (1992), EC Methods Part C1
GLP:	Yes
Deviations:	None of relevance
Validity:	Not acceptable

Material and methods:

Test substance:	Captan techn., CAS no. : 133-06-2, batch no. : 70138427, purity : 95.4% w/w
Test species:	3-spined stickleback (*Gasterosteus aculeatus*)
Number of organisms:	7 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 35.8 mm
	Weight: mean 0.57 g
Loading	0.4 g/L fish loading per test vessel
Type of test:	Static, 96 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 23.3, 51.2, 113, 248, 545 and 1200 µg ai/L
Solvent:	DMF (0.1 mL/L)

Test conditions:

Water quality:	Dilution water, hardness: 140 - 144 mg /L as $CaCO_3$
Temperature:	13.2 - 14.7 °C
pH:	7.94 - 8.43
O_2 content:	94 - 104 % air saturation
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with transition periods
Feeding	The fish were not fed during the 96 hours study period.

Methods: The test was carried out in glass aquaria with a working volume of 10 litres to a depth of 13 cm.

At the initiation of the study, 7 fish were allocated at random to each test vessel.

Test parameters: All test vessels were monitored for mortality and sub-lethal effects after 2, 4, 24, 48, 72 and 96 hours.

Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness was determined.

Analytical measurements: Samples of the test medium were taken from each control or test vessel at the start of the test (0 hours, fresh media) and after approximately 48 hours; no samples were taken at 96 hours because no test substance was found in the samples removed at 48 hours.

The analysis of the test solutions were measured using a gas chromatographic method of chemical analyses.

Statistics: LC_{10} and LC_{50} values were estimated by non-linear interpolation using angular transformed % mortality versus log 10 dose using SAS 8.2. The 95% confidence limits were derived using the likelihood ratio method described by Williams (1986), based on the Probit regression model.

Findings:

Analytical data: The mean captan concentration in the test media (at 0 hours) ranged from 64 to 91 % of nominal values.

Table B. 9.2.1-28: Analytical measurements in the test medium

Nominal concentrations [µg/L]		Measured concentrations [µg/L], 0 hours (% of nominal concentration)			Measured concentrations [µg/L], 48 hours	
Captan techn.	**Captan**	**Captan**		**Mean**	**Captan**	
Control	-	nd	nd	-	nd	nd
Solvent control	-	nd	nd	-	nd	nd
23.3	22.2	14.2 (64)	15.0 (68)	14.6	nd	nd
51.2	48.8	36.5 (75)	39.7 (81)	38.1	nd	nd
113	108	87.5 (81)	94.3 (87)	90.9	nd	nd
248	237	162.8 (69)	180.7 (76)	172	nd	nd
545	520	362.9 (70)	399.3 (77)	381	nd	nd
1200	1145	1030.4 (90)	1044.7 (91)	1038	nd	nd

nd…not determined

Biological : The highest nominal concentration resulting in 0% mortality was 180 µg ai/L. The lowest nominal concentration resulting in 100% mortality was 1088 µg ai/L.

In the solvent control group, one fish died at the end of the 96 h exposure period.

Sub-lethal effects were observed at test concentrations of 95.3 µg ai/L and higher.

In the 15.3 µg ai/L treatment group one fish showed aggressive behaviour. However, this effect is not considered treatment related.

Table B. 9.2.1-29: Mortality and sub-lethal effects

Test concentration [µg ai/L], initial measured		Cumulative mortality (initial number = 7 fish/concentration)						
Captan techn. *	Captan	2 h	4 h	24 h	48 h	72 h	96 h	%
Control	-	0	0	0	0	0	0	-
Solvent control	-	0	0	0	0	0	1	14
15.3	14.6	0 [d]	0	0 [d]	0 [d]	0 [d]	0 [d]	-
39.9	38.1	0	0	0	0	0	0	-
95.3	90.9	0	0	0 [a]	0 [a]	0	0	-
180	172	0 [ac]	0 [ac]	0 [ac]	0 [ac]	0	0	-
399	381	0 [ae]	1 [a]	2 [a]	4 [a]	4 [a]	4	57
1088	1038	0 [a]	5 [abe]	7	7	7	7	100

[a] Hyperventilation, [b] Darkened pigmentation, [c] Lethargy, [d] Aggression, [e] Loss of co-ordination
* Concentration of captan technical was calculated from the initial mean measured level of captan and purity of 95.4%

Conclusion: Based on these results a LC_{50} of 370 µg ai/L (95% C.I. = 285 - 532 µg ai/L) based on mean initial measured concentrations was determined. The LC_{10} was 231 µg ai/L (95% C.I. : 140 - 399 µg ai/L).

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

The concentration of the test substance was measured at the start of the test and after 48 hours. After 48 hours the measured concentrations were below the LOD. No further analytical measurements were conducted. Due to the missing analytical verification it could not be demonstrated that the test substance was satisfactorily maintained throughout the study. Hence, the study is not considered valid.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint based on mean measured concentrations could be calculated considering the static exposure system used in the test and the fast degradation of the active substance in the water sediment system. Considering the lack of appropriate analytical verification of the test substance in the test system no exposure profile could be determined. Hence, the use of the laboratory static study as a refined exposure study (Tier 2C) is not considered acceptable.

The RMS conducted a statistical re-evaluation of the results based on initial measured concentrations.
No reliable LC_x values could be determined based on Probit analysis as no confidence intervals could be calculated. Considering the steep dose-response the RMS conducted a statistical analysis based on Weibull using max. likelihood regression. Further, the endpoints were estimated by interpolation, i.e. Trimmed Spearman-Kärber procedure.
Based on initial measured concentrations a 96 h LC_{50} of 376.3 µg a.s./L (95% C.I. 268.84-526.662 µg a.s/L) was estimated. Using linear regression (Weibull) a LC_{50} of 380.2 µg ai/L (95% C.I. 269.569-579.743 µg ai/L) and a LC_{10} of 208.0 µg ai/L (95% C.I. 85.716-288.530 µg ai/L) were determined.
Further, a 96 h LC_{50} of 515.4 µg ai/L (95% C.I 388.766-692-192 µg ai/L) based on nominal concentrations was estimated by interpolation. Using linear regression (Weibull) a 96 h LC_{50} of 525.2 µg ai/L (95% C.I. 384.664-805.207 µg ai/L) and a LC_{10} of 341.5 µg ai/L (95% C.I. 81.402-439.673 µg ai/L) were determined.
The aggressive behaviour of one fish observed in the lowest test concentration is not considered treatment-related; hence, a NOEC of 38.1 µg ai/L based on initial measured concentrations and of 51.2 µg ai/L based on nominal concentrations was determined.
The differences in the endpoints derived by the RMS and the study author are because of the different statistical methods used. The RMS considers the linear regression (Weibull, max. likelihood) to be the more robust method than the non-linear interpolation used by the study author.
Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is not considered valid due to due to lack of analytical verification of the test substance in the test medium.

Metabolites:

Reference:	**THPI: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)**
Author(s), year:	1994
Report/Doc. number:	BL5237/B, R-8288, TMN-0025
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:

Test substance:	THPI (tetrahydrophthalimide), CAS no. 85-40-5, purity: 96%
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: 36 - 51 mm (mean: 46 mm)
	Weight: 0.60 - 1.97 g (mean: 1.51 g)
Loading	0.76 g/L fish loading per test vessel
Type of test:	Static, 96 hours, limit test

Applied concentrations:

Nominal:	0 (control and solvent control) and 120 mg THPI/L
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Water quality:	Dilution water, hardness: 27 mg /L as $CaCO_3$, alkalinity: 22.2 mg/L as $CaCO_3$, Conductivity: 171 µS/cm, pH: 7.56
Temperature:	14.8 - 15.0 °C
pH:	7.26 - 7.75
O_2 content:	8.6 - 9.8 mg/L (> 60 % air saturation)
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 20 litres. At the initiation of the study 10 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness, alkalinity and conductivity were determined.
Analytical measurements:	Samples of the test solutions were measured at 0, 48 and 96 hours using the high performance liquid chromatography method.
Statistics:	No mortalities or sub-lethal effects were observed in the limit test. Hence, no statistical analyses are required to determine the LC_{50} and NOEC.
Findings:	
Analytical data:	The mean concentration of the metabolite THPI in the test media (at 0, 48 and 96 hours) was 100% of the nominal concentration.

Table B. 9.2.1-30: Analytical measurements in the test medium

Nominal concentrations [mg/L]	Measured concentration of THPI [mg/L]			Mean measured concentration of THPI [mg/L]	% of nominal
	0 h	48 h	96 h		
Control	< 0.042	< 0.042	< 0.042	< 0.042	-
Solvent control	< 0.042	< 0.042	< 0.042	< 0.042	-
120	120	120	120	120	100

Limit of determination (LOD) = 0.042 mg/L

Biological :	No mortalities were observed in the control, the solvent control and the test concentration of 120 mg/L. Additionally, no sublethal effects were observed.

Table B. 9.2.1-31: Mortality and sub-lethal effects

Nominal concentrations [mg/L]	Cumulative mortality [%]			
	0 h	48 h	72 h	96 h
Control	0	0	0	0
Solvent control	0	0	0	0
120	0	0	0	0

Conclusion: Based on these results a LC_{50} of > 120 mg THPI/L (nominal concentrations) was determined. The NOEC was 120 mg/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

In addition, the following points deviated from the guidelines or were not reported in detail:

One of the fish in the dilution water control was smaller than recommended by the OECD guideline. The undersized fish was not considered to have affected the validity of the study.

The study is considered valid as all validity criteria according to the OECD test guideline were met.

Acceptability of the analytical methods used in the test: The analytical verification of the test item was conducted using HPLC/UV. No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: The RMS agrees on the endpoints given in the study report.

96 h LC_{50} > 120 mg THPI/L

NOEC = 120 mg THPI/L

based on nominal concentrations

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is considered valid.

Reference:	**THPAM: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)**
Author(s), year:	1995
Report/Doc. number:	BL5445/B, R-8749, TMN-0024
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:	
Test substance:	THPAM (*cis*-tetrahydropthalamic acid), CAS no. 1469-49-4, purity: 95%
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per control and test concentrations, 1 replicate per treatment
Length, weight:	Length: 35 - 44 mm (mean: 40 mm) Weight: 0.33 - 1.00 g (mean: 0.73 g)
Loading	0.73 g/L fish loading per test vessel
Type of test:	Semi-Static, re-newal after 48 hours, limit test
Applied concentrations:	
Nominal:	0 (control) and 120 mg THPAM/L
Solvent:	None
Test conditions:	
Water quality:	Dilution water, hardness: 33 mg /L as $CaCO_3$, alkalinity: 20.4 mg/L as $CaCO_3$, Conductivity: 159 µS/cm, pH: 7.64
Temperature:	14.9 - 15.6 °C
pH:	5.57 - 8.01
O_2 content:	9.4 - 10.2 mg/L (> 60 % air saturation)
Aeration:	Supplementary aeration
Light regime:	Light/dark cycle of 16/8, with transition periods
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria with a working volume of 10 litres. At the initiation of the study, 10 fish were allocated at random to each test vessel.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken throughout the 96 hour period. In addition, water hardness, alkalinity and conductivity were determined.
Analytical measurements:	Samples of the test solutions were measured at 0, 48 and 96 hours using the high performance liquid chromatography method.
Statistics:	No mortalities or sub-lethal effects were observed in the limit test. Hence, no statistical analyses are required to determine the LC_{50} and NOEC.

Findings:

Analytical data: The mean concentration of the metabolite THPAM in the test media (at 0, 48 and 96 hours) was 108% of the nominal concentration.

Table B. 9.2.1-32: Analytical measurements in the test medium

Nominal concentrations [mg/L]	Measured concentration of THPAM [mg/L]				Mean measured concentration of THPAM [mg/L]	% of nominal
	0 h	48 h		96 h		
	Fresh medium	Old medium	Fresh medium	Old medium		
Control	< 0.093	< 0.093	< 0.093	< 0.093	< 0.093	-
120	120 *	120	130 *	130	130	108

Limit of determination (LOD) = 0.093 mg/L
* Mean of triplicate analyses: 0 hour: 120, 120, 130 mg/L
48 hour: 130, 130, 130 mg/L

Biological : No mortalities were observed in the control and the test concentration of 120 mg/L. Additionally, no sublethal effects were observed.

Table B. 9.2.1-33: Mortality and sub-lethal effects

Nominal concentrations [mg/L]	Cumulative mortality [%]			
	0 h	48 h	72 h	96 h
Control	0	0	0	0
120	0	0	0	0

Conclusion: Based on these results a LC_{50} of > 120 mg THPAM/L (nominal concentrations) was determined. The NOEC was 120 mg/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992, updated 2009).

- The mortality in the controls should not exceed 10% (or 1 fish if less than 10 fish are used) at the end of the test.
- Constant conditions should be maintained throughout the test.
- The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.
- There must be evidence that the concentration of the test substance has been satisfactorily maintained, and preferably, it should be at least 80% of the nominal concertation throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

In addition, the following points deviated from the guidelines or were not reported in detail:

- Three of the fish in the dilution water control were smaller than recommended by the OECD guideline. The undersized fish were not considered to have affected the validity of the study.

- The pH in the treatment group was between 5.57 and 6.01 due to the acid properties of the metabolite. According to the test guideline, the pH should be in a range of 6.0 to 8.5. If there is evidence of marked change in the pH, it is proposed to repeat the test with adjustment of the pH of the stock solution. The study was conducted without any adjustment of the pH. However, no adverse effects on the fish could be observed during the test duration; hence, the lower pH might not have affected the results of the study.

All validity criteria were met; hence, the study is considered valid.

Acceptability of the analytical methods used in the test: The analytical verification of the test item was conducted using HPLC/UV. No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: The RMS agrees on the endpoints given in the study report.

96 h LC_{50} > 120 mg THPAM/L

NOEC = 120 mg THPAM/L

based on nominal concentrations

Conclusion of the RMS: Based on the evaluation of the study, the acute fish toxicity test is considered valid.

B.9.2.2. Long-term and chronic toxicity to fish

For the first EU peer-review of the active substance captan, one prolonged toxicity study with fish was submitted addressing the chronic risk to fish. The study was a 21 day prolonged toxicity test according to OECD 204. In addition, a 28 day prolonged toxicity test according to OECD 204 was conducted with the formulation Merpan 83 WP (study summary, refer to Vol.3, B.9 (PPP)).
However, according to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) prolonged toxicity tests are not the required tests to address the long-term risk to fish. Nevertheless, for the sake of completeness, the summaries of the prolonged toxicity studies are given below.

According to the Regulation 283/2013 a long-term toxicity study on fish shall be provided where the substance is deemed stable in water, that is to say there is less than 90% loss of the original substance over 24 hours via hydrolysis. A fish early life stage study shall be provided in these circumstances.
The rate of degradation in the water via hydrolysis was measured at pH 5, pH 7 and pH 9. At acidic conditions the rate of degradation is slower (DT_{50} = 11 - 18.8 hours) than at alkaline conditions (DT_{50} = 3.6-8.3 minutes). At a neutral pH value, the DT_{50} is in a range of 2.6 to 4.9 hours. No DT_{90} at relevant pH values were determined in the laboratory studies, hence the trigger for the requirement of long-term toxicity data of DT_{90} greater than 24 hours cannot be checked.
However, under consideration of a maximal DT_{50} of 18.8 hours and also taken into account the high number of applications a fish early life stage study (ELS-test) was conducted with a 80 WDG formulation (study summary, refer to Vol.3, B.9 (PPP)).

a) Captan: the 21 day LC_{50} to rainbow trout (Salmo gairdneri). (1991c; IIA, 8.2.2.1/01)

The 21-day toxicity of captan technical active substance (purity 90% w/w) to the rainbow trout (Salmo gairdneri now known as Oncorhynchus mykiss) was determined in a continuous flow-through system without aeration. Groups of ten fish in 54 L glass tanks containing 45 L test solution (15°C, 16:8 hour light/dark regime) were exposed to nominal concentrations of captan (dissolved in dimethylformamide) at 5.6, 10, 18, 32, 56 and 100 µg/L in comparison with a dilution water only (dechlorinated filtered tap water, total hardness approx. 34 to 47 mg CaCO3/L) and solvent only (100 µL dimethylformamide/L) control for 21 days. The test solutions were renewed (95% tank volume exchange) every nine hours. It was noted during analytical method development that captan technical rapidly hydrolysed in dilute aqueous media. Therefore, only stock solutions were analysed. Samples of the solvent stock solutions were taken for analysis of the captan content (by HPLC) at the start of exposure and at two or three day intervals during exposure. Measurements of pH, dissolved oxygen and temperature were taken at one to three day intervals. Mortality was recorded daily and symptoms of toxicity 4, 7, 10, 14 and 21 days after the start of exposure.

The study met the essential criteria of OECD 204. The fish were slightly smaller (mean length 34 mm) than recommended (50 ± 10 mm) but this is not considered to have affected the validity of the study. It was conducted according to Good Laboratory Practice.

Mean measured concentrations of captan in the solvent stock solutions ranged from 82 to 100% of the nominal values based on calculated values from each respective solvent stock solution. The results are, therefore, presented as nominal values. The physical and chemical parameters of the test solutions remained at expected values during the study (pH 7.3 to 8.1 for all treatments).

No treatment-related symptoms of toxicity apart from mortality were observed. There was no treatment-related effect on fish weight or length.

Summary of effects of captan on rainbow trout following 21-day exposure

Captan concentration	Cumulative mortality [%]		Mean fish weight [g]		Mean fish length [mm]
[µg/L][1]	14 days	21 days	14 days	21 days	21 days
Water only	0	0	2.39	2.57	54.2
Solvent only	0	0	2.42	2.74	54.4
5.6	10	10	2.60	2.86	56.7
10	0	0	2.19	2.61	54.0
18	0	0	2.53	2.78	56.5
32	10	10	2.76	3.23	58.1
56	0	0	2.38	2.67	55.0
100	100	100	TD	TD	TD

[1] *Nominal concentrations*
TD…test terminated

The 21-day LC_{50} of captan to the rainbow trout under flow-through conditions was 75 µg/L. The NOEC was 56 µg/L based on the mortality recorded at 100 µg/L. The 4, 7, 10 and 14-day LC_{50} values were also 75 µg/L.

B.9.2.3. Bioconcentration in fish

The octanol-water partitioning coefficient (log K_{OW}) for captan and its metabolites is below the trigger of 3. The log K_{OW} for captan was experimentally determined to be 2.52. Information on the log K_{OW} of the metabolites THPAM (soil, surface water) and THPI (soil, surface water) were provided. However, no log K_{OW} was determined for the metabolite THPAI (sediment).

The log K_{OW} of the metabolites THPAM and THPI were -0.4 and 0.22, respectively. The log K_{OW} for the metabolite THPAI was estimated to be about 0.74, using the US EPA software KOWWIN (EPIWEB 4.1).

Table B. 9.2.3-1: Log K_{OW} estimation using EPIWEB

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KOWWIN Program (v1.68) Results:
===============================

                  Log Kow(version 1.68 estimate): 0.74

SMILES : OC(=O)C1CC=CCC1C(=O)(O)
CHEM   :
MOL FOR: C8 H10 O4
MOL WT : 170.17
-------+-----+-------------------------------------------+---------+--------
 TYPE  | NUM |        LOGKOW FRAGMENT DESCRIPTION        |  COEFF  |  VALUE
-------+-----+-------------------------------------------+---------+--------
 Frag  |  2  |  -CH2-   [aliphatic carbon]               | 0.4911  |  0.9822
 Frag  |  2  |  -CH     [aliphatic carbon]               | 0.3614  |  0.7228
 Frag  |  2  |  =CH- or =C<  [olefinc carbon]            | 0.3836  |  0.7672
 Frag  |  2  |  -COOH   [acid, aliphatic attach]         |-0.6895  | -1.3790
 Factor|  1  |  Multi-aliphatic carboxylic acids         |-0.5865  | -0.5865
 Const |     |  Equation Constant                        |         |  0.2290
-------+-----+-------------------------------------------+---------+--------
                                                        Log Kow   =   0.7357
```

Taking into account that the log K_{OW} for captan and its metabolites is below the trigger of 3 no evaluation of the bioconcentration potential in fish is required.

However, during the first EU peer-review of captan two bioconcentration studies in fish were conducted with bluegill sunfish (*Lepomis macrochirus*). These fish bioconcentration studies were summarised and evaluated by the RMS.

Taking into account the deficiencies identified for the two bioconcentration studies in fish the RMS conducted an estimation of the BCF value. The BCF of the active substance captan was estimated using the US EPA software BCFBAF (EPIWEB 4.1) based on the chemical structure and a measured log K_{OW} of 2.52. The BCF was estimated to be 21.4 L/kg wet wt. Further details on the estimation based on the measured log K_{OW} see below.

Taking into account the estimated log K_{OW} of 2.85 (using KOWWIN) a BCF of 35.3 L/kg wet wt was determined.

Table B. 9.2.3-2: BCF estimation using EPIWEB

```
SMILES : c1ccc2c(c1)C(=O)N(C2(=O))SC(CL)(CL)CL
CHEM   :
MOL FOR: C9 H4 CL3 N1 O2 S1
MOL WT : 296.56
--------------------------------- BCFBAF v3.01 ---------------------------------
Summary Results:
  Log BCF (regression-based estimate):  1.33  (BCF = 21.4 L/kg wet-wt)
  Biotransformation Half-Life (days) :  2.49  (normalized to 10 g fish)
  Log BAF (Arnot-Gobas upper trophic):  1.54  (BAF = 34.8 L/kg wet-wt)

============================
BCF (Bioconcentration Factor):
============================
Log Kow  (estimated)  :  2.84
Log Kow (experimental):  2.85
Log Kow used by BCF estimates:  2.52 (user entered)

Equation Used to Make BCF estimate:
   Log BCF = 0.6598 log Kow - 0.333 + Correction

      Correction(s):                    Value
       No Applicable Correction Factors

   Estimated Log BCF =  1.330  (BCF = 21.36 L/kg wet-wt)

============================================================
Whole Body Primary Biotransformation Rate Estimate for Fish:
============================================================
------+-----+---------------------------------------------+---------+---------
 TYPE | NUM | LOG BIOTRANSFORMATION FRAGMENT DESCRIPTION |  COEFF  |  VALUE
------+-----+---------------------------------------------+---------+---------
 Frag |  3  |  Aliphatic chloride   [-CL]                |  0.3608 |  1.0823
 Frag |  1  |  Carbon with 4 single bonds & no hydrogens | -0.2984 | -0.2984
 Frag |  4  |  Aromatic-H                                |  0.2664 |  1.0655
 Frag |  1  |  Number of fused acyclic rings             |  0.6477 |  0.6477
 Frag |  1  |  Number of fused 6-carbon aromatic rings   | -0.5779 | -0.5779
 L Kow|  *  |  Log Kow =   2.52 (user-entered   )        |  0.3073 |  0.7745
 MolWt|  *  |  Molecular Weight Parameter                |         | -0.7605
 Const|  *  |  Equation Constant                         |         | -1.5371
============+=============================================+=========+=========
   RESULT   |        LOG Bio Half-Life (days)            |         |  0.3961
   RESULT   |            Bio Half-Life (days)            |         |    2.49
   NOTE     |  Bio Half-Life Normalized to 10 g fish at 15 deg C   |
============+=============================================+=========+=========

Biotransformation Rate Constant:
 kM (Rate Constant):  0.2784 /day (10 gram fish)
 kM (Rate Constant):  0.1566 /day (100 gram fish)
 kM (Rate Constant):  0.08804 /day (1 kg fish)
 kM (Rate Constant):  0.04951 /day (10 kg fish)

Arnot-Gobas BCF & BAF Methods (including biotransformation rate estimates):
   Estimated Log BCF (upper trophic) =  1.542  (BCF = 34.81 L/kg wet-wt)
   Estimated Log BAF (upper trophic) =  1.542  (BAF = 34.81 L/kg wet-wt)
   Estimated Log BCF (mid trophic)   =  1.365  (BCF = 23.15 L/kg wet-wt)
   Estimated Log BAF (mid trophic)   =  1.365  (BAF = 23.17 L/kg wet-wt)
   Estimated Log BCF (lower trophic) =  1.310  (BCF = 20.42 L/kg wet-wt)
   Estimated Log BAF (lower trophic) =  1.311  (BAF = 20.46 L/kg wet-wt)

Arnot-Gobas BCF & BAF Methods (assuming a biotransformation rate of zero):
   Estimated Log BCF (upper trophic) =  1.560  (BCF = 36.27 L/kg wet-wt)
   Estimated Log BAF (upper trophic) =  1.582  (BAF = 38.21 L/kg wet-wt)
```

Reference:	**Bioconcentration and elimination of ^{14}C-residues by Bluegill (*Lepomis macrochirus*) exposure to Trichloromethyl ^{14}C-Captan**
Author(s), year:	1988a
Report/Doc. number:	T-13069, R-5268, TMN-0002
Guideline(s):	Conducted in accordance to in-house method
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Materials and Methods:

Test material	[^{14}C]-Captan
Lot/Batch #	CFQ4573 (lot number), J42893 (batch)
Specific activity	38 mCi/mMole, 126 mCi/g
Test organism:	*Lepomis macrochirus*
Size	47 - 64 mm
Body weight	1.19 - 3.74 g (mean 2.0 g)
Loading	0.28 g/L/day
Diet/Food	Fish were fed daily with a dry pelleted food, *ad libitum*, except during the 24 hours prior to testing
Acclimatisation period:	Min. 14 days prior to test initiation
Environmental conditions:	
Temperature	17-18 °C
Photoperiod	16 hours light : 8 hours dark
pH	6.9 - 7.0
Dissolved oxygen	84 - 87%
Total hardness	32 - 36 mg $CaCO_3$/L
Alkalinity	29-41 mg $CaCO_3$/L
Conductivity	120-180 µmhos/cm

Study design:

The bioaccumulation of [^{14}C]-Captan in bluegill sunfish (*Lepomis macrochirus*) was investigated in a flow-through system at a nominal exposure concentration of 5.0 µg/L. Following pre-equilibration of the test system for 3 days, 90 juvenile bluegill from a fish population with a mean wet weight of 2.0 g (range: 1.19-3.74 g) and mean total length of 53 mm (range: 47-64 mm) were transferred to the aquaria (treatment and control).

The biomass loading was 0.28 g/L of water flowing through the aquaria in 24 hours. The fish were fed a dry pelleted food daily.

The test vessels were measured 76 x 40 x 30 cm, the water level was maintained at a depth of 24.7 cm for a total water volume of 75 L. The system was calibrated to deliver 2.0 L of water to each aquaria at an average rate of 319 times per day during the exposure period. This is equivalent to 90% aquarium volume replacement per each

6-hour period and 8.5 aquarium volume turnover every 24 hours.

The exposure of bluegill to thrichloromethyl ^{14}C-Captan at a nominal rate of 5.0 µg/L was continuous until a steady-state tissue residue concentration was established. This was determined by subjecting the measured ^{14}C-tissue residue concentrations for 3 consecutive sampling intervals to analysis of variance to establish so significant differences existed.

After 29 days of exposure, 16 of the remaining fish in the treatment aquarium were transferred to a clean aquarium (biomass loading 0.09 g/L/day). The depuration period continued for 14 days to estimate the half life.

57 fish were removed from the treatment group for possible metabolite characterisation.

The water temperature and dissolved oxygen concentration in each aquarium were measured daily and the pH was measured 3-times per week in each aquarium during the study.

Observations:

The water temperature and dissolved oxygen concentration in each aquarium were measured daily and the pH was measured 3-times per week in each aquarium during the study.

Water samples were taken on days 1, 2 and 5 of equilibration and hour 0 and days 1, 3, 7, 10, 14, 21, 25 and 28 of exposure and on days 1, 3, 7, 10 and 14 of depuration.

In order to quantify the accumulation and elimination of ^{14}C-residues in the edible and nonedible tissue of bluegill, two fish were collected from the treatment aquarium, eviscerated and filleted on days 1, 3, 7, 10, 14, 21, 25, and 28 of exposure and on days 1, 3, 7, 10, and 14 of depuration. Two control fish were removed, eviscerated and filleted on day 28 of exposure and on day 14 of depuration. Two unexposed fish were analysed on day 0 to establish background ^{14}C-residues levels in fish tissue.

Calculations:

The bioconcentration factors for edible, non-edible and whole body fish tissue were determined by dividing the mean measured steady state or equilibrium ^{14}C-tissue concentration for each tissue type by the mean measured water concentration for the entire exposure period (c_{ss}/c_w).

In addition, an additional method of calculating bioconcentration factors where the ratio of the uptake constant (K_u) to the depuration constant (K_d) was used.

Growth dilution was considered in the calculation of the bioconcentrations factors by correcting the measured radioactivity (DMP) for the sample size (weight in grams or in mL).

Results and discussion:

Environmental conditions: The temperature in each aquarium ranged between 16 and 18°C throughout the test. The total hardness and alkalinity was measured to be between 26 and 36 mg $CaCO_3$/L and 27 and 30 mg $CaCO_3$/L, respectively. The dissolved oxygen in both aquaria ranged from 6.4 and 9.7 mg/L (66-98% of saturation). The pH in both aquaria ranged between 6.5 and 7.7.

Exposure concentration: The concentration of ^{14}C-residues measured in the water of the exposure aquarium during the 29 day exposure period was between 2.9 and 4.6 µg/L.

Toxicant delivery system problems occurring between exposure days 7 and 14 were responsible for variability in

the measured ^{14}C-residue concentrations in water and consequently in the fish tissue concentration during this period of the uptake phase of the study. At day 7 a water concentration of 14.2 µg/L was measured. This value was not used in the calculation of the mean water concentrations or the bioconcentration factor. At day 10 a concentration less than the detection limit (< 0.27 µg/L) was measured. This value was also not used in the calculation of the mean water concentrations or the bioconcentration factor.
The mean ^{14}C-residue concentration in the water of the treated system during the 29-day exposure period was 3.6 ± 1.1 µg/L. This was 72% of the nominal concentration.
Throughout the 14-day depuration period concentration in the water remained < 0.27 µg/L. Analysed of control water indicated that no ^{14}C-residues were present above detectable limits.

Residue in fish: The mean ^{14}C-residue concentrations measured in the edible tissue reached steady state on day 21, within 7 days of constant exposure and remained relatively constant throughout the remainder of the exposure (range: 190-220 µg/kg). Based on a mean equilibrium concentration of 200 ± 39 µg/kg measured in edible tissue and a mean concentration of 3.6 ± 1.1 µg/L in the water during the period from 0-29 days of exposure, the mean equilibrium bioconcentration factor for ^{14}C-Captan in bluegill edible tissue was 56X.
The mean ^{14}C-residue concentrations measured in the nonedible tissue reached steady state on day 1 and ranged from 400-1300 µg/kg. Based on a mean equilibrium concentration of 1000 ± 430 µg/kg measured in nonedible tissue and a mean concentration of 3.6 ± 1.1 µg/L in the water during the period from 0-29 days of exposure, the mean equilibrium bioconcentration factor for ^{14}C-Captan in bluegill nonedible tissue was 280X.
The pattern of accumulation and elimination of ^{14}C-residues on a whole fish basis was similar to that observed in the nonedible tissues. Based on a mean equilibrium concentration in whole fish of 500 ± 230 µg/kg (range: 200-740 µg/kg) and a mean concentration of 3.6 ± 1.1 µg/L in the water during the period from 0-29 days of exposure, the mean equilibrium bioconcentration factor for ^{14}C-Captan in bluegill (whole fish) was 140X.

Table B. 9.2.3-3: Measured ^{14}C-residue concentration in the water and the tissue of bluegill

Day	Water concentration [µg/L] [a]	^{14}C-tissue concentration [µg/kg] [b]		
		Edible	Non-edible	Whole fish
Exposure phase				
0	4.0	-	-	-
1	4.0	63	850	360
3	2.9	55	1300	540
7	14.2 [c]	111	890	470
10	< 0.27 [c]	64	940	470
14	4.2	65	400	200
21	4.0	190	1100	630
25	3.1	220	1200	700
28	4.6	200	1300	740
Depuration phase				
1	< 0.27	120	1500	650
3	< 0.27	110	400	250
7	< 0.27	38	130	77

Day	Water concentration [µg/L] [a]	^{14}C-tissue concentration [µg/kg] [b]		
		Edible	Non-edible	Whole fish
10	< 0.27	87	200	140
14	< 0.27	56	110 [d]	83 [d]

Limit of quantification < 0.27 µg/L
[a] Average based on radiometric analysis of duplicate samples.
[b] Average based on analysis of tissue portions of 2 fish.
[c] This value was not used in the calculation of mean water concentrations or the bioconcentration factor.
[d] This value represents the analysis of one fish due to the loss of the nonedible tissue portion of the duplicate fish sampled at this interval.

Variability in toxicant delivery affected the relative tissue concentration (edible and nonedible) of ^{14}C-Captan during the period of malfunction (day 7-14). Tissue concentrations decreased in the absence of toxicant (i.e. day 10) but returned quickly to pre-malfunction levels. The patterns of accumulation observed during the last 14 days of continuous exposure was similar to the pattern of accumulation observed during the first 7 days of continuous exposure.
Under condition of constant exposure, equilibrium conditions were generally achieved within 7 days.

The elimination half-life (clearing time, CT_{50}) was attained in the nonedible and whole body tissue between day 1 and 3 of the depuration period and between day 3 and 7 in the edible tissue. By day 14 of the depuration period the bluegill had eliminate 72, 92 and 89% of the ^{14}C-residues that had been present on the last day of exposure in the edible, nonedible and whole body tissue, respectively.

Table B. 9.2.3-4: Bioconcentration factors for edible, nonedible and whole body fish tissue

Tissue type	Uptake constant K_u	Depuration constant K_d	Estimated BCF	Measured BCF
Edible	22	0.20	110X	56X
Nonedible	269	0.39	690X	280X
Whole fish	88	0.35	250X	140X

No mortality occurred during the 43-day exposure and depuration phase. The bluegill during both the exposure and depuration period exhibited normal behaviour, fed readily and were in good physical condition.

Conclusion:

Table B. 9.2.3-5: Summary of results

Parameter	Description	Value
Cf_{SS}	Concentration in fish at steady state [µg/kg]	500
Cw	Concentration in water at steady state [µg/L]	3.6
BCF_{SS}	Steady-state bioconcentration factor [L/kg], whole fish	140 (measured) 250 (estimated)
CT_{50}	Clearance time [days]	3-7 d (edible) 1-3 d (nonedible, whole fish)
Depuration after 14 days	Depuration after 14 days [%]	72% (edible) 92% (nonedible) 89% (whole fish)

Comment RMS:

Validity: The study was conducted prior to adoption of the latest version of OECD 305 (2012). For a test to be valid the following conditions have to apply:

- Temperature variation less than ± 2°C (16-18 °C).
- The concentration of dissolved oxygen does not fall below 60% saturation (69-98% of saturation).
- The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase (mean 72% of nominal concentrations, excluding results of day 7 and 10).
- The concentration of the test substance is below its limit of solubility in water, taking into account the effect that the test water have no effective solubility (solubility in water 2.67 mg/L (pH 7, 15 °C) – 5.2 mg/L (pH 7, 20°C)).
- The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical (No mortalities of adverse effects were observed in the control and treatment group).

All validity criteria except for one (stability of the test substance during the uptake phase) were met. The concentration of the test substance in the test chamber was not maintained because of delivery system problems occurring between exposure days 7 and 14. If the measurements on day 7 and 10 were excluded from the calculations, the concentrations were maintained within ± 20% of the mean of the measured values during the uptake phase.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- No information on residues of metabolites or impurities is given in the study report, even though it was mentioned that residues of metabolites were investigated.
- No detailed information on the water temperature, pH and dissolved oxygen throughout the study duration is given in the study report.
- No detailed information on the chemical characteristics of the dilution water is given in the study report.
- According to the OECD test guideline 305 the recommended range of test temperature for the bluegill fish should be between 20-25 °C. In the study, the temperature range was between 16 and 18°C.
- The number of fish per test concentration should be selected such that a minimum of four fish are available at each sampling point. In the study, only two fish per sampling point were used and analysed.
- The test was run with one concentration instead of two as recommended in the test guideline.
- No information on the lipid content of fish is given in the study report. Hence, it is not possible to express the BCF on a 5% lipid content basis.

Acceptability of the analytical methods used in the test: The Guidance Document SANCO/3029/99 rev. 4 is not applicable for radio-labelled substances.

Endpoints: The RMS agrees on the endpoints given in the study report.

BCF_{SS} = 250 (whole fish)

Conclusion of the RMS: Based on the evaluation, the fish bioconcentration study is considered valid.

Reference:	**Bioconcentration and elimination of ^{14}C-residues by Bluegill (*Lepomis macrochirus*) exposure to Cyclohexane ^{14}C-Captan**
Author(s), year:	1988b
Report/Doc. number:	T-13068, R-5269, TMN-0002A
Guideline(s):	Conducted in accordance to in-house method
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Materials and Methods:

Test material	Cyclohexane [^{14}C]-Captan Non-radiolabelled Captan technical (Lot number WRC 4921-26-17, purity: 89.7%)
Lot/Batch #	2099-252 (lot number)
Specific activity	16.6 mCi/mMole, 55.2 mCi/g
Test organism:	*Lepomis macrochirus*
Size	47 - 64 mm (mean 53 mm)
Body weight	1.19 - 3.74 g (mean 2.0 g)
Loading	0.28 g/L/day
Source	In-house culture
Diet/Food	Fish were fed daily with a dry pelleted food, *ad libitum*, except during the 24 hours prior to testing
Acclimatisation period:	Min. 14 days prior to test initiation
Environmental conditions:	
Temperature	17 - 18 °C
Photoperiod	16 hours light : 8 hours dark
pH	6.9 - 7.0
Dissolved oxygen	84 - 87%
Total hardness	32 - 36 mg $CaCO_3$/L
Alkalinity	29 - 41 mg $CaCO_3$/L
Conductivity	120 - 180 µmhos/cm

Study design:

The bioaccumulation of [^{14}C]-Captan in bluegill sunfish (*Lepomis macrochirus*) was investigated in a flow-through system at a nominal exposure concentration of 5.0 µg/L. Following pre-equilibration of the test system for 3 days, 90 juvenile bluegill from a fish population with a mean wet weight of 2.0 g (range: 1.19 - 3.74 g) and mean total length of 53 mm (range: 47 - 64 mm) were transferred to the aquaria (treatment and control).

The biomass loading was 0.28 g/L of water flowing through the aquaria in 24 hours. The fish were fed a dry pelleted food daily.

The test vessels were measured 75 x 39 x 39 cm, the water level was maintained at a depth of 25.4 cm for a total water volume of 75 L. The system was calibrated to deliver 2.0 L of water to each aquaria at an average rate of 319 times per day during the exposure period. This is equivalent to 90% aquarium volume replacement per each 6-hour period and 8.5 aquarium volume turnover every 24 hours.

The exposure of bluegill to thrichloromethyl ^{14}C-Captan at a nominal rate of 5.0 µg/L was continuous until a steady-state tissue residue concentration was established. This was determined by subjecting the measured ^{14}C-tissue residue concentrations for 3 consecutive sampling intervals to analysis of variance to establish so significant differences existed.

After 28 days of exposure, 16 of the remaining fish in the treatment aquarium were transferred to a clean aquarium (biomass loading 0.09 g/L/day). The depuration period continued for 14 days to estimate the half life.

57 fish were removed from the treatment group for possible metabolite characterisation.

The water temperature and dissolved oxygen concentration in each aquarium were measured daily and the pH was measured 3-times per week in each aquarium during the study.

Observations:

The water temperature and dissolved oxygen concentration in each aquarium were measured daily and the pH was measured 3-times per week in each aquarium during the study.

Water samples were taken on days 1, 2 and 5 of equilibration and hour 0 and days 1, 3, 7, 10, 14, 21 and 28 of exposure and on days 1, 3, 7, 10 and 14 of depuration.

In order to quantify the accumulation and elimination of ^{14}C-residues in the edible and nonedible tissue of bluegill, two fish were collected from the treatment aquarium, eviscerated and filleted on days 1, 3, 7, 10, 14, 21 and 28 of exposure and on days 1, 3, 7, 10, and 14 of depuration. Two control fish were removed, eviscerated and filleted on day 28 of exposure and on day 14 of depuration. Two unexposed fish were analysed on day 0 to establish background ^{14}C-residues levels in fish tissue.

Calculations:

The bioconcentration factors for edible, non-edible and whole body fish tissue were determined by dividing the mean measured steady state or equilibrium ^{14}C-tissue concentration for each tissue type by the mean measured water concentration for the entire exposure period (c_{ss}/c_w).

In addition, an additional method of calculating bioconcentration factors where the ration of the uptake constant (K_u) to the depuration constant (K_d) was used.

Growth dilution was considered in the calculation of the bioconcentrations factors by correcting the measured radioactivity (DMP) for the sample size (weight in grams or in mL).

Results and discussion:

Environmental conditions: The temperature in each aquarium ranged between 16-18°C throughout the test. The total hardness and alkalinity was measured to be between 26 and 36 mg $CaCO_3$/L and 27 and 30 mg $CaCO_3$/L, respectively. The dissolved oxygen in both aquaria ranged from 6.4-9.7 mg/L (66 - 98% of saturation). The pH in

both aquaria ranged between 6.5 and 7.7.

Exposure concentration: The concentration of ^{14}C-residues measured in the water of the exposure aquarium during the 28 day exposure period was between 4.2 and 5.0 µg/L.
The mean ^{14}C-residue concentration in the water of the treated system during the 28-day exposure period was 4.6 ± 0.3 µg/L. This was 94% of the nominal concentration.
Throughout the 14-day depuration period concentration in the water remained < 0.26 µg/L. Analysed of control water indicated that no ^{14}C-residues were present above detectable limits.

Residue in fish: The mean ^{14}C-residue concentrations measured in the edible tissue reached steady state on day 1, and remained relatively constant throughout the remainder of the exposure (range: 350-520 µg/kg). Based on a mean equilibrium concentration of 470 ± 70 µg/kg measured in edible tissue and a mean concentration of 4.6 ± 0.3 µg/L in the water during the period from 0-28 days of exposure, the mean equilibrium bioconcentration factor for cyclohexane ^{14}C-Captan in bluegill edible tissue was 102X.
The mean ^{14}C-residue concentrations measured in the nonedible tissue reached steady state on day 1 and ranged from 380-650 µg/kg. Based on a mean equilibrium concentration of 580 ± 120 µg/kg measured in nonedible tissue and a mean concentration of 4.6 ± 0.3 µg/L in the water during the period from 0-28 days of exposure, the mean equilibrium bioconcentration factor for cyclohexane ^{14}C-Captan in bluegill nonedible tissue was 126X.
The pattern of accumulation and elimination of ^{14}C-residues on a whole fish basis was similar to that observed in the edible and nonedible tissues. Based on a mean equilibrium concentration in whole fish of 520 ± 89 µg/kg (range: 360-580 µg/kg) and a mean concentration of 4.6 ± 0.3 µg/L in the water during the period from 0-28 days of exposure, the mean equilibrium bioconcentration factor for ^{14}C-Captan in bluegill (whole fish) was 113X.

Table B. 9.2.3-6: Measured ^{14}C-residue concentration in the water and the tissue of bluegill

Day	Water concentration [µg/L] [a]	^{14}C-tissue concentration [µg/kg] [b]		
		Edible	Non-edible	Whole fish
Exposure phase				
0	4.3	-	-	-
1	4.3	350	380	360
3	4.8	510	570	540
7	4.2	520	650	580
10	5.0	520	620	560
14	4.6	490	640	550
21	4.6	470	590	520
28	4.6	460	580	510
Depuration phase				
1	< 0.26	180	410	280
3	< 0.26	42	85	61
7	< 0.26	31	32	32
10	< 0.26	36	29	33
14	< 0.26	26	21	24

Limit of quantification < 0.27 µg/L
[a] Average based on radiometric analysis of duplicate samples.
[b] Average based on analysis of tissue portions of 2 fish.

The elimination half-life (clearing time, CT_{50}) was attained in the edible, nonedible and whole body tissue between day 0 and 3 days of the depuration period. By day 14 of the depuration period the bluegill had eliminate 94, 96 and 95% of the ^{14}C-residues that had been present on the last day of exposure in the edible, nonedible and whole body tissue, respectively.

Table B. 9.2.3-7: Bioconcentration factors for edible, nonedible and whole body fish tissue

Tissue type	Uptake constant K_u	Depuration constant K_d	Estimated BCF	Measured BCF
Edible	38	0.79	48X	102X
Nonedible	111	0.66	168X	126X
Whole fish	95	0.71	134X	113X

The bluegill during both the exposure and depuration period exhibited normal behaviour, fed readily and were in good physical condition. Three fish in the treatment and one fish in the control group died during the 42-day exposure and depuration phases.

Conclusion:

Table B. 9.2.3-8: Summary of results

Parameter	Description	Value
Cf_{SS}	Concentration in fish at steady state [µg/kg]	520
Cw	Concentration in water at steady state [µg/L]	4.7
BCF_{SS}	Steady-state bioconcentration factor [L/kg], whole fish	113 (measured) 134 (estimated)
CT_{50}	Clearance time [days]	0-3 d
Depuration after 14 days	Depuration after 14 days [%]	> 94%

Comment RMS:

Validity: The study was conducted prior to adoption of the latest version of OECD 305 (2012). For a test to be valid the following conditions have to apply:
- Temperature variation less than ± 2°C (16-18 °C).
- The concentration of dissolved oxygen dies not fall below 60% saturation (66-98% of saturation).
- The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase (mean 91% of nominal concentration).
- The concentration of the test substance is below its limit of solubility in water, taking into account the effect that the test water have no effective solubility (solubility in water 2.67 mg/L (pH 7, 15 °C) - 5.2 mg/L (pH 7, 20°C)).
- The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical (No mortalities of adverse effects were observed in the control and treatment group).

All validity criteria according to the OECD test guideline are met. Hence, the study is considered valid.
In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:
- No information on residues of metabolites or impurities is given in the study report, even though it was mentioned that residues of metabolites were investigated.
- No detailed information on the water temperature, pH and dissolved oxygen throughout the study duration is given in the study report.
- No detailed information of the chemical characteristics of the dilution water is given in the study report.
- According to the OECD test guideline 305 the recommended range of test temperature for the bluegill fish should be between 20-25 °C. In the study, the temperature range was between 16 and 18°C.
- The number of fish per test concentration should be selected such that a minimum of four fish are available at each sampling point. In the study, only two fish per sampling point were used and analysed.
- The test was run with one concentration instead of two as recommended in the test guideline.
- No information on the lipid content of fish is given in the study report. Hence, it is not possible to express the BCF on a 5% lipid content basis.

Acceptability of the analytical methods used in the test: The Guidance Document SANCO/3029/99 rev. 4 is not applicable for radio-labelled substances.
Endpoints: The RMS agrees on the endpoints given in the study report.
BCF_{SS} = 134 (whole fish)
Conclusion of the RMS: Based on the evaluation, the fish bioconcentration study is considered valid.

B.9.2.4. Acute toxicity to aquatic invertebrates

Two acute toxicity studies conducted with the active substance captan on *Daphnia magna* were conducted for the first EU peer-review of captan. A new acute toxicity test (Cockroft, 2016) with the active substance was submitted

by the applicant taking into account the identified deficiencies in the two existing studies regarding analytical measurements of the active substance.

All acute toxicity tests were conducted under static conditions. Due to the rapid hydrolysis of captan the measured concentrations were below the LOD at the end of the test.

According to the OECD test guideline 202 the endpoint shall be expressed in terms of mean measured concentrations if the test substance has not been satisfactorily maintained within ± 20% of the nominal or initial measured concentrations. In addition, according to the EFSA Technical Report (2015), the validity of studies should be considered questionable if the measured concentrations of all test concentrations are below the LOD at the end of the test. In conclusion, the RMS considered the studies as valid as the validity criteria according to the OECD test guideline are met. However, the reliability of the endpoints based on nominal concentrations is questionable. The RMS proposes to address the risk to aquatic invertebrates in a higher tier risk assessment, due to the lack of an acceptable tier 1 endpoint. For further information considering the aquatic risk assessment, refer to Volume 3, B.9 (PPP).

In addition, acute toxicity tests on *Daphnia magna* were conducted with the two metabolites THPI and THPAM.

Active substance:

Reference:	**Captan: Acute toxicity of the technical material to first instar *Daphnia magna***
Author(s), year:	1993
Report/Doc. number:	91JH187, RJ1116B, TMN-6
Guideline(s):	US EPA test guideline, subdivision E: 72-2 (1982)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:	
Test substance:	Captan, lot no. 25-MAR-91/07, purity: 93.5%, water solubility: 3.3 mg/L at 25 °C
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	3 replicates each with 10 daphnids per treatment and control
Age:	First instar, < 24 hours old
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	Not given
Initial measured (mean):	0 (control), 0.69, 1.1, 1.9 and 3.2 mg ai/L
Solvent:	None
Test conditions:	
Water quality:	Dilution water, total hardness: 160-180 mg/L as $CaCO_3$
Temperature:	19.9 - 20.2°C
pH:	7.2 - 8.0 (0 - 48 h)
O_2 content:	6.8 - 8.3 mg/L (> 74% saturation)
Light regime:	16 hours light / 8 hours darkness (800 lux light intensity)
Test parameters:	Immobility and sublethal effects were assessed after 3, 9, 24 and 48 hours. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test. Temperature was also recorded continuously. For chemical analysis (gas liquid chromatography) of captan in the test media samples were taken at test initiation (0 h) and termination (48 h).
Statistics:	The EC_{50}, and 95 % confidence limits were obtained by the technique of iteratively reweighted linear regression on the logit transformation of percent response on log_{10} (concentration).
Findings:	
Analytical data:	The test solutions were analysed for Captan at 0 and 48 hours. The initial measured concentrations (at 0 hours) were 0.893, 1.10, 1.93 and 3.25 mg ai/L. After 48 hours all test concentrations were below the limit of quantification (< 0.002 mg/L)

Effects: The estimated 24 and 48 hour EC_{50} values were 4.6 and 4.7 mg ai/L, respectively, based on initial measured concentrations. Adverse effects on daphnids were observed at the two highest test concentrations.

Table B. 9.2.4-1: Effects on daphnids (*D. magna*) exposed to technical Captan

Captan [mg ai/L] (initial measured)	Mean cumulative immobilized organisms [%]	
	3 hours	48 hours
Control	0	0
0.69	0	0
1.1	0	0
1.9	0	13.3
3.2	3.33	23.3

Conclusion: The acute toxicity of Captan to *Daphnia magna* has been investigated.
The 48-hour EC_{50} was calculated as 4.7 mg ai/L based on initial measured concentrations. The NOEC was determined to be 1.1 mg ai/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 202 (2004).

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised;
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.

All validity criteria are met; hence, the acute fish toxicity test is considered valid.

In addition, the following points deviated from the test guideline or were not reported in detail:

- The concentration of the test substance was measured at the start and the end of the test. No further analytical measurements were conducted. At test end of the test, all measured concentrations were below the LOD. Due to the results of the analytical measurements, it could not be shown that the active substance was satisfactorily maintained throughout the study.
- At least five concentrations should be used. They should be arranged in a geometric series with a separation factor preferably not exceeding 2.2. In the study, only 4 test concentrations were used.
- In the study protocol no nominal test concentrations are given, instead the initial measured test concentrations (0 hour) are reported.
- No information on the chemical characteristics (total organic carbon, chlorine, …) of the dilution water is given in the study report.
- No information on adverse effects as signs of stress or disease, for example, unusual behaviour was reported in the study report.

Taking into account the validity criteria the study is considered valid. The RMS is of the opinion that the identified deficiencies had no impact on the integrity of the study. However, based on the analytical

measurements a satisfactorily exposure of the test substance throughout the test duration could not be demonstrated.

Acceptability of the analytical methods used in the test: The analytical verification of the test item was conducted using HPLC/UV. No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: No reliable endpoint could be calculated based on the analytical measurements.

Conclusion of the RMS: Based on the evaluation of the study, the acute daphnid toxicity test is considered valid. However, no reliable endpoint could be derived.

Reference:	**Static acute toxicity of Captan technical (SX-1086) to *Daphnia magna***
Author(s), year:	1980
Report/Doc. number:	26633, TMN-10
Guideline(s):	US EPA test guideline (1975)
GLP:	No
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan, no information on the batch/lot number or purity of the technical active substance
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	3 replicates each with 10 daphnids per treatment and controls
Age:	Adult daphnids
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	5 and 10 mg ai/L (solubility test)
Measured (mean):	0 (control and solvent control) 3.18 and 7.27 mg ai/L using 0.5 mL acetone 3.07 and 6.91 mg ai/L using 1.0 mL acetone
Solvent:	Acetone
Test conditions:	
Water quality:	Well water, total hardness: 255 ppm as $CaCO_3$, alkalinity: 368 ppm as $CaCO_3$, conductivity: 50 µmhos/cm
Temperature:	20 ± 1°C
pH:	8.2 (0 h)
O_2 content:	9.3 ppm (0 h)
Light regime:	16 hours light / 8 hours darkness (800 lux light intensity)

Test parameters:	*Daphnia magna* were exposed to Captan for 48 hours in a solubility test. The solubility of technical Captan of 5 and 10 mg/L each in both 0.5 mL and 1.0 mL Acetone was tested. Immobility and sublethal effects were assessed after 24 and 48 hours. No information on the feeding of Daphnids was given in the study report. A chemical characterisation of the well water was conducted at the start of test. No information regarding the measurement of the temperature, pH and dissolved oxygen is given in the study report For chemical analysis (gas liquid chromatography) of captan in the test media samples were taken at test initiation (0 h) and termination (48 h).
Statistics:	No information on the statistical analyses is given in the study report. The LC_{50} was estimated based on the results of the study.
Findings:	
Analytical data:	The test solutions were analysed for Captan at 0 and 48 hours. The initial measured concentrations (at 0 hours) were 3.18 and 7.27 mg/L in 0.5 mL acetone and 3.07 and 6.91 mg/L in 1.0 mL acetone. After 48 hours the measured concentrations were 0.0028 and 0.0054 mg/L in 0.5 mL acetone and 0.0032 and 0.0052 mg/L in 1.0 mL acetone.
Effects:	No mortality was observed at any of the test concentrations at the end of the study (48 hours).
Conclusion:	From the results in the solubility study, the LC_{50} was estimated to be greater than 7.1 mg/L based on the mean of the initial measured concentrations.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 202 (2004).

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised;
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.

In addition, the following points deviated from the test guideline or were not reported in detail:

- The concentration of the test substance was measured at the start and the end of the test. No further analytical measurements were conducted. At test termination the measured concentrations of captan were below 1% of the nominal concentrations; hence, the results should have been expressed in terms of mean measured concentrations.

In addition, the following points deviated from the guidelines or were not reported in detail:

- At least five concentrations should be used. They should be arranged in a geometric series with a separation factor preferably not exceeding 2.2. In the study, only 2 test concentrations were used.
- No information on the feeding regime of daphnids during the study is given.
- No information on the measurements of temperature, pH and dissolved oxygen throughout the study duration is given.

- In general, the information given in the study report regarding test design, results and analytical measurements is poor.
- No information on adverse effects as signs of stress or disease, for example, discoloration or unusual behaviour was reported in the study report.
- The study was not conducted according to GLP (1980)

All validity criteria are met. However, taking into account the identified deficiencies of the study (number of test concentrations, study reporting) the RMS is of the opinion that the results of the study should not be considered for the risk assessment.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the identified deficiencies of the study.

Conclusion of the RMS: Taking into account the evaluation of the study, the acute daphnid toxicity test is not considered valid.

Reference:	**Captan: Acute immobilisation test on *Daphnia magna* under static exposure conditions.**
Author(s), year:	2016
Report/Doc. number:	ENV-16-016, R-37744
Guideline(s):	OECD test guideline 202
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:	
Test substance:	Captan, batch no.: 94138737, purity: 95.8%
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	3 replicates each with 10 daphnids per treatment and control
Age:	First instar, < 24 hours old
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	312.5, 625, 1250, 2500 and 5000 µg ai/L
Mean measured:	Not calculated.
Solvent:	DMF (0.1 mL/L)
Test conditions:	
Dilution medium:	Elendt M4 (according OECD 202, Annex 3)
Temperature:	19.3 - 20.1°C (mean: 19.7°C)
pH:	7.31 - 7.66 (0 h)

	7.82 - 7.88 (48 h)
O_2 content:	100 - 101 % of air saturation
Hardness:	231 mg/L as $CaCO_3$
Light regime:	16 hours light / 8 hours darkness (683 lux light intensity)
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. During the exposure, the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test. Hardness was determined at test start. For chemical analysis (gas chromatography with mass spectrometric detection, GC-MS) of captan in the test media samples were taken at test initiation (0 h) and termination (48 h).
Statistics:	The EC_{50}, and 95 % confidence limits were calculated using a 3P Log-Compertz EV model with non-linear regression (CETIS software).
Findings:	
Analytical data:	The test solutions were analysed for Captan at 0 and 4 hours. The measured concentrations after 4 hours were between 30 and 45% of the nominal concentrations.

Table B. 9.2.4-2: Analytical measurements for the test media at 0 h and 4 h

Nominal concentrations [µg test item/L]	Measured concentrations at 0 h		Measured concentrations at 4 h	
	Captan [µg ai/L]	% of nominal	Captan [µg ai/L]	% of nominal
Control	nd	-	-	-
Solvent control	nd	-	-	-
312.5	382	128	89	30
625	670	112	214	36
1250	1336	112	538	45
2500	2233	93	970	41
5000	5006	105	1889	39

nd…not detected

Biological effects:	The estimated 24 and 48 hour EC_{50} values were 1089 and 1007 µg ai/L, respectively, based on nominal concentrations. Adverse effects on daphnids were observed at all test concentrations, except for the lowest test concentration.

Table B. 9.2.4-3: Effects on daphnids (*D. magna*) exposed to technical Captan

Captan [µg ai/L] (nominal concentrations)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	5	0
Solvent control	0	0
312.5	0	0
625	5	10
1250	80	80
2500	100	100
5000	100	100

Conclusion: The 48-hour EC_{50} was determined to be 1007 µg ai/L (95% C.I. = 974.6 - 1039 µg ai/L) based on nominal concentrations. The NOEC based on immobilisation was 312.5 µg ai/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 202 (2004).

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised;
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.

In addition, the following points deviated from the guidelines or were not reported in detail:

- The concentration of the test substance was measured at the start of the test and after 4 hours. After 4 h the measured concentrations were below 80% of nominal concentrations. Hence, the endpoint shall be expressed in terms of mean measured concentrations. However, no further analytical measurements (e.g. at test end) were conducted. Due to the missing analytical verification it could not be demonstrated that the test substance was satisfactorily maintained throughout the study. Hence, no reliable endpoint could be calculated.

Acceptability of the analytical methods used in the test: The evaluation of the analytical method was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ of 10 µg ai/L.

Endpoints: Based on the results of the analytical measurements no reliable endpoint could be determined. The RMS does not agree on the determined endpoints based on nominal concentrations.

Conclusion of the RMS: Based on the evaluation of the study the acute daphnid toxicity test is considered valid. However, no reliable endpoint could be derived.

Metabolites:

Reference:	**THPI: Acute toxicity to *Daphnia magna***
Author(s), year:	1994
Report/Doc. number:	BL5239/B, R-8290, TMN-012
Guideline(s):	OECD 202 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:	
Test substance:	THPI (*cis*-1,2,3,6 tetrahydropthalamide), CAS no. 84-40-5, purity: 96%
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, 48 hours, limit test
Applied concentrations:	
Nominal:	0 (control) and 120 mg/L
Measured (mean):	0 (control), 120 mg/L
Solvent:	None
Test conditions:	
Water quality:	Elendt's M4 Daphnia medium, total hardness: 179 mg/L as $CaCO_3$, alkalinity: 40.2 mg/L as $CaCO_3$, conductivity: 553 µS/cm, pH 8.06
Temperature:	20 ± 1°C
pH:	7.61 - 8.18 (0 - 48 h)
O_2 content:	9.0 - 9.1 mg/L
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test. Temperature was also recorded continuously. For chemical analysis (high performance liquid chromatography) of THPI in the test media samples were taken at test initiation (0 h) and termination (48 h).
Statistics:	No statistical analyses were conducted considering that no adverse effects were observed at the limit test concentration.
Findings:	
Analytical data:	The mean measured concentration was 100% of the nominal concentrations.

Table B. 9.2.4-4: Analytical measurements

Nominal concentration	Measured concentrations of THPI [mg/L]		Mean measured concentrations of THPI [mg/L]	% of nominal concentrations
	0 hours	48 hours		
Control	< 0.04	< 0.04	< 0.04	-
120	120 *	110 *	120	100

* Mean of triplicate analyses

Effects: No effects on the mobility of Daphnids at a single concentration of 120 mg/L were observed. The estimated 24 and 48 hour EC_{50} values were greater than 120 mg/L based on nominal concentrations.

Table B. 9.2.4-5: Effects on daphnids (*D. magna*) exposed to the metabolite THPI

THPI [mg/L] (nominal concentrations)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
120	0	0

Conclusion: The acute toxicity of the metabolite THPI to *Daphnia magna* has been investigated. The 48-hour EC_{50} was calculated as greater than 120 mg/L based on nominal concentrations. The NOEC was determined to be 120 mg/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 202 (2004).

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised;
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.

In addition, the following points deviated from the test guideline or were not reported in detail:

- The concentration of the test substance was measured at the start and the end of the test. No further analytical measurements were conducted. In addition, the following points deviated from the guidelines or were not reported in detail:

- No information on the batch used in the study is given (e.g. batch or lot number).

Acceptability of the analytical methods used in the test: The analytical verification of the test item was conducted using HPLC/UV. No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: The RMS agrees on the endpoints given in the study report.

48 h EC_{50} > 120 mg THPI/L

NOEC = 120 mg THPI/L

based on nominal concentrations

Conclusion of the RMS: Based on the evaluation of the study, the acute daphnid toxicity test is considered valid.

Reference:	**THPAM: Acute toxicity to *Daphnia magna***
Author(s), year:	1995
Report/Doc. number:	BL5442B, R-8748, TMN-011
Guideline(s):	OECD 202 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	THPAM (*cis*-tetrahydropthalamic acid), CAS no. 1469-49-4, purity: 95%, sample identification WRC# 15184-16-1
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, 48 hours
Applied concentrations:	
Nominal:	0 (control), 32, 56, 100, 180 and 320 mg/L
Measured (mean):	0 (control), 29, 52, 98, 170 and 280 mg/L
Solvent:	None
Test conditions:	
Water quality:	Elendt's M4 Daphnia medium, total hardness: 249.7 mg/L as $CaCO_3$, alkalinity: 43.4 mg/L as $CaCO_3$, conductivity: 702 µS/cm, pH 7.97
Temperature:	20 ± 1°C
pH:	4.67 - 8.06 (0 - 48 h) The pH values measured in the exposure concentrations increased over the 48 hour test period with a maximum increase of 1.09 in the nominal 100 mg/L test concentration. The increases were considered to be due to the pH buffering capacity of the dilution water over the 48 hours test period. The changes in pH were considered not to have affected the validity of the test.
O_2 content:	8.8 - 9.0 mg/L
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test. Temperature was also recorded continuously. For chemical analysis (high performance liquid chromatography) of THPAM in the test media samples were taken at test initiation (0 h) and termination (48 h).
Statistics:	The EC_{50}, and 95 % confidence limits were calculated by the moving average angel and binominal methods were selected from the computer program and the

percentage immobility was plotted against concentration for the 24 and 48 hour data using a Hewlett-Packard graphics plotter.

Findings:

Analytical data: The mean measured concentrations ranged from 88 to 89% of the nominal concentrations.

Table B. 9.2.4-6: Analytical measurements

Nominal concentration [mg/L]	Measured concentrations of THPAM [mg/L]		Mean measured concentrations of THPAM [mg/L]	% of nominal concentrations
	0 hours	48 hours		
Control	< 0.19	< 0.19	< 0.19	-
32	29	29	29	91
56	51 *	53 *	52	93
100	95	100	98	98
180	170	170	170	94
320	270	280	280	88

* Mean of triplicate analyses

Effects: The estimated 24 and 48 hour EC_{50} values were 240 and 220 mg/L, respectively, based on nominal concentrations. Adverse effects on daphnids were observed at the two highest test concentrations.

Table B. 9.2.4-7: Effects on daphnids (*D. magna*) exposed to the metabolite THPAM

THPAM [mg/L] (nominal concentrations)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
32	0	0
56	0	0
100	0	0
180	0	10
320	100	100

Conclusion: The acute toxicity of the metabolite THPAM to *Daphnia magna* has been investigated. The 48-hour EC_{50} was calculated as 220 mg/L (95% C.I. 190 – 250 mg/L) based nominal concentrations. The NOEC was determined to be 100 mg/L.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 202 (2004).

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised;
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.

In addition, the following points deviated from the test guideline or were not reported in detail:

- The concentration of the test substance was measured at the start and the end of the test. No further analytical measurements were conducted. At the end of the test the mean measured concentrations were between 88 und 89% of the nominal concentrations.

In addition, the following points deviated from the guidelines or were not reported in detail:

- The pH should be in a range between 6 and 9 and should normally not vary by more than 1.5 units in any one test concentration. In the test the pH did not vary by more than 1.5 units within one test concentration. However, at the two highest test concentrations the pH was below pH 6. At the test concentration of 180 mg/L the pH was between 5.28 and 5.47 and at the test concentration of 320 mg/L the pH was between 4.67 and 4.76. According to the OECD test guideline a second test (with pH adjustment of the stock solution) should be carried out if the pH does not remain in the recommended range of pH 6-9.

Taking into account the observed adverse effects on Daphnids at the two highest test concentrations it is uncertain if the effects were due to the low pH values (which were already measured at the beginning of the test) or due to the toxicity of the metabolite THPAM.

Acceptability of the analytical methods used in the test: The analytical verification of the test item was conducted using HPLC/UV. No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: The RMS agrees on the endpoints given in the study report.

48 h EC_{50} = 220 mg THPAM/L

NOEC = 100 mg THPAM/L

based on nominal concentrations

Conclusion of the RMS: Based on the evaluation of the study, the acute daphnid toxicity test is considered valid; however, the endpoint should be considered with caution taking into account the identified uncertainty regarding the pH value.

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

For the first EU peer-review of the active substance captan a reproductive study with *Daphnia magna* was submitted addressing the long-term risk to aquatic invertebrates. The study was performed according to the OECD test guideline 202 under semi-static conditions.

Reference:	**Captan: Chronic toxicity to *Daphnia magna***
Author(s), year:	1991
Report/Doc. number:	BL4102/B, R-6412, TMN-009
Guideline(s):	OECD 202 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:	
Test substance:	Captan, CAS no. 133-06-2, batch no. RS309/H, purity: 90%
Test species:	Waterflea (*Daphnia magna*)
Number of organisms:	10 replicates each with 1 daphnids per treatment and control groups
Age:	First instar, < 24 hours old
Type of test, duration:	Semi-static, Medium renewal 3 times per week, 21 days
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.32, 0.56 and 1.0 mg/L
Solvent:	Dimethylformamid (DMF)
Test conditions:	
Water quality:	Reconstituted water medium, total hardness: 163.7-178.3 mg/L as $CaCO_3$ (0-21 d), alkalinity: 96.0 - 104.6 mg/L as $CaCO_3$ (0-21 d), Conductivity: 456 - 495 µS/cm (0-21 d), pH 8.0 - 8.4 (0-21 d)
Temperature:	19.0 - 20.3 °C
pH:	8.3 - 8.6 (new solutions) 7.9 - 8.7 (old solutions)
O_2 content:	7.7 - 10.0 mg/L
Light regime:	16 hours light / 8 hours darkness (average of 799 lux)
Test parameters:	The live and dead Daphnia of the "parental" (P_0) generation were counted daily and recorded together with observations on the general condition and size of the Daphnia as compared with the controls. At each test media renewal the numbers of live and dead "filial" (F_1) Daphnia were recorded. The number of Daphnia with eggs or young in the brood pouch plus the number of discarded unhatched eggs was also determined at this time.

	The daphnids were fed daily with cultured algae (*Chlorella vulgaris*) and a yeast suspension. Temperature was recorded daily for each flask. Dissolved oxygen, pH and temperature were measured before and after- each test media renewal.
Analytical measurements:	Verification of test concentration (high performance liquid chromatography) was carried out on each renewal occasion. It was not possible to analyse the test solutions in the test medium since captan hydrolyses rapidly in water. Hence, the stock solution was analysed for the concentration of the captan technical.
Statistics:	Reproduction and length: A nonparametric test, the Wilkoxon Rank Sum Test was used to determine significant effects on reproduction. To identify significant difference compared to the solvent control the Dunnett's procedure was used.
<u>Findings:</u>	
Analytical measurements:	No analytical measurements of the test medium were conducted. The measured concentrations for the nominal stock solutions are available. The mean measured stock concentrations ranged from 91 to 100% of the nominal value.
Lethal effects on $P_{0:}$	No mortalities were observed in the control and nominal test concentrations of 0.32 and 0.56 mg ai/L after 21 days. In the solvent control and nominal concentration of 1.0 mg ai/L, there was 10% mortality during the test. No other symptoms of toxicity were observed.
Sub-lethal effects on P_0:	The mean number if offspring produced in the control and solvent control were significantly different (P = 0.05, t-test, one-sided); therefore, the treatments were compared with the solvent control only. There was a significant decrease in mean number of offspring (F_1) produced at the 1.0 mg ai/L nominal concentration compared to the solvent control. In the control, solvent control and test concentrations up to and including 0.56 mg ai/L surviving individual P_0 Daphnids except one, released the first offspring by day 8 and completed five broods od offspring. One animal in the 0.32 mg ai/L test concentration released its first brood on day 11 and completed only 4 broods during the test. This was considered not to be a response to the test substance.
Length of P_0:	The mean length of Daphnids in the control and solvent control were not significantly different (P = 0.05, t-test, one-sided). At nominal concentrations up to and including 0.56 mg ai/L mean length was not significantly reduced. At a nominal rate of 1 mg ai/L the mean length of the surviving Daphnids was significantly less than that of the control and solvent control.

Table B. 9.2.5-1: Effects on daphnids (*Daphnia magna*) exposed to technical captan

Captan [mg ai/L] (nominal)	Number of dead daphnids (%)	Total offspring (F_1) (mean per parent ± SD)
Control	0 (0)	2121 (212 ± 11.2)
Solvent control	1 (10)	1809 (199 ± 15.4)
0.32	0 (0)	1947 (195 ± 19.8) *
0.56	0 (0)	1838 (184 ± 16.8) *
1.0	1 (10)	1684 (171 ± 10.6) **

* No significant (P = 0.05) decrease compared with the solvent control.
** Significant (P = 0.05) decrease compared with solvent control.

Conclusion: Based on the statistically significant effects a NOEC of 0.56 mg ai/L (reproduction and length, P_0 generation) was determined. The endpoint is expressed in terms of nominal concentrations.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 211 (2009).

- the mortality of the parent animals (female Daphnia) does not exceed 20% at the end of the test;
- the mean number of living offspring produced per parent animal surviving at the end of the test is > 60.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The concentration of the test substance was measured in the stock solution but not in the test solutions. Due to the missing analytical verification of the test substance in the test media there is no evidence that the concentration of the substance has been satisfactorily maintained throughout the study. Hence, the study is not considered valid.

The validity criteria according to the OECD test guideline are met. However, no analytical measurements were conducted in the test media; hence, the study is not considered acceptable.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was conducted by the RMS because the study is not considered valid.

Endpoints: No reliable endpoint could be determined based on the lack of analytical verification.

Conclusion of the RMS: Based on the evaluation of the study the chronic daphnid toxicity test is not considered acceptable.

According to the data requirements of Regulation EU (No) 283/2013, EC_{10} and EC_{20} shall be estimated in *Daphnia magna* reproduction toxicity tests, where possible.

The applicant submitted a statistical re-evaluation of the results of the study by Stewart et al. (1991) for determination of the EC_x values.

Reference:	**Estimation of EC_x values for Captan *Daphnia magna* reproduction toxicity test**
Author(s), year:	2016
Report/Doc. number:	562961-CA-08251-01

The statistical analyses were conducted using the software ToxRat® Professional 2.10. For the estimations the data on the number of offspring per day and female were used.

Table B. 9.2.5-2: Number of offspring per day and female (at day 21)

Replicate No.	Solvent control	Nominal test concentration [mg ai/L]		
		0.32	0.56	1.0
1	175	182	187	165
2	192	172	178	160
3	189	175	158	168
4	197	178	186	187
5	186	208	197	164
6	- *	228	185	157
7	224	223	196	179
8	205	201	211	179
9	214	190	184	181
10	210	190	156	- *
Mean	199	195	184	171
SD	15.4	19.8	16.8	10.6
Total offspring	1809	1947	1838	1684

* Female died before Day 21

The Effective Concentrations (EC_x) for mean cumulative offspring per survived female at 21 d were calculated by Probit analyses using linear max. likelihood regression.

The following EC_x values were determined.

EC_{10} = 0.7 mg ai/L

EC_{20} = 1.4 mg ai/L

The 95% confidence limits were not determined by the programme.

The NOEC for mean cumulative offspring per female at day 21 was estimated to be higher or equal to 1.0 mg ai/L based on the Williams Multiple Sequential t-Test.

Comment RMS:

The chronic toxicity test by Stewart et al. (1991) was evaluated by the RMS to be not acceptable. Hence, the following discussion on the statistical re-evaluation of the study was not evaluated by the RMS.

Endpoints: The RMS does not agree with the conducted statistical re-evaluation of the reproduction endpoint.

- The statistical analyses conducted by the applicant are based on the solvent control only. The data from the control were not included in the statistical analyses.

No statistically significant differences between the control and the solvent control were observed considering the number of offspring. Hence, the RMS is of the opinion that the statistical re-evaluation should be based on the pooled control.

The RMS conducted a statistical re-evaluation of the raw data including the pooled control data by using the software ToxRat® Professional 2.1.

The Effective Concentrations (EC_x) for mean cumulative offspring per survived female and introduced female at 21 d were calculated by a 3-parametric normal cumulative distribution function. The NOEC was calculated using the Williams Multiple Sequential t-test procedure.

<u>Mean cumulative offspring per introduced female, 21 d:</u>

21 d EC_{10} = 0.69 mg ai/L (95% C.I. = 0.3335 - 1.421 mg ai/L)

21 d NOEC = 0.32 mg ai/L

Treatment	Mean	Med	n	J	J*	p(J)	Sign.
Pooled Control	195,7	206,5	20				
0,320	194,7	190,0	10	63,0	1,390	0,0820	-
0,560	183,8	185,5	10	206,0	2,630	0,0040	+
1,000	154,0	166,5	10	502,0	4,410	<0.001	+

+: significant; -: non-significant

A NOEC of 0,320 is suggested by the program.

<u>Mean cumulative offspring per survived female, 21 day:</u>

21 d EC_{10} = 0.542 mg ai/L (95% C.I. = 0.329 - 0.892 mg ai/L)

21 d NOEC < 0.32 mg ai/L

Treatment	Mean	s	df	LhM	%MDD	t	t*	Sign.
Pooled Control	205,9	15,67						
0,320	194,7	15,67	44	194,7	-5	-1,84	-1,68	+
0,560	183,8	15,67	44	183,8	-5,2	-3,62	-1,74	+
1,000	171,1	15,67	44	171,1	-5,4	-5,5	-1,76	+

+: significant; -: non-significant

The NOEC is lower than 0,320 .

Conclusion of the RMS: Based on the statistical re-evaluation of the study by Stewart et al. (1991) a NOEC of < 0.32 mg ai/L was determined. However, the study by Stewart et al. (1991) was considered not valid due to the missing analytical measurements in the test medium (see study evaluation above).

Reference:	**Captan: Reproduction test on *Daphnia magna*, with repeated semi-static exposure**
Author(s), year:	2017
Report/Doc. number:	ENV-16-015, R-37745
Guideline(s):	OECD 202 (1984)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:	
Test substance:	Captan, CAS no. 133-06-2, batch no. 94138737, purity: 95.8%
Test species:	Waterflea (*Daphnia magna*)
Number of organisms:	1 daphnid per test vessel, 10 replicates per treatment, 20 replicates per control groups
Age:	First instar, < 24 hours old
Type of test, duration:	Semi-static, 4 pulsed exposures, 28 days
Applied concentrations:	
Nominal:	0 (control and solvent control), 25, 50, 100, 200 and 400 µg ai/L
Mean measured:	- (control and solvent control), 21.8, 40.9, 78.1, 188.6 and 352.1 µg ai/L
Solvent:	Dimethylformamid (DMF), 0.1 mL/L
Test conditions:	
Dilution medium:	Elendt M4 (OECD guideline, Annex 3) Total hardness: 228 - 240 mg/L as $CaCO_3$, Medium TOC: 7 mg/L
Temperature:	18.2 - 21.7 °C
pH:	7.23 - 8.04
O_2 content:	8.05 - 10.3 mg/L
Light regime:	16 hours light / 8 hours darkness (average of 1109 lux)
Test parameters:	Inert glass jars of 60 mL capacity had 50 mL of test item solution, dilution medium with solvent vehicle or dilution medium only dispensed into them. Single daphnids were added to each test vessel. The daphnids were fed daily with *Chlorella vulgaris* for the duration of the test period. At each dose or medium renewal, the dissolved oxygen concentration, temperature, and pH of freshly-prepared medium was determined. Temperature was also monitored continually in a surrogate vessel. Hardness was determined in each batch of the bulk dilution medium before used and total organic carbon (TOC) was determined in a single representative sample of dilution medium. Observational assessments were made daily. The health of the parental daphnids and the number of neonates removed at each time point was recorded.

Analytical measurements: The concentration of captan in all exposure media (and controls) was determined at the beginning of the test (Day 0) and at each exposure pulse, on freshly-prepared media sampled immediately after introduction (or transfer) of the experimental organisms. Further samples were taken at 0.5, 1, 4, 10 and 24 h from the surrogate vessels prepared on Day 7 at the 100 µg test item/L concentration.

Statistics: Fecundity: The control groups were compared using an equal-variance t two-sample test with Grubbs outlier detection. The statistical comparisons of reproduction for treated groups against the solvent control were carried out using Dunnett's multiple comparison test.

Survival: The control groups were compared using a Wilcoxon rank sum two-sample test. The statistical comparisons of parental survival for treated groups against the solvent control were carried out using a Wilcoxon/Bonferroni adjusted test.

Growth: The control groups, excluding the surviving solvent control adult which was considered likely to be male, were compared using an unequal variance t two-sample test with Grubbs outlier detection. The statistical comparisons of parental growth for treated groups against the solvent control were carried out using a Wilcoxon/Bonferroni adjusted test with Grubbs outlier detection.

The 10, 20 and 50% effect concentrations were not calculable as no treatment-related effects on reproduction of ≥ 10% were observed at any test concentration.

Findings:

Analytical measurements: Measured concentrations of captan were determined in the fresh medium (day 0, 7, 14 and 21) only. No analytical measurements were conducted for the old test media, except for the 100 µg/L treatment group.

Table B. 9.2.5-3: Concentrations of captan measured in the exposure solutions during 21 d exposure

Nominal concentration [µg/L]	Measured concentrations [µg/L]					% of nominal
	Day 0	Day 7	Day 14	Day 21	Mean	
Control	nd	nd	nd	nd	-	-
Solvent control	nd	nd	nd	nd	-	-
25	22.4	25.1	17.0	23.5	21.8	87
50	37.1	43.0	40.0	43.8	40.9	82
100	78.4	85.9	64.5	85.6	78.1	78
200	181	200	190	184	188.6	94
400	410	409	290	316	352.1	88

nd…not detected

No analytical measurements were conducted for the old test media, except for the 100 µg/L treatment group. Degradation profiling on Day 7 at 100 µg/L showed minimal degradation in the first hour and substantial degradation thereafter. The captan concentration at 10 hours was below the Limit of Quantification (LOQ < 10 µg ai/L) and at 24 hours no captan was detected (< LOD, i.e. 5 µg ai/L).

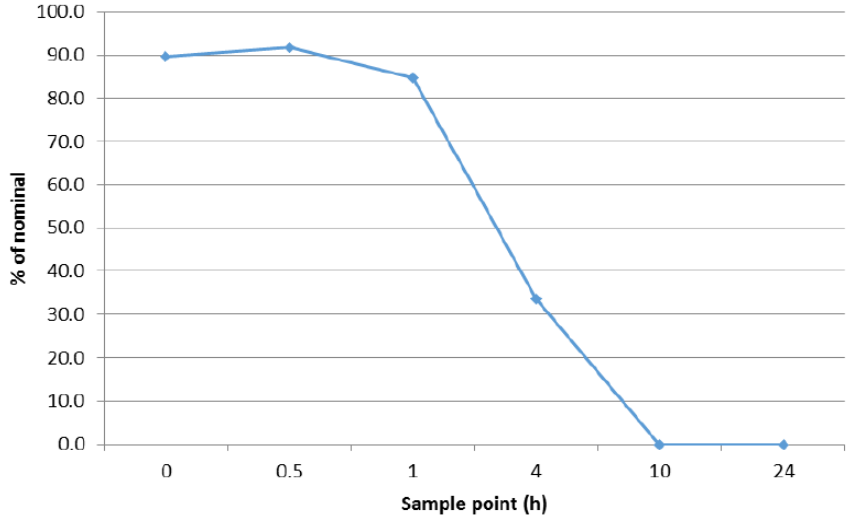

Figure 1: Degradation profile for 100 µg/L treatment group

Parental survival: No mortalities were observed in the control and in the 50 µg ai/L treatment group. In the solvent control and treatment groups 25, 100 and 400 µg/L 10% mortality was observed. In the 200 µg ai/L treatment group mortality of 20% was determined.

Reproduction: The mean number of offspring produced in the control and solvent control were not significantly different; therefore, the treatments were compared with the pooled control.

There were no significant effects on the mean number of neonates per female at the end of the test (day 28). In the highest test concentrations, an increase of reproduction compared to the control was observed.

In the control, solvent control and test concentrations surviving parental daphnids, released the first offspring by day 8.

Table B. 9.2.5-4: Effects on parental survival and reproduction

Captan [µg ai/L] (nominal)	Parental survival [%]	Mean neonate production/female at Day 28 (SD)	Compared to control [%]
Control	100	135.2 (9.0)	-
Solvent control	90	133.2 (8.4)	-
25	90	127.8 (7.6)	4.1
50	100	135.4 (10.0)	-1.7
100	90	135.9 (9.3)	-2.0
200	80	136.4 (9.8)	-2.4
400	90	142.6 (10.1)	-7.0

Parental body length: The mean length of Daphnids in the control and solvent control were not significantly different. No statistically significant effects on the parental body length were observed. In the highest test concentrations, an increase of body length compared to the control was shown.

Table B. 9.2.5-5: Effects on parental body length

Captan [µg ai/L] (nominal)	Mean adult body length at Day 28 [mm]	Compared to control [%]
Control	3.96	-
Solvent control	4.04	-
25	4.01	0.6
50	3.99	1.0
100	4.13	-2.3
200	4.15	-2.8
400	4.09 (4.14*)	-1.3 (-2.6*)

* Values in parentheses are exclusive of the outlier in vessel 89

Conclusion: The NOEC for survival, reproduction and body length was determined to be the highest tested concentration, 400 µg ai/L. The toxicity endpoint is based on nominal concentrations.

Comment RMS:

The applicant modified the study design to represent an environmentally-realistic "worst-case" pulsed exposure pattern as proposed in the EFSA guidance document (EFSA Journal 2013;11(7):3290)[1]. Each group of daphnids received four pulses of exposure, with 7 days between each pulse and 7 days of assessment after the 4th pulse. The study duration was 28 days, 7 days longer than specified in OECD test guideline 211. This modification was made to provide the maximum number of pulses, whilst respecting the practical-constraint of reproductive life-span.

Validity: The study was conducted according to the OECD test guideline 211 (2012).

- the mortality of the parent animals (female Daphnia) does not exceed 20% at the end of the test;
- the mean number of living offspring produced per parent animal surviving at the end of the test is > 60.

Taking into account the recommendations of the test guideline according to OECD 211 (2012), the chronic daphnid study is considered acceptable.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The study plan states that the total organic carbon (TOC) of a representative sample of the Elendt M4 dilution medium, measured once at the start of the study, should be < 2 mg/L. In this case, determination of TOC was carried out on the dilution medium used at the final media-exchange in the study. TOC was determined to be 7

[1] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 186 pp. doi:10.2903/j.efsa.2013.3290

mg/L (i.e. > 2 mg/L). The determination of TOC was carried out on bulk medium which was used in equal proportions for all treatment and control groups. Hence, the TOC being > 2 mg/L cannot have caused any experimental bias. Also, all validity criteria were met. This deviation is not considered to have affected the the outcome of the study.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ of 10 µg ai/L.

Endpoints: The applicant determined a NOEC of 400 µg ai/L (4 peaks. 7 day interval) based on nominal concentration. No statistically significant effects were observed on the survival, the reproduction and the growth.

A check of the statistical power of the test was not performed as the study design was in line with the cited guideline.

According to the test guidelines, ECx values should be determined for lethal and sub-lethal effects. In the study the percent reduction parental survival, length and growth as well as number of neonates for all test substance treatments was < 10% as compared to the control; therefore, an EC_{10} and EC_{20} was not calculated.

Conclusion of the RMS: Based on the evaluation of the study, the pulsed exposure chronic daphnid toxicity test is considered valid.

B.9.2.5.1. Reproductive and development toxicity to an additional aquatic invertebrate species

No chronic studies with an additional aquatic invertebrate species are required since captan is not an insecticide/insect growth regulator and does not show an insecticidal mode of action.

B.9.2.5.2. Development and emergence in Chironomus species

No chronic study on the development and emergence in Chironomus species was provided during the first EU approval of the active substance, since captan is not an insect growth regulator. The active substance captan does not interfere with insect moulting hormones nor has other effects on insect growth and development.

B.9.2.5.3. Sediment dwelling organisms

No studies to determine the effects of captan or the metabolite THPI and THPAM on sediment dwelling organisms have been conducted, since these compounds are not expected to persist in surface waters or partition to sediments. The hydrolytic DT_{50} of the active substance captan is smaller than 3.84 hours; the whole system DT_{90} is smaller than 24 hours in a water-sediment study. The metabolite THPAM is only found in the water phase, and the metabolite THPI which is found in the sediment has a DT_{50} of approximately 5 days in sediment. In addition, the active substance captan and its metabolites THPI and THPAM are not considered toxic to daphnids.

Concerning the metabolite THPAI (tetrahydrophthalic acid), this compound was only found in the sediment reaching a maximum level of 11.3% AR. Its DT_{50} in this compartment was estimated to be 17.8 days.

According to the data requirement (Reg. No. 283/2013) the chronic risk to sediment dwelling organisms as *Chironomus riparius* or *Lumbriculus spp*. should be determined. In order to assess the potential risk of THPAI to sediment-dwelling invertebrates a toxicity test with the metabolite on *Lumbriculus spp*. is submitted.
The PPR recommends *Lumbriculus spp*. for active substances with fungicidal activity.

Reference:	**THPAI: Sediment-water Lumbriculus toxicity test using spiked sediment under laboratory conditions**
Author(s), year:	2015
Report/Doc. number:	S15-00594, R-36061
Guideline(s):	OECD 225 (2007)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:	
Test substance:	THPAI (tetrahydrophthalic acid), batch no.: 516-143-00, purity: 96.8% w/w, analysed
Test species:	*Lumbriculus variegatus* (MÜLLER), Oligochaeta: Lumbriculidae
Number of organisms:	6 control replicates and 4 replicates per test concentration, 10 organisms per replicate.
Age:	Adult worms, 11 days before test start (i.e. application) worms were synchronised by artificial fragmentation
Type of test, duration:	Static test, 28 days
Applied concentrations:	
Nominal:	0 (control), 62.5, 125, 250, 500 and 1000 mg/kg dw
Measured (mean):	0 (control), 45.4, 92, 179, 378 and 726 mg/kg dw
Solvent:	None
Test substrate:	
Aqueous medium:	Reconstituted test water according OECD 203. The ratio of Ca:Mg and Na:K was 4:1 and 10:1, respectively, based on molarity. The pH of the aerated water was within a range of 6-9. The total hardness was between 90-300 mg/L (as $CaCO_3$), corresponding to 5-17°dH.
Artificial sediment:	4 - 5% (dry weight) sphagnum peat 20% ± 1% (dry weight) kaolin clay (kaolinite content ≥ 30%) 75 - 76% (dry weight) quartz sand (grain size ≤ 2 mm, but > 50% of particles in the range of 50 - 200 µm) Calcium carbonate of chemically pure quality is added to adjust the pH of the final mixture of the sediment

	0.4-0.5% powdered leaves of Stinging Nettle (*Urtica sp.*) as food source The TOC content of the final mixture was 3.3%.
Test conditions:	
Temperature:	19.2 - 19.8 °C
pH:	7.61 - 8.31
O_2 content:	7.6 - 9.3 mg/L (84-102% air saturation)
Ammonium:	0.0 5.0 mg/L
Light regime:	16 hours light / 8 hours darkness (approximately 320 lux)
Feeding:	None
Test parameters:	250 mL glass vessel, filled with 50 g wet sediment and 150 mL test medium. The ratio of the depth of the sediment layer of the depth of the overlying water was about 1:4 (1.5 cm sediment and 6 cm test medium).
Observations:	During the exposure, test vessels were observed in order to assess visually any behavioural differences in worms (e.g. sediment avoidance, faecal pellets visible on the sediment surface) compared with controls. At the end of the test, the total number of living and dead individuals per replicate was assessed. Likewise, dry weight of worms per replicate was recorded. Alive worms were also assigned to one of the three groups: a) large complete worms (adults) without regenerated body regions b) complete worms with regenerated, lighter-coloured body regions (i.e., with new posterior part, with new anterior part, or with both new posterior and anterior parts) c) incomplete worms (i.e., recently fragmented worms with non-regenerated body regions) The oxygen concentration (mg/L), water temperature and pH were recorded in one representative test vessel per test concentration and control on test start and on day 7, 14, 21 and at the end of the test. Total ammonium was measured three times a week for the duration of the test in a representative test vessel per concentration and control. Hardness was measured at the beginning and at the end of the test in the control and highest test item concentration level. Light intensity was measured once at the level of the water surface at test start.
Analytical measurements:	Additional test vessels for analytical sampling on the scheduled sampling date were prepared: one replicate per concentration for the sampling dates -2 d (day of application, only sediment before adding the overlying water), 0 d (introduction of organisms and 28 d (test end) after introduction of the animals. The content of THPAI in the treatment solution samples was determined by analysing with HPLC-MS/MS.
Statistics:	For the calculation of NOEC and LOEC a multiple comparison method was used. A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic, a test for homogeneity of the data was performed according to Levene. The

NOEC and LOEC were determined using a multiple comparison method. For total number of alive worms and dry weight Dunnett's t-test was used. The calculation of EC_{10}, EC_{20} and EC_{50} for total number of worms and dry weight was done with probit analysis following Gompertz procedure. SAS® Proprietary Software 9.3 was used.

Findings:

Analytical measurements: The validity criteria for untreated control were fulfilled. The measured content of THPAI of all concentration levels in the test vessels was between 64 and 74% at the day of application (t = -2 d) and between 74 and 78 % at start of exposure (t = 0 d). At the end of the test (t = 28 d) measured concentrations ranged from below the detection limit and 68% of nominal. Since the measured contents of THPAI at start of exposure (t = 0 d) were below 80% of nominal values the toxicological endpoints were evaluated using initially measured concentrations at start of exposure phase.

Table B. 9.2.5-6: Concentration of THPAI in the overlaying water and sediment

Time [d]	THPAI nominal [1]	THPAI nominal per vessel [2]	THPAI found				THPAI actual [mg/kg dw]
			Overlaying water [mg/L]	Sediment [mg/kg dw]	mg per vessel (Sediment + water)	% of nominal	
- 2	0	0	-	0	n.d.	n.a.	-
	60.5	2.24	-	40.1	1.45	65	
	121	4.48	-	81.9	2.87	64	
	242	8.97	-	174	6.42	72	
	484	17.94	-	376	13.19	74	
	968	35.87	-	675	23.91	67	
0	0	0	n.d.	n.d.	n.d.	n.a.	-
	60.5	2.24	9.13	9.51	1.67	75	45.4
	121	4.48	17.8	22.1	3.41	76	92.0
	242	8.97	33.2	48.8	6.67	74	179
	484	17.94	74.3	90.1	14.03	78	378
	968	35.87	120	266	27.02	75	726
28	0	0	n.d.	n.d.	n.d.	n.a.	-
	60.5	2.24	n.d.	n.d.	n.d.	n.a.	< LOD
	121	4.48	n.d.	n.d.	n.d.	n.a.	< LOD
	242	8.97	12.0	3.6	1.74	19	46.0
	484	17.94	26.2	8.23	4.13	23	111
	968	35.87	150	73.8	24.26	68	658

LOQ (Limit of Quantification): 0.01 mg THPAI/L in water, 0.823 mg THPAI/kg dw in sediment
n.d…not detectable, n.a…not applicable
[1] Considering purity in test item of 96.8% w/w
[2] Based on 37.06 g dry sediment per test vessel

Survival and growth: No significant effects of THPAI for the total number of surviving worms were determined at 45.4 and 92.0 mg/kg dw (actual). Significant effects for dry weight of surviving worms were determined at 179 and 726 mg/kg dw (actual).

No behavioural differences in worms were observed during the test. The worms were found to burrow into the sediment and no visual faecal pellets were observed.

Table B. 9.2.5-7: Total number of alive worms per test vessel and treatment and inhibition compared to control per treatment (definitive test)

Nominal Test item Conc. [mg/kg dw]	Number of alive worms/vessel						Mean	Inhibition compared to control [%]
	1	2	3	4	5	6		
Control	25	27	25	25	27	25	25.7	0
62.5	23	20	25	27	-	-	23.8	7.4
125	26	21	20	24	-	-	22.8	11.3
250	26	21	20	17	-	-	21.0*	18.3
500	19	13	15	16	-	-	15.8*	38.5
1000	9	10	13	16	-	-	12.0*	53.3

* Significant differences compared to control ($p < 0.05$).

Table B. 9.2.5-8: Dry weight of worms per test vessel and treatment and inhibition compared to control per treatment

Nominal Test item Conc. [mg/kg dw]	Dry weight of worms/vessel [g]						Mean	Inhibition compared to control [%]
	1	2	3	4	5	6		
Control	0.0138	0.0133	0.0117	0.0128	0.0126	0.0124	0.0128	0
62.5	0.0105	0.0109	0.0126	0.0126	-	-	0.0117	8.6
125	0.0126	0.0103	0.0120	0.0120	-	-	0.0117	8.6
250	0.0114	0.0113	0.0124	0.009	-	-	0.0110*	14.1
500	0.0111	0.0113	0.0135	0.0115	-	-	0.0118	7.8
1000	0.0088	0.0101	0.0114	0.0104	-	-	0.0102*	20.3

* Significant differences compared to control ($p < 0.05$).

Table B. 9.2.5-9: Classification of the worms (mean)

Nominal Concentration [mg/kg dw]	Large complete worms (adults)	Complete worms with regenerated body regions	Incomplete worms
Control	3.33	22.17	0.17
62.5	1.5	22.25	0.0
125	0.75 *	22.0	0.0
250	1.25	19.50	0.25
500	4.25	11.50 *	0.0
1000	7.0	5.0 *	0.0

* Significant differences compared to control ($p < 0.05$).

Conclusion: According to the results of the test, the 28-day NOEC for total number of surviving worms and dry weight of worms for the test item was determined to be 92.0 mg/kg dw (actual). The LOEC was determined to be 179 mg/kg dw (actual). The EC_{10} for total number of surviving worms was determined to be 74.4 mg/kg dw (actual). The EC_{20} for total number of surviving worms was determined to be 175 mg/kg dw (actual).

The EC_{50} for total number of surviving worms was determined to be 633 mg/kg dw (actual). For dry weight of worms the EC_{50} was determined to be > 726 mg/kg dw (actual).

Table B. 9.2.5-10: Overall NOEC, LOEC and EC_{50} actual concentrations

THPAI actual*					
	NOEC [mg/kg dw]	**LOEC [mg/kg dw]**	**EC_{10} [mg/kg dw]**	**EC_{20} [mg/kg dw]**	**EC_{50} [mg/kg dw]**
Total number of alive worms	92.0	179	74.4	175	633
95 % confidence limits	na	na	43.6 - 105	128 - 219	508 - 850
Dry weight of worms	92.0	179	111 [a]	> 726 [b]	> 726
95 % confidence limits			0.0313 - 266		

* Based on initially measured content of THPAI at start of exposure phase.
[a] No clear dose-response observed.
[b] 20% inhibition was observed at the highest test item concentration level.
dw…dry weight, na…not applicable

Comment RMS:

Validity: The study was conducted according to the currently valid test guideline, OECD test guideline 225 (2007).

- The average number of living worms per replicate in the controls should have increased by a factor of at least 1.8 at the end of exposure compared to the number of worms per replicate at the start of exposure.
- The pH of the overlying water should be between 6 and 9 throughout the test.
- The oxygen concentration in the overlying water should not be below 30% of air saturation value (ASV) at test temperature during the test.

The pH of the overlying water was in a range of 7.61 and 8.31 (mean: 8.16 ± 0.17). The oxygen concentrations in the overlying water was between 7.6 and 9.3 mg/L (mean: 8.7 ± 0.4 mg/L), corresponding to > 80% air saturation.

At the end of the study (28 days) the mean total number of alive worms in the control was 25.7 (range: 25 - 27 alive worms). Taking into account the number of worms at test start (10 individuals) the number of alive worms in the control group increased by a factor of 2.5 - 2.7.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The TOC content of the artificial sediment already containing powdered leaves of Stinging Nettle was analysed instead of the final mixture without the food source. Therefore the TOC content of the mixture without the food source is missing. The TOC of the final sediment already containing powdered leaves of Stinging Nettle was determined to be 3.3 %. There is no impact on the results of the study. TOC contents around 2 % were determined during analysis of artificial sediment charges with nearly the same sediment composition used for other tests.

In conclusion, the study is considered valid taking into account that all validity criteria were met.

The calculated endpoints are based on initially measured concentrations taking into account the recommendations given in the OECD guideline 225. However, according to PRAS 133 endpoints shall be based on mean measured concentrations if the concentrations cannot be maintained throughout the study duration (± 80% of nominal concentrations).

Taking account the mass balance (considering the amount of sediment of 50 g and the volume of water of 150 mL) it was shown that the concentrations of the metabolite THPAI were not maintained. The metabolite THPAI partition to the water up to 80% in the first hour. At the end of the test the concentrations were below the LOQ in the two lowers test concentrations. In the other test concentrations, THPAI was found in higher concentrations in the overlying water.

Mass balance:

Nominal [mg/L]	0 d			28 d		
	Overlying water [%]	Sediment [%]	Total	Overlying water [%]	Sediment [%]	Total
Control	-	-	-	-	-	-
62.5	43.8	45.7	89.5	0.05 [a]	3.95 [a]	4.00
125	42.7	53.0	95.8	0.02 [a]	1.98 [a]	2.00
250	39.8	58.6	98.4	14.4	4.32	18.7
500	44.6	54.1	98.6	15.7	4.94	20.7
1000	36.0	79.8	115.8	45.0	22.1	67.1

[a] For calculation of the mass balance a concentration of 0.01 mg/Lin water (LOQ) and 0.823 mg/kg in sediment (LOQ) was considered.

In conclusion, the RMS is of the opinion that the endpoint shall be expressed in terms of mean measured concentrations. However, considering that the measured concentrations were below the LOQ at the two lowest concentrations no mean measured concentrations could be calculated. Hence, the RMS considers a concentration of LOQ/2 for the statistical evaluation of the study results.

The results of the statistical re-evaluation based on mean measured concentrations are the following:

28 d NOEC = 3.02 mg/kg (number of worms and weight), Williams Multiple Sequential t-test

No reliable EC_{10} value for number of worms and worm weight could be determined because of the high normalised width of the confidence intervals (number of worms: EC_{10} = 2.67 mg/kg, 95% C.I. 0.677-10.558 mg/kg, NW = 3.67; weight of worms: EC_{10} = 6.05 mg/kg, 95% C.I. 0.038-962.543 mg/kg).

The 28 d NOEC was also expressed in terms of mg/L taking into account the partition to the overlying water.

28 d NOEC = 0.30 mg/L.

However, the reliability of the endpoints is questionable considering that the NOEC is based on concentrations below the LOQ. A comparison of the exposure profile of the metabolite THPAI as recommended in the PRAS 133 meeting was not provided by the applicant.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ for sediment (0.8 mg/kg) and water (10 µg/L).

Endpoints: No reliable endpoint could be determined based on the results of the analytical measurements. At the test concentrations relevant for the NOEC determination (nominal test concentration 125 mg/kg) the measured concentrations were below the LOQ.
Conclusion of the RMS: Based on the evaluation of the study the chronic *Lumbriculus* test is considered valid; however, the reliability of the endpoint is questionable.

B.9.2.6. Effects on algal growth

Effects of captan and its major metabolites (THPI and THPAM) on algae growth were investigated in three studies. The studies have already been submitted for the first EU peer-review of the active substance captan.
In addition, new algae studies (Scheerbaum, 2016) according to the currently valid test guideline were conducted with the active substance captan and the two metabolites THPI and THPAM for confirmation of the old endpoints.

All algae toxicity test were conducted under static conditions as recommended in the OECD test guideline 201. Due to the rapid hydrolyses of captan the measured concentrations were below the LOD at the end of the test.
According to the OECD test guideline 201 the endpoint shall be expressed in terms of mean measured concentrations if the test substance has not been satisfactorily maintained within ± 20% of the nominal or initial measured concentrations. In addition, according to the EFSA Technical Report (2015), the validity of studies should be considered questionable if the measured concentrations of all test concentrations are below the LOD at the end of the test. In conclusion, the RMS considered the studies as valid as all validity criteria were met. However, the reliability of the endpoints based on nominal concentrations is questionable. Nevertheless, the RMS proposed a tier 1 risk assessment based on endpoints expressed in terms of mean measured concentrations. Even though, this approach is not proposed in the EFSA Technical Report (2015) the RMS is of the opinion that this approach is valid taking into account that algae toxicity tests are generally conducted under static conditions. In addition, a higher tier risk assessment taking into account the realistic exposure profiles might be an additional approach addressing the risk to algae. For further information considering the aquatic risk assessment, refer to Volume 3, B.9 (PPP).

Active substance:

Reference:	**Captan: Toxicity to the green alga *Selenastrum capricornutum***
Author(s), year:	1991
Report/Doc. number:	BL3954/B, TMN-016
Guideline(s):	OECD test guideline 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Captan, batch no. RS309/H, purity: 90% w/w
Test species:	Green alga (*Raphidocelis subcapitata*, strain ATCC 22662, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata)*
Number of organisms:	1.07 x 10^4 cells/mL (control); 3 replicates per treatment and 6 replicates per control group
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control), 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mg ai/L
Solvent:	None
Test conditions:	
Water quality:	Algal nutrient medium (AAP medium, US EPA)
Temperature:	24 ± 1 °C
pH:	7.3-7.4 (0 h), 7.2-10.4 (96 h)
Incubation:	Continuous illumination at ~ 7730 lux, orbital shaking at 100 rpm
Analytical measurements:	For chemical analysis (High Performance Liquid Chromatography) of test the substance, samples of test solution were taken at test initiation. No chemical analyses were performed at the end of the test due to the rapid hydrolysis of captan technical in aqueous conditions.
Test parameters:	Test vessels (250 mL glass conical flasks) contained 100 mL test solution. Samples were taken at 24, 48, 72 and 96 hours to determine the cell densities by electronic particle counting using a Coulter counter model ZB. Measurements of pH and temperature were made at initiation and at termination.
Findings:	
Analytical data:	The measured concentrations at the start of the test ranged from 86-104% of the nominal values. No analytical measurements were conducted at the end of the test (96 h).

Table B. 9.2.6-1: Concentration of captan technical at test start

Nominal concentrations of captan [mg/L]	Measured concentration at test start [mg/L]	% of nominal
Control	< 0.0079	-
0.2	0.2	100
0.4	0.38 *	95
0.8	0.83	104
1.6	1.5	94
3.2	2.9	91
6.4	5.8	91
12.8	11.0	86

* Mean of triplicate analyses

Biological results:

Table B. 9.2.6-2: Areas under the curve and growth rates

Nominal concentrations of captan [mg/L]	Biomass		Growth rate	
	Mean area under growth curve (0-96 h)	% of control	Mean growth rate (0-96 h)	% of control
Control	287.2	100	1.431	100
0.2	273.1	95	1.419	99
0.4	252.5*	88	1.400	98
0.8	241.5*	84	1.395	97
1.6	157.9*	55	1.308*	91
3.2	75.7*	26	1.142*	80
6.4	25.8*	9	0.847*	59
12.8	7.7*	3	0.576*	40

* Significant difference compared to the control (P = 0.05)

Conclusion:

96 h E_rC_{50} = 11 mg ai/L (95% C.I. 6.4-18 mg ai/L)

NOEC = 0.8 mg ai/L (growth rate)

96 h E_bC_{50} = 1.6 mg ai/L (95% C.I. 1.3-2.0 mg ai/L)

NOEC = 0.2 mg ai/L (biomass)

based on nominal concentrations

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 201 (2011).

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the growth rate was 1.43 per day, which corresponds to an increase of biomass by a factor of 94.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%. In the study the mean coefficient of variation for section-by-section specific growth rates was 16.8%.

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%. In the study the coefficient of variation of average specific growth rates was 1.9, which is below the required 7% for *Raphidocelis subcapitata*.

Taking into account the recommendations of the currently valid test guideline according to OECD (2011) the algae study is considered acceptable.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The concentration of the test substance was measured in the test media at test start only. Hence, an exposure over the whole study period (96 hours) could not be verified. According to the OECD test guideline for unstable test substance, sampling for analysis should be conducted at 24 hour intervals during the exposure period.

Acceptability of the analytical methods used in the test: The analytical verification of the test item was conducted using HPLC/UV. No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: Based on the results of the analytical measurements the determination of a reliable endpoint is questionable.

Conclusion of the RMS: Based on the evaluation of the study the algae toxicity test is considered valid. However, the reliability of the endpoint is low considering that the concentration of captan was measured only at the start of test.

Reference:	**Captan technical: Algal, Growth inhibition test with *Pseudokirchneriella subcapitata*, 72 hours including recovery phase**
Author(s), year:	2016c
Report/Doc. number:	150423AF / SPO16612
Guideline(s):	OECD test guideline 201 (2011), Council Regulation (EC) No. 761/2009, Method C.3 (2009)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Captan, CAS-No.: 133-06-2, batch no. 95138738, purity: 95.8%
Test species:	Green alga (*Raphidocelis subcapitata*, formerly known as *Pseudokirchneriella subcapitata* HINDÁK, SAG 61.81)
Number of organisms:	6153 cells/mL (initial cell density); 3 replicates per treatment and 6 replicates per control group
Type of test, duration:	Static test, 72 hours

Applied concentrations:

Nominal: 0 (control), 0.75, 1.5, 3.0, 6.0 and 12 mg ai/L

Mean measured: 0 (control), 0.217, 0.381, 0.458, 0.614 and 1.21 mg ai/L

Solvent: None

Toxic reference: Potassium dichromate

Test conditions:

Water quality: Algal nutrient medium (AAP medium)

Temperature: 21.5 - 22.5°C (mean: 22.0°C)

pH: 7.48 - 7.58 (0 h), 7.56 - 8.31 (72 h)

Incubation: Continuous illumination at 60.8 - 77.9 µE/m²/s (mean: 71.3 µE/m²/s), orbital shaking at 70 rpm

Analytical measurements: For chemical analysis (HPLC-DAD) of test the substance, samples of test solution were taken at the start (0 h) and the end (72 h) of the test.

Test parameters: Test vessels (250 mL glass conical flasks) contained 100 mL test solution.

After 72 hours algae were transferred from the three highest concentrations (3.0, 6.0 and 12.0 mg ai/L) and the control into fresh untreated medium. Algae suspension from the test concentrations and the control replicates were filled up with dilution water to 100 mL and allowed to grow for further 3 days to determine whether the effect of the test item was reversible.

Samples were taken at 24, 48 and 72 hours to determine the cell densities. The cell density was measured daily via Chlorophyll-a-fluorescence, excitation at 436 nm, emission at 685 nm.

Measurement of pH was made at initiation and at termination. The temperature was measured continuously. Light intensity was measured prior to the start of exposure.

Statistics: The EC_x values with confidence intervals of growth rate and yield inhibition after 72 h were calculated by sigmoidal dose-response regression.

The NOEC and LOEC were determined by calculation of statistically significant differences of growth rates and yield.

Findings:

Analytical data: The measured concentrations at the start of the test ranged from 40% to 65% of the nominal values. At the end of the test (72 h) the measured concentrations were below of the LOQ (0.3 mg ai/L), except for the highest test concentration. In the 12 mg ai/L treatment group the measured concentration was 2.6% of the nominal test concentration.

Table B. 9.2.6-3: Analytical measurements for the test media at 0 h and 72 h

Nominal concentrations [mg test item/L]	Measured concentrations Captan [mg/L]		Measured concentrations THPI [mg/L]		Geometric mean measured [mg ai/L] [a]
	0 h	72 h	0 h	72 h	
Control	< LOQ	< LOQ	< LOQ	< LOQ	-

Nominal concentrations [mg test item/L]	Measured concentrations Captan [mg/L]		Measured concentrations THPI [mg/L]		Geometric mean measured [mg ai/L] [a]
	0 h	72 h	0 h	72 h	
0.75	0.313	< LOQ	< LOQ	< LOQ	0.217
1.5	0.970	< LOQ	< LOQ	0.283	0.381
3.0	1.40	< LOQ	< LOQ	0.546	0.458
6.0	2.51	< LOQ	< LOQ	1.15	0.614
12.0	4.75	0.310	< LOQ	2.26	1.21

LOQ…Limit of quantification of the analytical method (0.3 mg/L of the test item)
[a] Values < LOQ were taking with ½ LOQ into account

Biological results: Microscopic evaluation of the cells at the start and end of exposure showed no morphological abnormalities.

Table B. 9.2.6-4: Yield and growth rate

Nominal concentrations of captan [mg/L]	Yield		Growth rate	
	Yield [cells/mL] (0-72 h)	Inhibition of yield [%]	Mean growth rate [d^{-1}] (0-72 h)	Inhibition of growth rate [%]
Control	1600281	-	1.85	-
0.75	1470832	8	1.83	1
1.5	1073720 *	33	1.72 *	7
3.0	671570 *	58	1.56 *	16
6.0	138157 *	91	1.05 *	43
12.0	28638 *	98	0.572 *	69

* Significant difference compared to the control (P = 0.05)

Recovery: Cell growth was shown at all tested concentrations (control, 3.0, 6.0 and 12 mg ai/L). Potential for recovery following exposure up to 12 mg ai/L was shown.

Table B. 9.2.6-5: Recovery cell growth

Nominal concentrations [mg test item/L]	Incubation time [d]	Cell density [cells/mL]		Growth rate after incubation time [d^{-1}]
		Initial	Final	
Control	3 d	8032	788152	1.53
			976657	1.60
3.0		3389	419610	1.61
			401327	1.59
			590020	1.72
6.0		7216	1611791	1.80
			1016422	1.65
			984215	1.64
12.0		1740	402267	1.81
			269176	1.68
			336286	1.75

Conclusion: The endpoints are based on geometric mean measured concentration

72 h E_rC_{50} = 0.660 mg ai/L (95% C.I. = 0.630 - 0.698 mg ai/L)

72 h E_rC_{20} = 0.483 mg ai/L (95% C.I. = 0.462 - 0.503 mg ai/L)

72 h E_rC_{10} = 0.412 mg ai/L (95% C.I. = 0.386 - 0.439 mg ai/L)

NOE_rC = 0.217 mg ai/L

72 h E_yC_{50} = 0.432 mg ai/L (95% C.I. = 0.412 - 0.453 mg ai/L)

72 h E_yC_{20} = 0.336 mg ai/L (95% C.I. = 0.290 - 0.368 mg ai/L)

72 h E_yC_{10} = 0.260 mg ai/L (95% C.I. = < 0.217 - 0.326 mg ai/L)

NOE_yC = 0.217 mg ai/L

Comment RMS:

Validity: The study was assessed according to the OECD test guideline 201 (2011) and the EC guideline C3 (2009).

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the growth rate was 1.854 per day, which corresponds to an increase of biomass by a factor of 261.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%. In the study the mean coefficient of variation for section-by-section specific growth rates was 16.4%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. In the study the coefficient of variation of average specific growth rates was 1.4%, which is below the required 7% for *Raphidocelis subcapitata*.

Taking into account the recommendations of the currently valid test guideline according to OECD (2011) the algae study is considered acceptable.

In addition to the validity criteria, the following points deviated from the guideline or were not reported in detail:

- The concentration of the test substance was measured in the test media at the start and the end of the test. According to the OECD test guideline for unstable test substance, sampling for analysis should be conducted at 24 hour intervals during the exposure period.

However, after 72 hours the measured concentrations were below the LOQ for all treatment groups except for the highest test concentrations. The study author calculated geometric mean concentrations using LOQ/2 as recommended by the OECD document on testing difficult substances (OECD 23).

However according to the EFSA technical guidance document (EFSA Supporting publication 2015:EN-924) this approach is not acceptable. In the EFSA technical guidance document it is stated that*:"…When only initial and final measurements are available and no concentrations were detected at study end, the use of the LOD or half of LOQ is not supported. This is because it is not known when the concentrations decreased to practically zero (< LOD)."*

In addition, according to the test guideline (OECD) there must be evidence that the concentration of the substance has been maintained, and preferably, it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see B.5 (AS), B.5.1.2.4) the analytical method is considered acceptable covering the LOQ of 0.3 mg ai/L.

For the metabolite THPI, no full validation is available, only linearity was determined and no further validation was given.

Endpoints: Based on the results of the analytical measurements the determination of a reliable endpoint is questionable. However, taking into account that the algae toxicity test has to be conducted under static conditions the RMS agrees on the proposed endpoint expressed in terms of mean measured concentrations.

Conclusion of the RMS: Based on the evaluation of the study the algae toxicity test is considered valid.

Metabolites:

Reference:	**THPI: Toxicity to the green alga *Selenastrum capricornutum***
Author(s), year:	1994
Report/Doc. number:	BL5238/B, TMN-018
Guideline(s):	OECD test guideline 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:	
Test substance:	THPI (tetrahydrophthalimide), CAS no. 85-40-5, purity: 96%
Test species:	Green alga (*Raphidocelis subcapitata*, strain ATCC 22662, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata)*
Number of organisms:	1.01 x 10^4 cells/mL (control); 3 replicates per treatment and 6 replicates per control group
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (control), 18, 32, 56, 100 and 180 mg/L
Solvent:	None
Test conditions:	
Water quality:	Algal nutrient medium (according Miller et al., 1978)
Temperature:	24 ± 1 °C
pH:	6.71 - 7.47 (0 h), 8.57 - 9.79 (72 h)

Incubation: Continuous illumination at ~ 7680 lux, orbital shaking at 160 rpm

Analytical measurements: For chemical analysis (High Performance Liquid Chromatography) of test the substance, samples of test solution were taken at test initiation. At the end of the test each blank solution (without algal inoculum) was analysed.

Test parameters: Test vessels (250 mL glass conical flasks) contained 100 mL test solution.
Samples were taken at 24, 48 and 72 hours to determine the cell densities by electronic particle counting using a Coulter counter model ZB.
Measurements of pH and temperature were made at initiation and at termination.

Findings:

Analytical data: The mean measured concentrations determined ranged from 94 and 110% of the nominal values. On the basis of these determinations nominal THPI concentration were used.

Table B. 9.2.6-6: Measured concentrations of THPI

Nominal concentrations of THPAM [mg/L]	Measured concentration [mg/L]		Mean measured concentration [mg/L]	% of nominal
	0 h	72 h		
Control	< 0.079	< 0.079	< 0.079	-
18	17	17	17	94
32	32*	32*	32	100
56	59	57	58	104
100	110	110	110	110
180	180	180	180	100

* Mean of triplicate analyses

Biological results:

Table B. 9.2.6-7: Areas under the curve and growth rates

Nominal concentrations of THPI [mg/L]	Biomass		Growth rate	
	Mean area under growth curve (0-72 h)	% of control	Mean growth rate (0-72 h)	% of control
Control	174.8	-	1.845	-
18	177.9	102	1.848	100
32	179.4	103	1.850	100
56	182.8	105	1.858	101
100	175.1	100	1.841	100
180	170.7	98	1.833	99

Conclusion: 96 h E_rC_{50} and E_bC_{50} > 180 mg/L
NOEC = 180 mg/L (biomass and growth rate)
based on nominal concentrations

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 201 (2011).

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the growth rate was 1.844 per day, which corresponds to an increase of biomass by a factor of 253.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%. In the study the mean coefficient of variation for section-by-section specific growth rates was 9.4%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%. In the study the coefficient of variation of average specific growth rates was 0.9.

Taking into account the recommendations of the currently valid test guideline according to OECD (2011) the algae study is considered acceptable.

In addition, the following points deviated from the guidelines or were not reported in detail:

- The test media prepared for analyses of exposure concentrations during the test should be treated identically to those used for testing, i.e. they should be inoculated with algae and incubated under identical conditions. In the study, the test media for analytical measurements were incubated under identical conditions. However, they were not inoculated with algae, a blank test media was used for analytical measurements.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: The RMS agrees on the endpoints given in the study report.

96 h E_rC_{50} and E_bC_{50} > 180 mg/L

NOEC = 180 mg/L (biomass and growth rate)

Conclusion of the RMS: Based on the evaluation of the study, the algae toxicity test is considered valid.

Reference:	**1,2,3,6-Tetrahydrophthalamide (THPI): Alga, growth inhibition test with *Pseudokirchneriella subcapitata*, 72 hours**
Author(s), year:	2016a
Report/Doc. number:	150423AF, SPO16614
Guideline(s):	OECD test guideline 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	THPI (1,2,3,6-tetrahydrophthalimide), CAS no. 85-40-5, batch no. 279-071-02, purity: 98.5%

Test species:	Green alga (*Raphidocelis subcapitata*, strain SAG 61.81, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata)*
Number of organisms:	6153 cells/mL (0 h); 6 replicates per treatment and control group
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (control) and 100 mg/L (limit test)
Solvent:	None
Reference:	Potassium dichromate (CAS no. 7778-50-9), tested twice a year as reference item, 72 h E_rC_{50} = 0.564 mg/L (95% C.I. = 0.546 - 0.582 mg/L) 72 h E_yC_{50} = 0.322 mg/L (95% C.I. = 0.239 - 0.324 mg/L)
Test conditions:	
Water quality:	Algal nutrient medium (nutrient medium Z according Lüttge et al., 1994)
Temperature:	22 °C (mean)
pH:	7.47 - 7.53 (0 h), 7.96 - 8.07 (72 h)
Incubation:	Continuous illumination at 60 - 120 µE/m²/s, rotary shaker at approximately 70 rpm
Analytical measurements:	The analytical determination of the test item THPI was carried out via HPLC-DAD from the limit concentration and the control at the start of the exposure (0 hours) and the end of the exposure (72 hours).
Test parameters:	The study was conducted under static conditions with an initial cell density of 6153 cells/mL. Based on a preliminary range finding test a limit concentration of 100 mg test item/L was tested. Six replicates were tested for the limit concentration and the control.
Observations:	Chlorophyll-a-fluorescence: The cell density was measured daily via Chlorophyll-a-fluorescence, excitation at 436 nm, emission at 685 nm. Dilution water was used as background signal. No self-fluorescence was found in the preliminary range finding test at the test concentration of 100 mg/L at the start of the exposure. Microscopic evaluation: Microscopic evaluation of the cells at the start and end of the incubation was carried out. The cells were checked for any unusual cell shapes, colour differences, differences in chloroplast morphology, flocculation, adherence of algae to test containers or aggregation of algae cells. The pH-value at the beginning of the exposure was measured in one additional replicate of the limit concentration level and the control. At the end of the exposure, it was measured from pooled replicates of the limit concentration level and the control, respectively. The room temperature was measured continuously. Light intensity was measured prior to the start of the test.
Statistics:	EC_{10}-, EC_{20}- and EC_{50}-values of the growth rate and yield inhibition after 72 hours were estimated empirically based on the results of the only treatment level (limit test design).

NOEC, LOEC and statistical NOEC/LOEC were determined by calculation of statistical significance of growth rate and yield. As a standard a t-test was used for NOEC/LOEC calculations. When running a t-test a Normality test and an Equal Variance test were done first. The SHAPIRO-WILK-Test was used to test for normally distributed populations. The Levene median test was used for equal variance. P-values for both Normality and Equal Variance tests are 0.05. The α-value (acceptable probability of incorrectly concluding that there is a difference) is α=0.05.

The data for the tables in this report were computer generated and have been rounded for presentation from the full derived data. Consequently, if calculated manually based on the given data minor deviations may occur from these figures.

Findings:

Analytical data: The measured concentration of THPI in the limit concentration at the start of the exposure was 94 % and at the end of the exposure 95% of the of the nominal test item concentration. Therefore, all effect levels are given based on the nominal test item concentration.

Biological results:

Table B. 9.2.6-8: Growth rates and yield

Nominal concentrations of THPI [mg/L]	Replicate	Growth rate [/d]	Growth rate inhibition [%]	Yield [cells/mL]	Yield inhibition [%]
Control	1	1.80	-	1350832	-
	2	1.86		1601524	
	3	1.85		1577722	
	4	1.84		1549968	
	5	1.88		1726995	
	6	1.88		1728940	
	Mean	1.85	-	1589330	-
100	1	1.92	- 4	1939240	- 22
	2	1.92	- 4	1959686	- 23
	3	1.98	- 7	2332055	- 47
	4	1.85	0	1568847	1
	5	1.90	- 2	1810036	- 14
	6	1.90	- 2	1817124	- 14
	Mean	1.91 *	- 3	1904498 *	- 20

* Statistically significant difference of growth rates and yield compared to the control values

Conclusion: 96 h E_rC_{50} and E_bC_{50} > 100 mg/L

NOEC = 100 mg/L (yield and growth rate)

LOEC > 100 mg/L (yield and growth rate)

based on nominal concentrations

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 201 (2011).

The validity criteria according to the OECD guideline 201 are the following:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the growth rate was 1.85 per day, which corresponds to an increase of biomass by a factor of 259.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%. In the study the mean coefficient of variation for section-by-section specific growth rates was 21.8%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%. In the study the coefficient of variation of average specific growth rates was 1.6.

Taking into account the recommendations of the currently valid test guideline according to OECD (2011) the algae study is considered acceptable.

Acceptability of the analytical methods used in the test: See section on physical-chemical properties.

Endpoints: The endpoints are based on nominal concentrations.

E_rC_{50} and E_yC_{50} > 100 mg/L

NOEC = 100 mg/L (growth rate and yield)

Based on nominal concentrations

Conclusion of the RMS: Based on the evaluation of the study the algae toxicity test is considered valid.

Reference:	**THPAM: Toxicity to the green alga *Selenastrum capricornutum***
Author(s), year:	1995
Report/Doc. number:	BL5428/B, R-8747, TMN-017
Guideline(s):	OECD test guideline 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	THPAM (*cis*-tetrahydropthalamic acid), CAS no. 1469-49-4, sample identification WRC# 15184-16-1, purity: 95% w/w
Test species:	Green alga (*Raphidocelis subcapitata*, strain ATCC 22662, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata)*
Number of organisms:	1.03 x 10^4 cells/mL (control); 3 replicates per treatment and 6 replicates per control group

Type of test, duration: Static test, 72 hours

Applied concentrations:

Nominal: 0 (control), 10, 18, 32, 56, 100 and 180 mg/L

Solvent: None

Test conditions:

Water quality: Algal nutrient medium (according Miller et al., 1978)

Temperature: 24 ± 1 °C

pH: 4.3 - 7.4 (0 h), 4.7 - 8.9 (72 h)

A maximum increase of 2.2 pH units was observed over the test duration. This pH shift was considered to be a function of the very high growth factors observed. The pH shift occurred despite a high orbital shaking rate of 160 rpm to improve mass transfer of carbon dioxide into the test solutions.

Incubation: Continuous illumination at ~ 8010 lux, orbital shaking at 160 rpm

Analytical measurements: For chemical analysis (High Performance Liquid Chromatography) of test the substance, samples of test solution were taken at test initiation. At the end of the test each blank solution was analysed.

Test parameters: Test vessels (250 mL glass conical flasks) contained 100 mL test solution.

Samples were taken at 24, 48 and 72 hours to determine the cell densities by electronic particle counting using a Coulter counter model ZB.

Measurements of pH and temperature were made at initiation and at termination.

Findings:

Analytical data: The mean measured concentrations determined ranged from 93 and 120% of the nominal values. On the basis of these determinations nominal THPAM concentration were used.

Table B. 9.2.6-9: Measured concentrations of THPAM

Nominal concentrations of THPAM [mg/L]	Measured concentration [mg/L]		Mean measured concentration [mg/L]	% of nominal
	0 h	72 h		
Control	< 0.11	< 0.11	< 0.11	-
10	12	11	12	120
18	19*	20*	20	111
32	34	36	35	109
56	60	54	57	102
100	100	85	93	93
180	190	140	170	94

* Mean of triplicate analyses

Biological results:

Table B. 9.2.6-10: Areas under the curve and growth rates

Nominal concentrations of captan [mg/L]	Biomass		Growth rate	
	Mean area under growth curve (0-72 h)	% of control	Mean growth rate (0-72 h)	% of control
Control	150.5	-	1.797	-
10	156.9	104	1.818	101
18	145.5	97	1.799	100
32	122.1*	81	1.733	96
56	0.2*	0	0.014*	1
100	-0.1*	0	-0.065*	-4
180	-0.2*	0	-0.105*	-6

* Significant difference from the culture medium control (P = 0.05)

Conclusion: 72 h E_rC_{50} = 41 mg/L (95% C.I. 32 - 53 mg/L)

NOE_rC = 32 mg/L

72 E_bC_{50} = 33 mg/L (95% C.I. 24 - 44 mg/L)

NOE_bC = 18 mg/L

based on nominal concentrations

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guidelines, the OECD test guideline 201 (2011).

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the growth rate was 1.797 per day, which corresponds to an increase of biomass by a factor of 220.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%. In the study the mean coefficient of variation for section-by-section specific growth rates was 10.1%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%. In the study the coefficient of variation of average specific growth rates was 0.7.

Taking into account the recommendations of the currently valid test guideline according to OECD (2011) the algae study is considered acceptable.

In addition, the following points deviated from the guidelines or were not reported in detail:

- The test media prepared for analyses of exposure concentrations during the test should be treated identically to those used for testing, i.e. they should be inoculated with algae and incubated under identical conditions. In the study, the test media for analytical measurements were incubated under identical conditions. However, they were not inoculated with algae, a blank test media was used for analytical measurements.

- A maximum increase of 2.2 pH units was observed over the test duration. This pH shift was considered to be a function of the very high growth factors observed. The pH shift occurred despite a high orbital shaking rate of 160 rpm to improve mass transfer of carbon dioxide into the test solutions.

The applicant provided a statement addressing the shift of the pH in the study. The detailed pH data from the study report are presented in the table below.

THPAM (nominal) [mg/L]	pH		
	Day 0	Day 3	
		Replicate A	Replicate B
Control	7.4	8.7	8.9
10	6.7	8.5	8.9
18	6.4	8.4	8.6
32	5.7	7.5	7.7
56	5.0	5.3	5.3
100	4.6	4.9	4.9
180	4.3	4.7	4.7

The pH of this medium is 7.5. As seen in the table above this pH was achieved on day 0 in the control. In addition, the increase of pH in controls was not more than 1.5 units during the 72 hours of the study. The pH deviations were observed only in treatments with THPAM. There is a positive correlation of a pH decrease with increasing concentrations of THPAM. The substance made the solutions more acidic.

At the three highest concentrations, the algae did not grow at all, possibly due to the very low initial pH values (all ≤5), and thus the pH did not increase with time in these solutions. In the other three treatments, the pH increased by ~ 2 units. The solutions on day 0 had pH values lower than those in controls, but due to the growth of algae they reached after 72 hours pH levels similar to those in the control treatments at the end of the study. The fact that the presence of the test-item had an effect on pH in the initial solutions may have had an impact on the growth of algae in treatments. But as the controls were in line with the pH requirements for this test, then any toxic effect of these lower pH values can be attributed directly to the test-item without any concerns.

Acceptability of the analytical methods used in the test: No evaluation of the analytical methods according to the Guidance Document SANCO/3029/99 rev. 4 was possible due to lack of information.

Endpoints: The RMS agrees on the EC_{50} values for growth rate and biomass given in the study report.

72 h E_rC_{50} = 41 mg/L

72 h E_bC_{50} = 33 mg/L

based on nominal concentrations

However, the RMS does not agree on the NOEC values proposed in the study report. Taking into account the statistical significant effects observed on biomass and growth rate the RMS proposed the following NOEC values.

72 h NOE_bC = 18 mg/L

72 h NOE_rC = 32 mg /L

based on nominal concentrations

Conclusion of the RMS: Based on the evaluation of the study, the algae toxicity test is considered acceptable.

Reference:	**1.2.3.6-Tetrahydrophthalamic acid (THPAM) Alga, Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 hours**
Author(s), year:	2016b
Report/Doc. number:	150423AF, SPO16615
Guideline(s):	OECD test guideline 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	THPAM (1,2,3,6-tetrahydrophthalamic acid), CAS no. 2028-12-8, Batch no. 516-156-00, purity: 98.1%
Test species:	Green alga (*Raphidocelis subcapitata*, strain SAG 61.81, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata)*
Number of organisms:	6153 cells/mL (0 h); 3 replicated per treatment group and 6 replicates per control group
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (control), 10, 25, 62.5, 156 and 390 mg/L
Solvent:	None
Reference:	Potassium dichromate (CAS no. 7778-50-9), tested twice a year as reference item, 72 h E_rC_{50} = 0.564 mg/L (95% C.I. = 0.546 - 0.582 mg/L) 72 h E_yC_{50} = 0.322 mg/L (95% C.I. = 0.239 - 0.324 mg/L)
Test conditions:	
Water quality:	Algal nutrient medium (nutrient medium Z according Lüttge et al., 1994)
Temperature:	22 °C (mean)
pH:	4.62 - 8.21 (0 h), 4.84 - 8.92 (72 h)
Incubation:	Continuous illumination at 60 - 120 µE/m²/s, rotary shaker at approximately 70 rpm
Analytical measurements:	All concentration levels, the pH control and the control were analytically verified via HPLC-DAD after 0 hours (start of exposure) and 72 hours (end of exposure). Separate replicates for the test item analysis at the beginning of exposure were prepared with algae. For the test item analysis after 72 hours, samples were taken out of the test replicates.
Test parameters:	The study was conducted under static conditions with an initial cell density of 6153 cells/mL. A stock solution of 390 mg/L was freshly prepared with dilution water. Five concentrations were tested in a geometrical series with a dilution factor of 2.5 (nominal): 10.0 - 25.0 - 62.5 - 156 - 390 mg/L, corresponding to the geometric mean

measured test item concentration: 11.3 - 26.6 - 63.7 - 149 - 334 mg/L. Additionally, three replicates of the highest test concentration (nominal: 390 mg/L, corresponding to the geometric mean measured test item concentration: 398 mg/L) were tested with a pH value adjusted to 8.21. Three replicates were tested for each test item concentration and six replicates for the control.

Observations: Chlorophyll-a-fluorescence: The cell density was measured daily via Chlorophyll-a-fluorescence, excitation at 436 nm, emission at 685 nm. Dilution water was used as a background signal. No self-fluorescence was found in the definitive test.

Microscopic evaluation: Microscopic evaluation of the cells at the start and the end of exposure was carried out. The cells were checked for unusual cell shapes, colour differences, differences in chloroplast morphology, flocculation, adherence of algae to test containers and agglutination of algae cells.

The pH-value at the start of exposure was measured in one additional replicate of each test item concentration and the control. At the end of exposure, the pH-value was measured from pooled samples of each test item concentration and the control after measurement of the cell density. The room temperature was measured continuously. Light intensity was measured prior to the start of exposure.

Statistics: EC_{10}-, EC_{20}- and EC_{50}-values with confidence intervals of growth rate and yield inhibition after 72 hours were calculated by sigmoidal dose-response regression.

The NOEC and LOEC were determined by calculation of statistically significant differences of growth rates and yield.

As a standard, One Way Analysis of Variance (ANOVA) and DUNNETT's test were used for NOEC/LOEC calculations. When running a One Way Analysis of Variance, a Normality test and an Equal Variance test were done first. P-values for both Normality and Equal Variance tests are 0.05. The α-value (acceptable probability of incorrectly concluding that there is a difference) is $\alpha = 0.05$.

The data for the tables in this report were computer-generated and have been rounded for presentation from the full derived data. Consequently, if calculated manually based on the given data minor deviations may occur from these figures.

Findings:

Analytical data: The measured concentrations of 1,2,3,6-Tetrahydrophthalamic acid (THPAM) in the fresh media (0 h) were in the range of 101 to 113% of the nominal values at all tested concentration levels. The measured concentrations in the old media (72 h) were in the range of 71 to 114% of the nominal values of all concentrations.

Therefore, all effect values given are based on the geometric mean measured of THPAM.

Table B. 9.2.6-11: Measured concentrations of THPAM

Nominal concentrations of THPAM [mg/L]	0 h		72 h		Mean measured concentration [mg/L]
	Measured concentration [mg/L]	% of nominal	Measured concentration [mg/L]	% of nominal	
Control	< LOQ		< LOQ		-
10	11.3	113	11.4	114	11.3
25	26.7	107	26.5	106	26.6
62.5	63.9	102	63.6	102	63.7
156	158	101	140	90	149
390	405	104	276 [1]	71	334
390 (pH adjusted)	408	105	389	100	398

LOQ…Limit of quantification (10 m/L test item)
[1] Reanalysed, mean value of 3 replicates and two injections each

Biological results: The two highest concentrations caused a change of the pH value to be below 5.5. The toxic effects are clearly related to the pH value.

Table B. 9.2.6-12: Growth rates and yield

Mean measured concentrations of THPAM [mg/L]	Replicate	Growth rate [/d]	Growth rate inhibition [%]	Yield [cells/mL]	Yield inhibition [%]
Control	1	1.88	-	1704410	-
	2	1,83		1478555	
	3	1.86		1641502	
	4	1.96		2216330	
	5	1.90		1848917	
	6	1.90		1907246	
	Mean	1.89	-	1799493	-
11.3	1	1.88	1	1713567	5
	2	1.86	2	1614908	10
	3	1.90	0	1821194	-1
	Mean	1.88	1	1716556	5
26.6	1	1.97	- 4	2246499	- 25
	2	1.93	- 2	1995807	- 11
	3	1.91	- 1	1898089	- 5
	Mean	1.94	- 2	2046798	- 14
63.7	1	1.91	- 1	1899281	- 6
	2	1.88	1	1734954	4
	3	1.91	- 1	1873566	- 4
	Mean	1.90	0	1835934	- 2
149	1	1.09	42	157452	91
	2	1.03	45	129862	93
	3	1.01	46	136742	93
	Mean	1.05*	45	136742*	92
334	1	- 0.064	100	- 1082	100
	2	- 0.081	100	- 1333	100
	3	- 0.047	100	- 803	100
	Mean	- 0.064*	100	- 1073*	100
398 (pH adjusted to 8.21)	1	1.90	0	1808399	0
	2	1.85	2	1589695	12

Mean measured concentrations of THPAM [mg/L]	Replicate	Growth rate [/d]	Growth rate inhibition [%]	Yield [cells/mL]	Yield inhibition [%]
	3	1.93	- 2	2006595	- 12
	Mean	1.89	0	1801563	0

* Statistically significant difference of growth rates and yield compared to the control values

Recovery: After 72 hours algae were transferred from the two highest concentrations (149 and 334 mg test item/L) and the control into fresh untreated medium. The test item effect was observed to be reversible at these concentrations. Therefore, there is potential for recovery following exposure up to the geometric mean measured concentration 334 mg test item/L (highest test concentration).

Table B. 9.2.6-13: Cell densities for recovery of algae cells

Mean measured concentrations of THPAM [mg/L]	Incubation time [d]	Cell density [cells/mL]		Growth rate after incubation time [/d]
		Initial	final	
Control	4	9028	2549819	1.41
			2832686	1.44
149		7145	2807724	1.49
			3070771	1.52
			3304466	1.53
334		254	1214542	2.12
			542497	1.92
			610736	1.95

Conclusion: 72 h E_rC_{50} = 154 mg/L (95% C.I. = 151 – 199 mg/L)
72 h E_yC_{50} = 107 mg/L (95% C.I. = 80.8 – 127 mg/L)
NOEC = 63.7 mg/L (yield and growth rate)
LOEC = 149 mg/L (yield and growth rate)
based on mean measured concentrations

Comment RMS:

Validity: The study was conducted according to the OECD test guideline 201 (2006, updated 2011).

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the growth rate was 1.89 per day, which corresponds to an increase of biomass by a factor of 293.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%. In the study the mean coefficient of variation for section-by-section specific growth rates was 20%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For

other less frequently tested species, the value should not exceed 10%. In the study the coefficient of variation of average specific growth rates was 2.5%.

Taking into account the recommendations of the currently valid test guideline according to OECD (2011) the algae study is considered acceptable.

Acceptability of the analytical methods used in the test: See section on physical-chemical properties.

Endpoints: The RMS agrees on the endpoints given in the study report.

E_rC_{50} = 154 mg/L

E_yC_{50} = 107 mg/L

NOEC = 63.7 mg/L (growth rate and yield)

Based on mean measured concentrations

Conclusion of the RMS: Based on the evaluation of the study the algae toxicity test is considered acceptable.

B.9.2.7. Effects on aquatic macrophytes

No data on aquatic macrophytes is required since the active substance captan is not an herbicide or plant growth regulator.

B.9.2.8. Further testing on aquatic organisms

In addition, two literature studies were found by the literature search.

Reference:	**Assessment of acute toxicity and histopathology of the fungicide captan in rainbow trout**
Author(s), year:	Boran, H., Capkin, E.m Altinok, I. and Terzi, E., 2012
Report/Doc. number:	Experimental and Toxicologic Pathology 64 (2012) 175-179
Guideline(s):	Not applicable
GLP:	No
Deviations:	Not applicable
Validity:	Not applicable

Abstract:

Acute toxicity of the fungicide, captan, to juvenile rainbow trout was evaluated under static-renewal test conditions. Actual concentrations of captan ranged from 0.05 to 1.00 mg/L. The concentrations of captan that killed 50% of the rainbow trout (3.11 ± 0.8 g) within 24 (24 h; LC_{50}), 48, 72 and 96 h were 0.57 ± 0.09, 0.49 ± 0.10, 0.44 ± 0.11 and 0.38 ± 0.13 mg/L (95% confidence limits), respectively. None of the unexposed control fish died and the first fish died 6 h after exposure to captan (≥ 0.65 mg/L). Hypertrophy, separation of epithelium from lamellae, lamellar fusion, and epithelial cell necrosis were observed on captan exposed fish. Gills also had scattered areas of focal lamellar hyperplasia. Fish exposed to fungicide had inflammation and necrosis in liver, trunk kidney and spleen. In order, the most affected organs were gill, trunk kidney and liver.

Comment RMS:

Materials and Methods: For the test, juvenile rainbow trout (3.11 ± 0.8 g, 5.8 ± 0.3 cm) were exposed to captan at environmental relevant test concentrations. The fish were exposed to a commercial formulation containing 50% active substance (Safa Tarim, Konya). The test item was renewed every day, 80% of the test solution. Throughout the acclimation period (15 days) and the actual test the fish were held under a photoperiod of 12 h of light and darkness. The fish were fed 5% body weight twice a day with commercial trout pellets, during the acclimation period and toxicity tests.

The fish were held in a glass aquaria containing 25 L of static well water. Triplicate random groups of 10 fish were exposure for 96 h to 7 test concentrations (0.05-1.00 mg/L) and a negative control. The environmental conditions were measured daily and are according to the OECD guideline (Dissolved oxygen: 8.13 ± 0.25 mg O_2/L, Temperature: 13.4 ± 0.6 °C, pH: 7.85 ±0.29, total hardness: 31.2 ± 2.1 mg/L as $CaCO_3$, total alkalinity: 17.5 ±0.8 mg/L as $CaCO_3$).

At the end of the test 19 dead fish from each of the 0.35 or 0.65 mg/L exposed fish and 15 control fish were histopathologic examined. Therefore, the second gill arch, liver, spleen and the trunk kidney were removed and analysed.

Results: The study is considered valid taking into account the validity criteria according to OECD 203.

The LC_{50} values were determined by Probit analyses according to the maximum-likelihood procedure.

24 h LC_{50} = 0.57 ± 0.09 mg ai/L

48 h LC_{50} = 0.49 ± 0.10 mg ai/L

72 h LC_{50} = 0.44 ± 0.11 mg ai/L

96 h LC_{50} = 0.38 ± 0.13 mg ai/L

At test concentrations of 0.35 mg/L or higher fish showed sub-lethal effects (restlessness, dark pigmentation, flashing, increasing respiratory rates,…).

Histopathological findings could be observed in both test concentrations, 0.35 and 0.65 mg/L; however, at 0.65 mg/L the histopathological findings were more severe. Most severe histopathological findings were tubular necrosis (trunk kidney), cytoplasmic vacuolation (liver) and epithelial lifting and hyperplasia (gill). Further findings in the gill were epithelial necrosis and lamellar fusion (moderate) and hypertrophy (mild).

Reliability: The study was conducted according to the OECD test guideline 203 for static-renewal test conditions (OECD 1992). The reporting of the study was acceptable and relevant information (environmental test conditions, information on the test species, exposure regime and results) were included in the report. However, no information on the analytical verification of the test substance is reported. Further, information on the test item is brief (50% WP formulation) and no information on the composition of the formulation or the batch is given.

Relevance: The study was conducted according to the OECD test guideline 203 with one of the most sensitive test species, the rainbow trout. No information is given in the report if the endpoints are based on nominal, initial measured or mean measured concentrations. Further, the fish were fed during the toxicity tests.

Hence, the endpoints might not be relevant for the risk assessment. The 96 h LC_{50} of 0.38 mg ai/L is slightly higher than the 96 LC_{50} derived from the acute toxicity test with captan (Jenkins, 2004a) of 0.1736 mg/L based on initial measured concentrations and 0.2113 mg/L based on nominal concentrations. Considering the sub-lethal effects observed at test concentrations of > 35 mg/L a NOEC of 0.2 mg/L can be determined.

The information on the histopathological findings on the trunk kidney, liver, spleen and ecspecially on the gills might be considered as additional information, e.g. relevant for the discussion on the toxicological dependence/independence of peaks.

The conclusion of the authors regarding adverse effects on the gills:*"Gills damage due to toxic agents causes a chain of destructive event, which may lead to respiratory distress (Magare and Patil, 2000). In the present study, exposure of rainbow trout to captan results in strucutiral alteration of the gill lamellae including edema, separation of epithelium from lamellae, lamellar fusion, and swelling of the epithelial cells. Similar finding were also observed by Sunitha and Sahai (1983), Roy and Munshi (1991) and Bhavan and Geraldine (2000). The results of these studies clearly indicate that sublethal concentrations of captan have diverse effects on fish gills.*

In the present study, gills were found to be the most seriously affected organs compared to liver, spleen and trunk kidney, perhaps because of the direct contact with the compound."

Reference:	**Influence of Bioassay Volume, Water Column Height, and Octanol-Water Partition Coefficient on the Toxicity of Pesticides in Rainbow Trout**
Author(s), year:	Altinok, I., Capkin, E. and Boran, H., 2011
Report/Doc. number:	Bull. Environ Contam Toxicol (2011) 86: 596-600
Guideline(s):	Not applicable
GLP:	No
Deviations:	Not applicable
Validity:	Not applicable

Abstract:

Effects if water volume and water column height on toxicity of cypermethrin, carbaryl, dichlorvos, tetradifon, maneb, captan, carbosulfan, endosulfan and $HgCl_2$ to juvenile rainbow trout (Oncorhynchus mykiss, 3.2 ± 0.7 g) were evaluated in different glass aquaria under static conditions. When fish were exposed to the chemical compounds in 23 cm water column height (25 L), their mortality ranged between 0% and 58%. At the same water volume, but lower water column height (9 cm), mortality of fish increased significantly and was in a range from 60% to 95%. At the same water column height, toxic effects of chemicals were significantly higher in 25 L water volume than that of 8.5 L, water except maneb which has lowest (- 0.45) octanol-water partition coefficient value. Mortality rates ratio of 9 and 23 cm water column height ranged between 1.12 and 90 while morality rates of 9 and 25 L water volume ranged between 1.20 and 4.0.

Because of actual exposure concentrations were not affected by either water volume or water water column height, we propose that increased pesticides' toxicity was related to an increase in bioassay volume, since more pesticide molecules were able to interact with or accumulate the fish. However, there seem to be no relationshop between the effects of water volume, water column height and K_{OW} value of chemicals with regard to toxicity in juvenile rainbow trout.

Comment RMS:

Materials and Methods: For the test, juvenile rainbow trout (2.2 ± 0.7 g, 5.1 ± 0.4 cm) were exposed to captan at environmental relevant test concentrations. The fish were exposed to a commercial formulation containing 50% active substance (Hektas). The test item was renewed every day, 80% of the test solution.

Throughout the acclimation period (3 weeks) and the actual test the fish were held under a photoperiod of 12 h of light and darkness. The fish were fed 5% body weight twice a day with commercial trout pellets, during the acclimation period. Fish were not fed 48 h before exposure and during the toxicity tests.

The fish were held in glass aquaria of different volume and height of water column. Quadruplicate random groups of 10 fish were exposure for 96 h to one test concentration (0.2 ± 0.01 mg/L mg/L) and a negative control. The environmental conditions were measured daily and are according to the OECD guideline

(Dissolved oxygen: 7.87 ± 0.21 mg O_2/L, Temperature: 17.4 ± 0.33 °C, pH: 7.58 ±0.19, total hardness: 32.2 ± 1.9 mg/L as $CaCO_3$, total alkalinity: 26.12 ± 2.4 mg/L as $CaCO_3$). For the analytical verification the GC-ECD method was used.

Results: The study is considered valid taking into account the validity criteria according to OECD 203. Two experiments were conducted. The first experiment with a water volume of 25 L and 9 L and a water column height of 9 cm. The second experiment with a water volume of 25 L and a water column of 9 and 23 cm. In both experiments the fish loading was below 1 g/L.

It was shown that captan was the most affected substance. The mortality rates ratio for the water column heights (9 and 23 cm) was 90 and for the water volume 4.5.

The toxicity of pesticides was significantly affected by different bioassays volume (9 and 25 L) with the same water column height (9 cm).

Reliability: The study was conducted according to the OECD test guideline 203 (1992). The reporting of the study was acceptable and relevant information (environmental test conditions, information on the test species, exposure regime, analytical measurements and results) were included in the report. Information on the test item is brief (50% WP formulation) and no information on the composition of the formulation or the batch is given.

Relevance: The study was conducted according to the OECD test guideline 203 with one of the most sensitive test species, the rainbow trout. The results are interesting for interpreting the results as it was shown that the water volume and water column height used in the study might have significant effects on the results of an acute toxicity fish study.

However, no toxicity endpoints were determined in the study. Hence, the study is not relevant for the risk assessment but might be considered as additional information.

B.9.3. REFERENCES RELIED ON

List of data submitted in support of the evaluation

Data point	Author(s)	Year	Title Owner Report N. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.1		2016a	Captan: Acute toxicity to brown trout ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., ENV-16-013, R-37526, Agrochemex Ltd, Manningtree, Essex, UK GLP: Y Published: N	Y	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.1		2016b	Captan: Acute toxicity to common carp ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., ENV-16-021, R-37527 Agrochemex Ltd, Manningtree, Essex, UK GLP: Y Published: N	Y	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.1		2017a	Captan: Acute toxicity to bream ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., ENV-16-022, R-37528 Agrochemex Ltd, Manningtree, Essex, UK GLP/GEP: Y Published: N	Y	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY

Data point	Author(s)	Year	Title Owner Report N. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.1		2017b	Captan: Acute toxicity to roach ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., ENV-16-023, R-37529 Agrochemical Experimentation Ltd. Essex, UK GLP/GEP: Y Published: N	Y	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.1		2004a	Captan: Acute toxicity to rainbow trout Huntingdon Life Sciences Ltd., Huntingdon, UK ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S. Report-no. MAK/836, R-17596 GLP: yes Published: no	Y	N		ADM/ARY
KCA 8.2.1		2004b	Captan: acute toxicity to 3-spined stickleback Huntingdon Life Sciences Ltd., Huntingdon, UK ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S. Report-no. MAK/837, R-17597 GLP: yes Published: no	Y	N		ADM/ARY
KCA 8.2.1		1995a	THPAM: acute toxicity to rainbow trout (*Oncorhynchus mykiss*). Zeneca Agrochemicals UK, Report BL5445/B. (Company file: R-8749/TMN-0024). GLP, Unpublished.	Y	N		ADM/ARY

Data point	Author(s)	Year	Title Owner Report N. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.1		1994a	THPI: acute toxicity to rainbow trout (*Oncorhynchus mykiss*). Zeneca Agrochemicals UK, Report BL5237/B. (Company file: R-8288/TMN-0025). GLP, Unpublished.	Y	N		ADM/ARY
KCA, 8.2.2.3		1988a	Bioconcentration and elimination of ^{14}C-residues by bluegill (*Lepomis macrochirus*) exposed to trichloromethyl ^{14}C-captan. ICI Agrochemicals UK, Report T-13069. (Company file: R-5268/TMN-0002). GLP, Unpublished.	Y	N		ADM/ARY
KCA 8.2.2.3		1988b	Bioconcentration and elimination of ^{14}C-residues by bluegill (*Lepomis macrochirus*) exposed to cyclohexane ^{14}C-captan. ICI Agrochemicals UK, Report N. T-13068. (Company file: R-5269/TMN-0002A). GLP, Unpublished.	Y	N		ADM/ARY
KCA 8.2.3		2013	Captan - Amphibian metamorphosis Assay with african clawed frog (*Xenopus laevis*) following OPPTS Test Guideline 890.1100 and OECD test Guideline N. 231 ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., 14017.6109, R-28281 Smithers Viscient, Massachusetts, USA GLP: Y Published: N	Y	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY

Data point	Author(s)	Year	Title Owner Report N. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.4.1		2012	Captan - Short-term reproduction assay with fathead minnow (*pimephales promelas*) following OPPTS 890.1350 and OECD 229 guidelines ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., 14017.6110, R-28286 Smithers Viscient, Massachusetts, USA GLP: Y Published: N	Y	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.4.1		1993	Captan: acute toxicity of the technical material to first instar *Daphnia magna*. ICI Americas, Report RJ1116B. (Company file: TMN-006). GLP, Unpublished	N	N		ADM/ARY
KCA 8.2.4.1		2016	Captan: Acute immobilisation test on *Daphnia magna* under static exposure conditions ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., ENV-16-016, R-37744 AgroChemex Environmental Ltd. UK GLP: Y Published: N	N	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.4.1		1995b	THPAM: acute toxicity to *Daphnia magna*. Zeneca Agrochemicals UK, Report N. BL5442/B. (Company file: R-8748/TMN-011). GLP, Unpublished.	N	N		ADM/ARY

Data point	Author(s)	Year	Title Owner Report N. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.4.1		1994b	THPI: acute toxicity to *Daphnia magna*. Zeneca Agrochemicals UK, Report N. BL5239/B. (Company file: R-8290/TMN-012). GLP, Unpublished.	N	N		ADM/ARY
KCA 8.2.5.1		2017	Captan: Reproduction test on *Daphnia magna*, with repeated semi-static exposure ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., ENV-16-015, R-37745 AgroChemex Environmental Ltd. UK GLP/GEP: Y Published: N	N	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.5.1/03		2016	Estimation of ECx values for Captan. *Daphnia magna* reproduction toxicity test ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., 562961-CA-08251-01 GAB Consulting Agrociencias S.L.U. GLP/GEP: no Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.5.4		2015	THPAI: Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment under Laboratory Conditions ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., S15-00594, R-36061 Eurofins Agroscience Services EcoChem GmbH GLP: Y Published: N	N	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY

Data point	Author(s)	Year	Title Owner Report N. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.6.1		2016c	CAPTAN TECHNICAL Alga, Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 hours, including recovery phase DR.U.NOACK-LABORATORIEN, Sarstedt, Germany ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S. Report-N. SPO16612 GLP: Y Published: N	N	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.6.1		1995	THPAM: toxicity to the green alga *Selenastrum capricornutum*. Zeneca Agrochemicals UK, Report BL5428/B. (Company file: R-8747/TMN-0017). GLP, Unpublished.	N	N		ADM/ARY
KCA 8.2.6.1		1994c	THPI: toxicity to the green alga *Selenastrum capricornutum*. Zeneca Agrochemicals UK, Report BL5238/B. (Company file: R-8289/TMN-0018). GLP, Unpublished.	N	N		ADM/ARY
KCA 8.2.6.1		2016a	1.2.3.6-Tetrahydrophthalimide (THPI) Alga, Growth Inhibition Test with Pseudokirchneriella subcapitata, 72 hours DR.U.NOACK-LABORATORIEN, Sarstedt, Germany ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S. Report-N. SPO16614 GLP: Y Published: N	N	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY

Data point	Author(s)	Year	Title Owner Report N. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.6.1		2016b	1.2.3.6-Tetrahydrophthalamic acid (THPAM) Alga, Growth Inhibition Test with Pseudokirchneriella subcapitata, 72 hours DR.U.NOACK-LABORATORIEN, Sarstedt, Germany ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S. Report-N. SPO16615 GLP: Y Published: N	N	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCA 8.2.9	Boran, H., Capkin, E.m Altinok, I. and Terzi, E.	2012	Assessment of acute toxicity and histopathology of the fungicide captan in rainbow trout Experimental and Toxicologic Pathology 64 (2012) 175-179 Not GLP Published	Y	N		Public
KCA 8.2.9	Altinok, I., Capkin, E. and Boran, H.	2011	Influence of Bioassay Volume, Water Column Height, and Octanol-Water Partition Coefficient on the Toxicity of Pesticides in Rainbow Trout Bull. Environ Contam Toxicol (2011) 86: 596-600 Not GLP Published	Y	N		Public

ADM stands for ADAMA Agricultural Solutions. The study owner (sponsor) of the studies is ADAMA Makhteshim Ltd (previously known as Makhteshim Chemical Works "**MCW**"), a member of ADAMA Agricultural Solutions (ADM)."