Annex II Ecotoxicology (PPP) 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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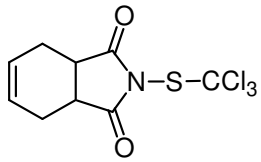
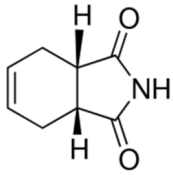
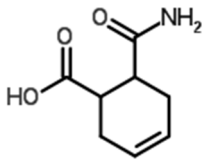
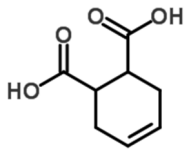
B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

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

Risk assessment for the intended greenhouse use:

According to the EFSA Technical Report (PRAS 133) "*It is rarely indicated in the GAP table, under what type of structure the representative use will be made. It is suggested that clarification is sought from the applicant. If it is not clearly indicated in the GAP table, then it will be assumed that the representative use can be made under all types of structures*."

On request by the RMS the definition of the greenhouse proposed for the use in strawberries was specified by the notifier as a closed walk-in structure. That means it differs from the definition for greenhouses as included in regulation 1107/2009. The technical report states that "*walk-in tunnels may be open or have the sides of the tunnel rolled up. It was therefore agreed that exposure to non-target organisms is likely. It was acknowledged that if the tunnels remained closed during and after the application then exposure would be different*."

The term “closed” was interpreted as closed for the whole application period up to harvest. Following this definition, no specific risk assessment was considered necessary for birds and mammals, bees, arthropods and non-target plants. Though also aquatic organisms are considered to be less exposed in comparison the field use, there are currently only accepted calculation tools for either field uses or high tech glasshouses. Therefore, the approach for field uses has to be followed. As the aquatic risk assessment could not be finalised for various reasons in general, the assessment for the indoor use is also still open.

The definition of the indoor use is also included under the point restrictions in Volume I. Any use deviating from this specification would require an additional risk assessment for all groups.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

The active substance captan was notified by two companies, ADAMA Makhteshim Ltd. and Arysta LifeScience Corporation. In addition to the Annex II data (see Volume 3, B.9 (AS)) aquatic toxicity studies with the EU representative formulations "Merpan 80 WDG" and "Captan 80 WG" were submitted.

Most of the studies were conducted under semi-static or static conditions addressing the risk to aquatic organisms under more realistic conditions taking into account the rapid degradation of captan in the water sediment system. 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 RMS agrees with the applicant that the exposure to aquatic organisms is short-timed due to the fast hydrolysis of captan in aquatic systems. Nevertheless, in the laboratory studies an appropriate exposure should be maintained throughout the whole test period according to the OECD test guidelines. In the available semi-static and static tests an appropriate exposure could only be demonstrated for the first hours of the study period.
Hence, even though the studies might not be assessed as invalid, no reliable tier 1 endpoints could be determined for studies conducted under semi-static and static test conditions.

The validity and reliability of the static and semi-static toxicity tests was discussed at the Pesticide Peer Review Meeting (PREV 12). It was agreed that acute toxicity studies in which exposure was not maintained throughout the test period, should be considered not valid (fish) or valid but not reliable (aquatic invertebrates and algae).
The use of standard Tier 1 studies to be used as a refined exposure study is only acceptable for those studies with a clear exposure profile, i.e. sufficient analytical measurements within the first hours of exposure.

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Reference:	**Captan: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) of an 83% WP formulation**
Author(s), year:	1993
Report/Doc. number:	BL5005/B, TMN-0021, X278/B
Guideline(s):	OECD test guideline 203 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:	
Test substance:	83% WP formulation of captan, batch no. : A00634, purity : 84.62% w/w
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per test concentrations and 9 fish per dilution water control, 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: 49 - 55 mm (mean: 52 mm) Weight: 1.59 - 2.51 g (mean: 2.04 g)
Loading	0.41 g/L fish loading per test vessel
Type of test:	flow-through, 96 hours
Applied concentrations:	
Nominal:	0 (control), 32, 56, 100, 180, 320, 560 and 1000 µg formulation/L 0 (control), 27, 47, 85, 150, 270, 470 and 850 µg ai/L
Solvent:	None
Test conditions:	
Water quality:	Dilution water, hardness: 27.3 – 34.4 mg /L as $CaCO_3$, alkalinity: 17.6 - 18.0 mg/L as $CaCO_3$ Free and combined residual chlorine: < 4 µg/L
Conductivity:	140 - 219 µS/cm
Temperature:	14.7 - 15.1 °C
pH:	7.4 - 7.62
O_2 content:	9.4 - 10.0 mg dissolved oxygen/L (> 60% air saturation)
Light regime:	Light/dark cycle of 16/8, with 20 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, so that a 95% exchange of the test solutions was calculated to occur within 9 hours. The nominal flow rate of the stock solutions was 1.25 mL per minute. 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 concentrations of captan in the test vessels were measured at 0, 24, 48, 72 and 96 hours. The concentrations of captan in the stock solutions were measured at 0 and 72 hours.

The analysis of the test solutions were conducted by high pressure 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 concentrations of captan in the stock solution measured during the study ranged from 57 to 65% of the nominal values. The mean measured concentrations of captan in the 180 to 560 µg/L test solutions ranged from 11 to 24% of the nominal values. The measured concentrations of captan observed in the 1000 µg/L nominal concentration of formulation on day 0 was 260% of its nominal value. This value was considered anomalous. The stock solution analysis of this concentration showed no deviation from the expected results and the dilution water and toxin flow rates at this time were noted to be normal.

The measured concentrations for the remaining test solutions (32 - 100 µg/L) were below the limit of quantification of captan (< 20 µg/L) in this study.

Table B. 9.3.1-1: Analytical measurements of captan in test vessels

Nominal concentration of formulation [µg/L]	Nominal concentration of captan [µg/L]	Measured concentration of captan [µg/L]					Mean measured concentration	
		0 h	24 h	48 h	72 h	96 h	[µg/L]	% of nominal
Control	-	< 20	< 20	< 20	< 20	< 20	< 20	-
32	27	< 20	< 20	< 20	< 20	< 20	< 20	-
56	57	< 20	< 20	< 20	< 20	< 20	< 20	-
100	85	< 20*	< 20*	< 20*	< 20*	< 20*	< 20	-
180	150	73	20	27	31	31	36	24
320	270	140	22	39	ND	ND	67	25
560	470	62	40	58	ND	ND	53	11
1000	850	2200[1]	70	ND	ND	ND	70	7

The limit of quantification (LOQ) of captan in this study was 20 µg/L

ND…not determined because of the mortality (100%) observed in the treatment group.
[1] Value considered anomalous
* Mean of triplicate analyses

Biological results: The symptoms of toxicity noted in this study were rapid and laboured respiration, quiescence, sounding, loss of balance and dark discolouration.
At 24 hours, it was noted that there were only nine fish present in the dilution water control which was assumed to be an operator error at the start of the test. The validity of the test was considered not affected.

Table B. 9.3.1-2: Mortality and sub-lethal effects

Nominal concentration of formulation [µg/L]	Cumulative percentage of mortality observed [%]			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
32	0	0	0 [d]	0 [d]
56	0	0	0	0
100	0	0	0	0
180	0	10 [d]	20 [adf]	30 [abcdf]
320	50 [abcdf]	100	100	100
560	10 [abdef]	100	100	100
1000	100	100	100	100

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

Conclusion: A LC_{50} of 190 µg formulation/L (95% C.I. = 140 - 280 µg/L) based on nominal concentrations was determined. The NOEC of 100 µg formulation/L is based on behavioural effects, i.e. quiescent behaviour and mortality.

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 every 24 hours thereafter.
In addition, the following points deviated from the guidelines or were not reported in detail:

- The fish were fed until 48 hours instead of 24 hours before the test is started.

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 study author proposed an endpoint based on nominal concentrations. However, the RMS is of the opinion that the endpoint should be based on mean measured concentrations taking into account that the mean measured concentrations were between 7 and 25% of the nominal test concentration.

Taking into account the lack of analytical measurements at the highest test concentrations and the measurements to be below the LOQ at the lowest test concentrations a calculation of an endpoint is difficult. For the nominal test concentration of 150 µg ai/L reliable analytical measurements are available till the end of the study. The geomean measured concentrations were 24% of the nominal (36 µg ai/L). At this concentration mortalities of 30% were observed at 96 h. At the next higher test concentrations (nominal 270-850 µg ai/L) 100% mortality was shown. Hence, the RMS is of the opinion that a LC_{50} of greater than 36.0 µg ai/L is considered acceptable.

No reliable NOEC could be determined taken into account that the NOEC is below the LOQ. In the lowest test concentrations (nominal 27-85 µg ai/L) the measured concentrations were below the LOQ at test start till the end of the test. At the next test concentration (150 µg ai/L) lethal and sub-lethal effects could be observed.

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

Reference:	**Captan 80 WG: A 96-hour static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*)**
Author(s), year:	1999
Report/Doc. number:	TMN-0024A
Guideline(s):	OECD test guideline 203 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Captan 80% WDG, formulation of captan, batch no. : 16940-4, purity : 76.45% w/w
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per test concentrations and dilution water control, 2 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: 43 - 59 mm (mean: 47 mm) Weight: 0.79 - 1.3 g (mean: 1-0 g)
Loading	0.5 g/L fish loading per test vessel
Type of test:	Static, 96 hours

Applied concentrations:

Nominal:	0 (control), 0.10, 0.17, 0.29, 0.48 and 0.80 mg formulation/L
Solvent:	None
Test conditions:	
Water quality:	Dilution water, hardness: 140 mg /L as $CaCO_3$, alkalinity: 176 mg/L as $CaCO_3$, conductivity: 300 µmhos/cm
Conductivity:	140 - 219 µS/cm
Temperature:	13.5 - 14.2°C
pH:	7.9 - 8.4
O_2 content:	8.2 - 11.0 mg dissolved oxygen/L (> 60% air saturation)
Light regime:	Light/dark cycle of 16/8, with 30 min transition periods (483 lux)
Feeding	The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria of ca. 38 litres capacity with and a working volume of 20 litres. The depth of water was approximately 16 cm. 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 6, 24, 48, 72 and 96 hours. Measurements of temperature were undertaken throughout the 96 hour period. Dissolved oxygen and pH were measured at test start and at approximately 24 hours intervals thereafter. In addition, water hardness, alkalinity and conductivity and was determined at test start.
Analytical measurements:	Mean measured test concentrations were determined form samples of test waster collected from each treatment and control group at the beginning of the test, at approximately 48 hours and at test termination.
Statistics:	The LC_{50} was calculated at various time intervals by binominal probability with non-linear interpolation using Stephan's method. The NOEC was determined by visual interpretation of the mortality and sublethal effects.
Findings:	
Analytical data:	Samples collected at test initiation had measured values that ranged from 98 to 111% of nominal values. Due to the rapid hydrolysis of captan, measured values for samples taken at 48 and 96 h were all less than the limit of quantification. Based on the decline in test substance concentrations during the test, the results of the study were based on nominal concentrations.

Table B. 9.3.1-3: Analytical measurements of captan in test vessels

Nominal concentration of formulation [mg/L]	Measured concentration of captan 80 WG [mg form./L] [1] (% of nominal)		
	0 h	48 h	96 h
Control	< LOQ	< LOQ	< LOQ
0.10	0.102 (102)	< LOQ	< LOQ
0.17	0.170 (100)	< LOQ	< LOQ
0.29	0.286 (99)	< LOQ	< LOQ
0.48	0.488 (102)	< LOQ	< LOQ
0.80	0.866 (108)	< LOQ	< LOQ

The limit of quantification (LOQ) of captan in this study was 0.0375 mg ai/L. The LOQ was equivalent to 0.0491 mg form./L.
[1] Measured concentrations (mg ai/L) were corrected to test substance concentrations (mg form./L) based on an active ingredient of 76.45%.

Biological results: No mortality or sublethal effects were observed in the treatment groups 0.10 - 0.48 mg form./L. In the highest test treatment group (0.8 mg form./L) 100% of mortality was observed after 24 hours of exposure.

Table B. 9.3.1-4: Mortality and sub-lethal effects

Nominal concentration of formulation [mg/L]	Cumulative percentage of mortality observed [%]				
	6 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
0.10	0	0	0	0	0
0.17	0	0	0	0	0
0.29	0	0	0	0	0
0.48	0	0	0	0	0
0.80	30 [ab]	100	100	100	100

[a] Lethargy, [b] Loss of equilibrium

Conclusion: Based on the results of the study a 96 h LC_{50} value for rainbow trout of 0.62 mg form/L (95% C.I. = 0.48 - 0.80 mg form./L), corresponding to 0.47 mg ai/L was determined. The 96 h NOEC was 0.48 mg form./L (corresponding to 0.37 mg ai/L) based on mortality and sublethal 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 concentration 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, after 48 hours and at the end of the test. After 48 and 96 hours, the measured concentrations were below the LOQ. The measured concentrations indicated that the test substance was not 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 measured concentrations of the test item in the control were below the LOQ, but not below the LOD. Hence, an exposure of the control fish could not be excluded. However, no adverse effects on fish were observed in the control groups.

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.

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

Considering nominal concentrations no reliable LC_x values could be determined based on Probit analysis using max. linear likelihood regression as no confidence intervals could be calculated.

Hence, a 96 h LC_{50} of 473.7 µg ai/L (95% C.I. 366.960-611.600 µg ai/L) was estimated based on interpolation (Binominal distribution). The NOEC was determined to be 367 µg ai/L based on mortality.

Considering initial measured concentration no reliable LC_x values could be determined based on Probit analysis using max. linear likelihood regression as no confidence intervals could be calculated.

Hence, a 96 h LC_{50} of 497.0 µg ai/L (95% C.I. 373.076-662.057 µg ai/L) was estimated based on interpolation (Binominal distribution). The NOEC was determined to be 373.1 µg ai/L based on mortality.

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:	**Merpan 80 WDG: Flow-through acute toxicity test with rainbow trout (*Oncorhynchus mykiss*)**
Author(s), year:	1998
Report/Doc. number:	R-10155, 98-038-1026
Guideline(s):	OECD test guideline 203 (1984), EC methods C.1, US EPA FIFRA 72-1 (1982)
GLP:	Yes
Deviations:	None of relevance
Validity:	Not acceptable

Material and methods:

Test substance:	Merpan 80 WDG, batch no. : 6C01-AA, purity : 79.4% w/w
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	Preliminary test: 4 fish per replicate, one replicate per test concentration and control Definitive test: 5 fish per replicate, two replicates per test concentration and control
Length, weight:	The weight and length of fish in the dilution water control was measured at the end of the exposure period. Length: 35 - 50 mm (mean: 44.1 mm) Weight: 0.45 - 1.35 g (mean: 0.89 g)
Loading	0.41 g/L fish loading per test vessel
Type of test:	flow-through, 96 hours

Applied concentrations:

Nominal:	Preliminary test: 0 (control), 80, 128, 205, 328 and 524 µg formulation/L Definitive test: 0 (control), 50, 83, 137, 225 and 371 µg formulation/L
Solvent:	None

Test conditions:

Water quality:	Dilution water, hardness: 246 - 250 mg /L as $CaCO_3$, alkalinity: 38 - 41 mg/L as $CaCO_3$, conductivity: 530 - 610 µS/cm, pH 7.50 - 7.84, dissolved oxygen: 8.84 - 9.43 - 10.97 mg/L, temperature: 13.8 – 14.5 °C
Temperature:	12.2 - 14.1 °C
pH:	7.11 - 7.78
O_2 content:	9.54 - 10.12 mg dissolved oxygen/L (> 60% air saturation)
Light regime:	Light/dark cycle of 16/8 (210 - 500 lux)
Feeding	The fish were not fed during the 96 hours study period.
Methods:	Prior to initiation of the final test, two preliminary tests were performed under static renewal and static conditions to assess differences in toxicity between these two different regimes. The final test was carried out in glass aquaria of ca. 15 litres capacity with and a working volume of 10.9 litres. The test solutions were renewed at a nominal rate of 50 mL/minutes, which provided approximately 6.6 solution volume replacement per

day. This corresponds to a 90% test solution replacement rate within approximately 8 hours.

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 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 and conductivity was determined daily.

Analytical measurements: The concentrations of captan in the freshly prepared stock solutions were measured at 0, 24, 48, 72 and 96 hours.

The analysis of the test solutions were conducted by high pressure 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 concentrations of captan were verified in the freshly prepared test solutions at hour 0, 24, 48 and 72. At 0, 24, 48 and 71 hours, the recovery of the samples was 112.7, 98.2, 104.2 and 110.3% of the nominal, respectively. Recovery of the old stock solutions (after 24 hours at room temperature) was 110.7, 108.5, 109.5 and 81.4% of the nominal. Based on these result the test concentrations were expressed as nominal concentrations.

Table B. 9.3.1-5: Analytical measurements of captan in the stock solution

Nominal concentration of formulation [mg/L]	Nominal concentration of captan [m/L]	Measured concentration of captan [µg/L] (% of nominal)				
		0 h	24 h	48 h	72 h	96 h
Control	-	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
1500 (freshly prepared)	1191	1342 (112.7)	1169 (98.2)	1241 (104.2)	1313 (110.3)	NA
1500 (old)	1191	NA	1318 (110.7)	1292 (108.5)	1305 (109.5)	969.6 (81.4)

The stock solutions were analysed twice, immediately after preparation (freshly prepared stock solution) and after 24 hours of storage (old stock solution)
NA…not applicable

Biological results: Preliminary test under static exposure conditions: The highest test concentration at which no mortality or sublethal effects occurred was 205 µg form./L and the lowest at which there was 100% mortality was 524 µg form./L.

Preliminary test under static-renewal conditions: The highest test concentration at which no mortality or sublethal effects occurred was 128 µg form./L and the lowest at which there was 100% mortality was 205 µg form./L.

Definitive test under flow-through exposure conditions: The highest test concentration at which no mortality or sublethal effects occurred was 50 µg form./L and the lowest at which there was 100% mortality was 225 µg form./L.

Sub-lethal effects were observed at a test concentration of 137, 225 and 371 µg form./L. Sub-lethal effects did not occur in the control and at test concentrations of 50 and 83 µg form./L.

Table B. 9.3.1-6: Mortality and sub-lethal effects – Preliminary test (static and static renewal exposure regime)

Nominal concentration [µg form./L]	Cumulative mortality [%] - Static exposure regime				Cumulative mortality [%] – Static-renewal exposure regime			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0
128	0	0	0	0	0	0	0	0
205	0	0	0	0	25 [d]	50 [e]	100	100
328	0 [a]	0 [b]	0 [b]	0 [c]	100	100	100	100
524	100	100	100	100	100	100	100	100

[a] One fish showed dark pigmentation and lethargy
[b] Two fish showed dark pigmentation, rapid respiration and lethargy
[c] Three fish showed dark pigmentation, rapid respiration and lethargy
[d] Three fish showed rapid respiration
[e] Two fish showed rapid respiration

Table B. 9.3.1-7: Mortality and sub-lethal effects – Definitive test (flow-through exposure regime)

Nominal concentration [µg form./L]	Cumulative mortality [%]						
	0 h	2 h	4 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0
83	0	0	0	0	0	0	10
137	0	0	0	0	0	10 [ab]	30
225	0	0	0	80 [c]	100	100	100
371	0	0 [d]	0 [cd]	100	100	100	100

[a] lethargic, [b] fish on bottom of aquarium, [c] partial loss of equilibrium, [d] rapid respiration

Conclusion: Based on these results a LC_{50} of 153 µg formulation/L (95% C.I. = 83 - 225 µg/L) based on nominal concentrations was determined. The NOEC of 50 µg formulation/L is based on mortality.

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 satisfactory maintained throughout the study. Hence, the study is not considered valid.

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

- The length of fish was not within the recommendations given in the OECD test guideline (50 mm ± 10 mm).
- The fish were fed daily before the test is started. According to the OECD test guideline, feeding should be stopped 24 hours before the test is started.

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.

Reference:	**Acute toxicity of Merpan (Captan) 83 WP in rainbow trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae) under flow-through conditions**
Author(s), year:	1995
Report/Doc. number:	R-8249, 94131/02-AsOm
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:

Test substance:	Merpan (Captan) 83 WP, batch no.: 720977, content ai: 83% (nominal)
Test species:	Rainbow trout (*Oncorhynchus mykiss*)

Number of organisms:	8 fish per controls and test concentrations, 1 replicate per treatment
Length, weight:	Length: mean 40 - 50 mm Weight: mean 0.8 g
Loading	0.64 g/L fish loading per test vessel
Type of test:	Flow-through, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.0043, 0.0094, 0.021, 0.045 and mg test item/L
Solvent:	Acetone
Test conditions:	
Water quality:	Mixture of dechlorinated tap water and deionised water, hardness: 178 mg/L as $CaCO_3$)
Temperature:	15.6 - 16.5°C
pH:	7.89 - 8.07
O_2 content:	90 - 109% 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 10 litres. At the initiation of the study, 8 fish were allocated at random to each test vessel. The flow rate of the test medium was 140 mL/min, resulting in an entire exchange per test vessel of 8.4 L/hour.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 3, 6, 24, 48, 72 and 96 hours. Measurements of temperature, pH and dissolved oxygen were undertaken in 24 h intervals.
Analytical measurements:	Samples of the test medium were taken at the start of the test and thereafter in 24 h intervals. The analytical measurements were conducted for the active substance captan and the metabolite THPI. The analysis of the test solutions (captan and metabolite THPI) were conducted by gas chromatographic analysis
Statistics:	LC_{50} values were estimated by Probit regression analysis.
Findings:	
Analytical data:	The mean measured concentrations of captan were in a range of 68 to 83%.

Table B. 9.3.1-8: Analytical measurements - captan

Nominal concentrations [µg ai/L]	Measured concentrations [µg ai/L]					Mean measured concentrations [µg ai/L]	% of nominal
	0 h	24 h	48 h	72 h	96 h		
3.57	3.6	3.1	2.3	2.3	3.1	2.9	81%
7.80	6.2	5.6	6.3	6.8	7.6	6.5	83%

Nominal concentrations [µg ai/L]	Measured concentrations [µg ai/L]					Mean measured concentrations [µg ai/L]	% of nominal
	0 h	24 h	48 h	72 h	96 h		
17.43	13.4	11.2	13.4	9.5	15.5	12.6	72%
37.35	21.1	27.6	25.4	29.0	16.4	28.8	77%
83.0	71.6	49.2	48.5	*	ND	56.4	68%

* The measured concentration was considered an outlier.
ND…not determined

Table B. 9.3.1-9: Analytical measurements – metabolite THPI

Nominal concentrations [µg ai/L]	Measured concentrations of THPI [µg/L]					Mean measured concentrations of THPI [µg/L]
	0 h	24 h	48 h	72 h	96 h	
3.57	1.7	1.7	2.1	2.2	1.6	1.86
7.80	3.8	4.0	4.5	4.1	3.8	4.04
17.43	8.6	8.0	8.6	9.1	11.1	12.6
37.35	14.6	16.2	14.4	16.9	16.6	15.74
83.0	42.4	28.1	33.0	*	ND	34.43

* The measured concentration was considered an outlier.
ND…not determined

Biological effects: At a mean measured concentration of 56.4 µg ai/L, a mortality of 100% was observed within 72 h. No mortality was observed in the 6.5 µg ai/L treatment group and in the control and solvent control group.

No mortalities, abnormalities or behavioural effects were found in the 6.5 µg ai/L test group. The mortality of 1 fish in the lowest test concentrations (2.9 µg ai/L) was assessed as accidently.

Table B. 9.3.1-10: Mortality and sub-lethal effects

Mean measured concentration [µg ai/L]	Cumulative mortality [%]					
	3 h	**6 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
2.9	0	0	0	12.5	12.5	12.5
6.5	0	0	0	0	0	0
12.6	0	0	0	12.5	37.5	50.0
28.8	0	0	0	25.0	37.5	62.5
56.4	0	0	37.5	75.0	100	100

Conclusion: Based on these results a LC_{50} of 17.7 µg test item/L (95% C.I. = 11.3-31.1 µg test item/L) based on mean measured concentrations was determined. The NOEC was observed at a concentration of 7.80 µg test item/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 every 24 hours thereafter.

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

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.

Method: GC-ECD (captan) and GC-MSD (THPI)

Linearity: Calibration is based on single determination at 5 concentrations (captan) and 10 concentrations (THPI). The calibration function was linear within the range from 0.01-0.5 µg/mL for captan and 0.01 - 10.0 µg/mL THPI with $r^2 > 0.9996$.

Accuracy: 4 fortifications levels for captan and 7 fortification levels for THPI (each 2-3 measurements): 0.1 - 5000 µg/L (captan and THPI). Recoveries: 91-106% (captan) and 85-103% (THPI)

Precision: The relative standard deviation per fortification level ≤ 20 %

LOQ: The limit of quantification (LOQ) = 0.1 µg/L (captan) and 0.2 µg/L (THPI)

LOD: The limit of detection (LOD) = 0.03 µg/L (captan) and 0.1 µg/L (THPI)

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints: The study author proposed an endpoint based on mean measured concentrations. The RMS agrees on the use of the arithmetic mean measured concentrations for calculation of the endpoint taking into account the analytical measurements (< 80% of nominal concentrations). Nevertheless, the RMS conducted a statistical re-evaluation of the study based using the software ToxRat®.

Taking into account the non-significance of the slope curve using Probit analysis the RMS conducted a statistical analysis based on Weibull using linear max. likelihood regression.

The LC_{50} was determined to be 17.91 µg ai/L (95% C.I. 10.099-28.813 µg ai/L) and the LC_{10} was 4.65 µg ai/L (95% C.I. 0.810-8728 µg ai/L).

The derived endpoints are well in line with the calculated endpoint in the study report. Hence, the RMS agrees on the endpoints given in the study report.

96 h LC_{50} = 17.7 µg test item/L (95% C.I. = 11.3-31.1 µg test item/L) corresponding to 14.7 µg ai/L

NOEC = 6.5 µg ai/L (based on mortality)

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

Reference:	**Captan: Acute toxicity to *Daphnia magna* of an 83% WP formulation**
Author(s), year:	1993
Report/Doc. number:	BL4962/B
Guideline(s):	OECD 202 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	83% WP formulation of captan, batch no. A00634, purity: 84.62% w/w
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control, 20 daphnids per treatment and control
Age:	First instar, < 24 hours old
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	0 (control), 0.56, 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 mg form./L
Solvent:	None
Test conditions:	
Water quality:	Dilution water (reconstituted water medium), total hardness: 202.6 mg/L as $CaCO_3$, alkalinity: 127.2 mg/L as $CaCO_3$, pH 8.19, conductivity: 660 µS/cm
Temperature:	20 ± 1 °C
pH:	8.7 - 9.1 (0 - 48 h)
O_2 content:	7.85 - 8.45 mg/L (> 70% saturation)
Light regime:	16 hours light / 8 hours darkness, 15 min transition period
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.
Analytical measurements:	The concentrations of the active substance in the test solutions were measured at the start and end of the test. The concentrations were measured using high performance liquid chromatography. At the start of the test the excess test solutions and stock solution were sampled and at the end of the test one replicate of the dilution water control and each test concentrations was sampled.
Statistics:	The EC_{50}, and 95 % confidence limits were calculated using Stephan's method.
Findings:	
Analytical data:	The measured concentration of captan in the 100 mg form./L stock solution was 86 mg/L (102% of nominal) thus confirming that the correct nominal concentration had been prepared. The measured concentrations of captan at the start of the test ranged

from 12 to 39% of the nominal values. At the end of the test the concentration of captan in the top exposure concentration was 0.08% of nominal and all lower concentrations were below the limit of detection.

Table B. 9.3.1-11: Analytical measurements of captan

Nominal concentration of formulation [mg/L]	Nominal concentration of captan [mg/L]	Measured concentration of captan [mg/L]		Mean measured concentration of captan	
		0 h	48 h	[mg/L]	% of nominal
Control	-	< 0.048	< 0.01	< 0.048	-
0.56	0.47	0.096	< 0.01	-	-
1.0	0.85	0.10 *	< 0.01 *	-	-
1.8	1.5	0.27	< 0.01	-	-
3.2	2.7	0.45	< 0.01	-	-
5.6	4.7	1.3	< 0.01	-	-
10	8.5	3.0	< 0.01	-	-
18	15	5.8	< 0.01	-	-
32	27	9.7	0.021	4.9	18

Limit of quantification (LOQ) = 0.048 mg ai/L, Limit of detection (LOD) = 0.01 mg ai/L
* Mean of triplicate analyses

Effects: The lowest concentration at which where was 100% immobilisation was 18 mg form./L. The 48 h NOEC was determined to be 0.56 mg form./L based on immobility.

Table B. 9.3.1-12: Effects on daphnids (*D. magna*) exposed to an 83% WP formulation of captan

Nominal concentration of formulation [mg/L]	Nominal concentration of captan [m/L]	Total number of immobilised daphnids / total number tested		% of immobilised daphnids	
		24 h	48 h	24 h	48 h
Control	-	0 / 20	0 / 20	0	0
0.56	0.47	1 / 20	1 / 20	5	5
1.0	0.85	2 / 20	3 / 20	10	15
1.8	1.5	2 / 20	4 / 20	10	20
3.2	2.7	7 / 20	8 / 20	35	40
5.6	4.7	12 / 20	14 / 20	60	70
10	8.5	15 / 20	17 / 20	75	85
18	15	20 / 20	20 / 20	100	100
32	27	20 / 20	20 / 20	100	100

Conclusion: The 48 h EC_{50} was determined to be 3.3 mg form./L (95% C.I. = 2.4 – 4.2 mg form./L). The NOEC was 0.56 mg form./L. The endpoints are based on nominal concentrations.

Comment RMS:

Validity: The study was assessed according to the currently valid test guideline, OECD test guideline 202 (2004).
The validity criteria are met considering the immobilisation observed in the control group (should not exceed 10% at the end of the test) and the dissolved oxygen concentration (≥ 3 mg/L in control and test vessels at the end of the test).
All validity criteria are met; hence, the acute toxicity test is considered valid.
In addition, the following points deviated from the guidelines or were not reported in detail:
- The concentration of the test substance was measured in the test media. However, an exposure over the whole study duration (48 hours) could not be achieved, except for the highest test concentration (mean measured concentration at test end was measured to be 11% of nominal).
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: 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 determined based on the results of 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 determined.

Reference:	**Assessment of toxic effects on Merpan 80 WDG on *Daphnia magna* using the 48 h acute immobilisation test**
Author(s), year:	1996
Report/Doc. number:	R-8854
Guideline(s):	OECD 202 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Merpan 80 WDG, formation of captan, batch no.: 4722 AA, purity: 79.6%
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control, 20 daphnids per treatment and control
Age:	First instar, between 6 and 24 hours old
Type of test, duration:	Static-renewal test, 48 hours
Applied concentrations:	
Nominal:	0 (control), 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5.0 and 10.0 mg form./L

Solvent:	None
Reference substance:	Potassium dichromate, nominal test concentrations: 0.9 and 1.9 mg/L
Test conditions:	
Test medium:	M 4 medium, total hardness: 14.5 °dH, pH 7.9 ± 0.3, aerated for at least 30 min
Temperature:	20 ± 1 °C
pH:	7.62 - 8.15 (0 - 48 h)
O_2 content:	~ 6.0 mg/L (> 60% saturation)
Light regime:	16 hours light / 8 hours darkness, 15 min transition period
Test parameters:	The immobilisation test was performed semi-statically with replacing of solution after 24 hours. 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 after 24 and 48 h.
Analytical measurements:	Analytical measurements were performed at three concentration levels. The active substance and its first metabolite tetrahydrophtalimide were analysed during the test. Samples were taken at test start and after 24 h of ageing.
Statistics:	The EC_{50}, and 95 % confidence limits were determined by statistical analyses, i.e. Probit analyses.
Findings:	
Analytical data:	The test concentration at the start of the test was above 80% of nominal values at each concentration. After 24 hours of testing only a resting concentration of 0 - 4% was found due to a great hydrolytic instability of the test substance. Only a low amount of the first hydrolytic metabolite tretrayhydrophtalimide (THPI) was found after 24 h of testing.

Table B. 9.3.1-13: Analytical measurements of captan

Nominal concentration of formulation [mg/L]	Nominal concentration of captan [mg/L]	Measured concentration of captan [mg/L] (% of nominal)			
		0 h	24 h	24 h (fresh medium)	48 h
0.08	0.064	0.065 (102)	0.001 (2)	0.060 (94)	ND
0.63	0.501	0.433 (86)	0.004 (0.7)	0.470 (92)	0.002 (0.4)
5.0	3.98	3.86 (97)	0.06 (2)	4.07 (102)	0.15 (4)

ND…not detectable (LOD = 0.5 µg ai/L)

Table B. 9.3.1-14: Analytical measurements of the metabolite THPI

Nominal concentration of formulation [mg/L]	Nominal concentration of captan [mg/L]	Measured concentration of THPI [mg/L]			
		0 h	24 h	24 h (fresh medium)	48 h
0.08	0.064	0.013	0.02	0.012	0.08
0.63	0.501	0.068	0.084	0.068	0.084
5.0	3.98	0.29	0.61	0.29	0.60

ND…not detectable

Effects: The lowest concentration where all daphnids are immobilised was 10 mg form./L. The highest concentration with no immobilised daphnids was 0.31 mg form./L after 48 hours.

The EC_{50} for the reference substance was determined at 1.42 mg ai/L.

Table B. 9.3.1-15: Effects on daphnids (*D. magna*) exposed to an 80% WDG formulation of captan

Nominal concentration of formulation [mg/L]	Total number of immobilised daphnids / total number tested		% of immobilised daphnids	
	24 h	48 h	24 h	48 h
Control	0 / 20	0 / 20	0	0
0.04	0 / 20	0 / 20	0	0
0.08	1 / 20	1 / 20	5	5
0.16	0 / 20	0 / 20	0	0
0.31	0 / 20	0 / 20	0	0
0.63	1 / 20	3 / 20	5	15
1.25	1 / 20	2 / 20	5	10
2.5	5 / 20	8 / 20	25	40
5.0	5 / 20	11 / 20	25	55
10.0	15 / 20	20 / 20	75	100

Conclusion: The 48 h EC_{50} was determined to be 4.3 mg form./L. The NOEC based on immobility was 0.31 mg form./L. The endpoints are based on nominal concentrations.

Comment RMS:

Validity: The study was assessed according to the currently valid test guidelines, OECD test guideline 202 (2004).

The validity criteria are met considering the immobilisation observed in the control group (should not exceed 10% at the end of the test) and the dissolved oxygen concentration (≥ 3 mg/L in control and test vessels at the end of the test).

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

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

- The concentration of the test substance was measured in the test media, however only at three test concentrations. According to the OECD test guideline 202 the concentration of the test substance should be

measured, as a minimum, at the highest and lowest test concentration. The test concentrations which were analysed were 0.08, 0.64 and 5.0 mg form./L, the lowest and highest concentrations are not included, but the analysed test concentrations are in the range of the EC_{50}.

However, 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. Based on the outcome of the analytical measurements the toxicity endpoints shall be expressed based on initial measured concentrations or mean measured concentrations.

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: No reliable endpoint could be determined based on the results of the analytical measurements. After 24 and 48 hours the measured concentrations were below 80% of the nominal concentrations (between 0.4 and 4% of nominal concentrations). The analytical measurements were only performed in three test concentrations. The choice of the test concentrations which were analysed were not according to the recommendations given in the OECD test guideline. In addition, no analytical measurements were performed in the control.

The RMS conducted a statistical re-evaluation based on the available analytical measurements for three test concentrations. For the other test concentrations a measured concentration of 10.97% of nominal was assumed taking into account the mean degradation observed in the three test concentrations.

Nominal [mg/L]	0 h [mg ai/L]	24 h (old) [mg ai/L]	24 h (new) [mg ai/L]	48 h (old) [mg ai/L]	Geomean [mg ai/L]	% nominal
0.064	0.065	0.001	0.060	0.001	0.007	10.383
0.501	0.433	0.004	0.470	0.002	0.036	7.130
3.980	3.860	0.060	4.070	0.150	0.613	15.407
					Mean:	**10.97**

Considering a mean of 10.97% the following measured concentrations were determined.

Nominal [mg ai/L]	Geomean measured [mg ai/L]
0.032	0.003
0.064	0.007
0.127	0.014
0.247	0.027
0.501	0.036
0.995	0.109
1.990	0.218
3.980	0.613
7.960	0.873

A statistical re-evaluation of the results was conducted by the RMS using the software ToxRat.

Taking into account mean measured concentrations a 48 h EC_{50} of 0.289 mg ai/L (95% C.I. 0.125-1.145 mg ai/L) was determined based on Probit analysis using linear max. likelihood regression. The NOEC based on immobilisation was 0.027 mg ai/L (using Step-down Cochran-Armitage Test Procedure).

Conclusion of the RMS: Based on the evaluation of the study the acute daphnid toxicity test is considered valid. A 48 h EC_{50} of 0.289 mg ai/L was determined by the RMS based on geometric mean measured concentrations.

Reference:	**Captan 80 WDG: Algal, Growth inhibition test with *Pseudokirchneriella subcapitata*, 72 hours including recovery phase**
Author(s), year:	2016d
Report/Doc. number:	150423AF / SPO16613
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 80 WDG, CAS-No.: 133-06-2, batch no. 95131119/3, content of ai: 79.1%
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), 0.8, 2.53, 8.0, 25.3 and 80 mg test item/L corresponding to 0.633, 2.0, 6.33, 20 and 63.3 mg ai/L
Mean measured:	0 (control), 0.294, 0.458, 0.842, 1.4 and 16.5 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:	7.44 – 7.66 (0 h), 7.44 – 8.26 (72 h)
Incubation:	Continuous illumination at 60.8 – 77.9 µE/m²/s, orbital shaking at 70 rpm
Analytical measurements:	For chemical analysis (HPLC-DAD) of the test substance and the metabolite THPI, 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 four highest concentrations (2.0, 6.33, 20 and 63.3 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 and 6 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 60% to 90% 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 63.3 mg ai/L treatment group the measured concentration was 11% of the nominal test concentration.

Table B. 9.3.1-1: 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	-
0.8	0.572	< LOQ	< LOQ	< LOQ	0.293
2.53	1.40	< LOQ	< LOQ	0.415	0.458
8.0	4.73	< LOQ	< LOQ	1.66	0.842
25.3	13.1	< LOQ	< LOQ	5.33	1.40
80.0	37.9	7.19	< LOQ	17.0	16.5

LOQ…Limit of quantification of the analytical method (0.3 mg/L of the test item)
[a] Values < 70% 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. 9.3.1-2: Yield and growth rate

Nominal concentrations [mg ai/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	1910449	-	1.91	-
0.633	1589082 *	17	1.85	3
2.00	866872 *	55	1.65 *	14
6.33	72848 *	96	0.833 *	56
20.0	4406 *	100	0.174 *	91

Nominal concentrations [mg ai/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 [%]
63.3	- 2733 *	100	- 0.197 *	100

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

Recovery: Cell growth was shown at all tested concentrations (control, 2.0, 6.33, 20.0 and 63.3 mg ai/L). Potential for recovery following exposure up to 63.3 mg ai/L was shown.

Table B. 9.3.1-3: 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	9583	1829285 1573262	1.75 1.70
2.00		4365	1225637 1069683 1065858	1.88 1.83 1.83
6.33		3950	864464 1068868 644975	1.80 1.87 1.70
20.0		211	30501 67675 56166	1.66 1.92 1.86
63.3	6 d	34	147743 60268 131311	1.40 1.25 1.38

* calculated from the cell density of the definitive test after 72 hours

Conclusion: The endpoints are based on geometric mean measured concentration

72 h E_rC_{50} = 0.780 mg ai/L (95% C.I. = 0.741-0.817 mg ai/L)

72 h E_rC_{20} = 0.533 mg ai/L (95% C.I. = 0.491-0.579 mg ai/L)

72 h E_rC_{10} = 0.420 mg ai/L (95% C.I. = 0.365-0.470 mg ai/L)

NOE_rC = 0.293 mg ai/L

72 h E_yC_{50} = 0.440 mg ai/L (95% C.I. = 0.412-0.462 mg ai/L)

72 h E_yC_{20} = 0.314 mg ai/L (95% C.I. = < 0.293-0.354 mg ai/L)

72 h E_yC_{10} = < 0.293 mg ai/L

NOE_yC < 0.293 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 1.913 per day, which corresponds to an increase of biomass by a factor of 311.5.
- 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 7.8%.
- 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*. 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.

At the Pesticide Peer Review Meeting (PREV 12) the experts agreed to express the endpoint based on geometric mean measured concentrations using LOQ/2 for concentrations at which the measured concentrations were below the LOQ or LOD. It was stated that this approach should be considered as a pragmatic solution considering that flow-through or semi-static test design are not feasible with standard algae testing. Further, it was highlighted that the endpoint expressed in terms of geometric mean measured concentrations is considered a worst-case endpoint.

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 the conclusion of the PREV 12 meeting the endpoints given in the study summary are considered reliable to be used in the risk assessment.
Conclusion of the RMS: Based on the evaluation of the study the algae toxicity test is considered valid and the endpoints based on geometric mean measured concentrations are reliable to be used in the risk assessment.

Reference:	**Captan: Toxicity to the green alga *Selenastrum capricornutum* of an 83% WP formulation**
Author(s), year:	1994
Report/Doc. number:	BL5001/B
Guideline(s):	OECD test guideline 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	83% WP formation of captan, batch no.: A00634, purity: 84.62% w/w
Test species:	Green alga (*Raphidocelis subcapitata*, strain ATCC 22662, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata)*
Number of organisms:	1.05 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), 0.2, 0.4, 0.8, 1.5, 3.0, 6.0, 12 and 24 mg form./L
Solvent:	None
Test conditions:	
Water quality:	Culture medium
Temperature:	24 ± 1 °C
pH:	7.3 - 7.4 (0 h), 4.7 - 9.2 (72 h) A maximum increase of 1.8 units over the test duration was measured in the culture medium control. 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.

A marked decrease in pH, 2.6 pH units was measured in the nominal 24 mg/L test concentration and was believed to be due to the hydrolysis of captan in the test culture medium affecting the pH.

Incubation: Continuous illumination at ~ 7390 lux

Analytical measurements: For chemical analysis (High Performance Liquid Chromatography) the samples of test solution were taken at test initiation and at the end (blank solution).

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 were made at test initiation and at termination. The temperature was measured daily.

Findings:

Analytical data: The measured concentrations at the start of the test ranged from 74 to 85% of the nominal values. The measured concentrations in the blanks after 72 hours were below the limit of detection except the nominal 24 mg/L concentration where a 1.9% residual concentration was determined. Therefore, all results are reported using the nominal concentrations.

Table B. 9.3.1-16: Concentration of captan

Nominal concentrations of formulation [mg/L]	Nominal concentrations of captan [mg/L]	Measured concentration of captan [mg/L]		% of nominal	
		0 h	72 h	0 h	72 h
Control	-	< 0.061	< 0.047	-	-
0.2	0.17	0.14	< 0.047	82	-
0.4	0.34	0.25*	< 0.047*	74	-
0.8	0.68	0.52	< 0.047	76	-
1.5	1.3	0.96	< 0.047	74	-
3.0	2.5	2.1	< 0.047	84	-
6.0	5.1	4.0	< 0.047	78	-
12.0	10	8.0	< 0.047	80	-
24.0	20	17	0.37	85	1.9

* Mean of triplicate analyses
Limit of quantification (LOQ) = 0.047 mg ai/L

Biological results:

Table B.9.3.1-17: Areas under the curve and growth rates

Nominal concentrations of formulation [mg/L]	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	-	174.3	-	1.808	-
0.2	0.17	160.4	92	1.789	99
0.4	0.34	167.5	96	1.803	100
0.8	0.68	124.6*	71	1.712*	95

Nominal concentrations of formulation [mg/L]	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
1.5	1.3	89.3*	51	1.605*	89
3.0	2.5	30.0*	17	1.234*	68
6.0	5.1	11.9*	7	0.895*	50
12.0	10	4.8*	3	0.626*	35
24.0	20	1.2*	1	0.159*	9

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

Conclusion: 72 h E_rC_{50} = 6 mg form./L (95% C.I. = 1.7 - 21 mg form./L)
72 h E_bC_{50} = 1.4 mg form./L (95% C.I. = 0.42 - 3.1 mg form./L)
NOEC = 0.4 mg form./L (growth rate and 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 1.807 per day, which corresponds to an increase of biomass by a factor of 226.5.
- 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 12.7%.
- 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 0.8.

The validity criteria are met; hence, the algae toxicity test is considered valid.

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. However, an exposure over the whole study duration (48 hours) could not be achieved, except for the highest test concentration (mean measured concentration at test end was measured to be 1.9% of nominal). 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: 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 no reliable endpoint could be determined.

According to the EFSA Technical Report (PRAS 133) a calculation of a reliable endpoint is questionable if no test item was measured at the end of the test.

At the Pesticide Peer Review Meeting (PREV 12) the experts agreed to express the endpoint based on geometric mean measured concentrations using LOQ/2 for concentrations at which the measured concentrations were below the LOQ or LOD. It was stated that this approach should be considered as a pragmatic solution considering that flow-through or semi-static test design are not feasible with standard algae testing. Further, it was highlighted that the endpoint expressed in terms of geometric mean measured concentrations is considered a worst-case endpoint.

Hence, the RMS conducted a statistical re-evaluation of the data based on geometric mean measured concentrations.

Growth rate:

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,807	0,0150	6	
0,057	1,787	0,0417	3	1,1
0,077	1,802	0,0181	3	0,3
0,111	1,711	0,0231	3	5,3
0,150	1,604	0,0310	3	11,3
0,222	1,232	0,0338	3	31,8
0,307	0,894	0,0322	3	50,5
0,434	0,625	0,0154	3	65,4
2,508	0,158	0,0410	3	91,3

C_x concentrations were calculated by 3-parametric normal cumulative distribution function. The NOEC was determined using Williams Multiple Sequential t-Test Procedure.

72 h E_rC_{50} = 0.316 mg ai/L (95 % C.I. 0.310-0.321 mg ai/L)

72 h E_rC_{20} = 0.176 mg ai/L (95 % C.I, 0.174-0.179 mg ai/L)

72 h E_rC_{10} = 0.130 mg ai/L (95 % C.I. 0.128-0.132 mg ai/L)

NOE_rC = 0.077 mg ai/L

Yield:

Treatm.[mg/L]	Mean	Std. Dev.	n	%Decrease
Control	236,8	10,68	6	
0,057	223,6	27,23	3	5,6
0,077	233,3	12,86	3	1,5
0,111	177,3	12,22	3	25,1
0,150	128,3	11,72	3	45,8
0,222	41,5	4,20	3	82,5
0,307	14,4	1,51	3	93,9
0,434	5,8	0,32	3	97,5
2,508	0,6	0,21	3	99,7

C_x concentrations were calculated by 3-parametric normal cumulative distribution function. The NOEC was determined using Multiple Sequentially-rejective Welch- t-Test after Bonferroni-Holm.

72 h E_yC_{50} = 0.154 mg ai/L (95% C.I. 0.135-0.177 mg ai/L)

72 h E_yC_{20} = 0.108 mg ai/L (95% C.I. 0.097-0.121 mg ai/L)

72 h E_yC_{10} = 0.090 mg ai/L (95% C.I. 0.080-0.101 mg ai/L)

NOE_yC = 0.077 mg ai/L

Conclusion of the RMS: Based on the evaluation of the study the algae toxicity test is considered valid and the endpoints based on geometric mean measured concentrations are reliable to be used in the risk assessment.

Reference:	**Testing of toxic effects of Merpan 80 WDG on the single cell green alga *Scenedesmus subspicatus***
Author(s), year:	1996
Report/Doc. number:	R-8855, 95194/01-AASs
Guideline(s):	OECD test guideline 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Merpan 80 WDG, formulation of captan, batch no.: 4722.AA, purity: 79.6%
Test species:	Green alga (*Desmodesmus subspicatus* CHODAT, formerly known as *Scenedesmus subspicatus)*
Number of organisms:	1.0 x 10^4 cells/mL (control); 3 replicates per treatment and 6 replicates per control group, the cell numbers were determined twice (except at 24 hours)
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (control), 10, 19, 36.1, 68.6 and 130.3 mg form./L
Solvent:	None
Test conditions:	
Water quality:	Culture medium (according OECD guideline 201)
Temperature:	21 - 24 °C
pH:	7.84 – 7.95 (0 h), 7.20 – 8.91 (72 h)
Incubation:	Continuous illumination at ~ 8000 lux
Analytical measurements:	For chemical analysis of captan and the metabolite THPI (Gas Chromatography) the samples of test solution were taken at 0, 24, 48 and 72 hours (blank solution).
Test parameters:	Test vessels (1000 mL glass conical flasks) contained 500 mL test solution. Samples were taken at 0, 24, 48 and 72 hours to determine the cell densities by electronic particle counting using a Coulter counter model ZB. Measurements of pH were made at test initiation and at termination. The temperature was measured daily.
Statistics:	The EC_x values were calculated by log-linear regression. The percentage inhibition based on the area under the growth curve and based on the growth rate (according OECD test guideline).
Findings:	
Analytical data:	The measured concentrations at the start of the test ranged from 86 - 103% of the nominal values. The measured concentrations in the blanks after 72 hours were below the limit of detection except the nominal 103.3 mg/L concentration where

55% of the nominal values were determined. Therefore, all results are reported using the nominal concentrations.

Table B. 9.3.1-18: Analytical measurements of captan

Nominal concentration of formulation [mg/L]	Nominal concentration of captan [mg/L]	Measured concentration of captan [mg/L] (% of nominal)			
		0 h	24 h	48 h	72 h
10.0	7.96	7.66 (96)	0.015 (0.2)	ND (-)	0.002 (-)
36.1	28.7	24.6 (86)	3.71 (13)	0.054 (0.2)	0.003 (-)
130.3	103.7	107.2 (103)	85.0 (82)	71.1 (69)	56.9 (55)

ND…not detectable (LOD = 0.5 µg ai/L)

Table B. 9.3.1-19: Analytical measurements of the metabolite THPI

Nominal concentration of formulation [mg/L]	Nominal concentration of captan [mg/L]	Measured concentration of THPI [mg/L]			
		0 h	24 h	48 h	72 h
10.0	7.96	0.81	1.36	1.43	1.64
36.1	28.7	2.03	4.53	5.98	6.5
130.3	103.7	5.0	7.74	14.0	17.3

ND…not detectable

Biological results: Merpan 80 WDG caused significant inhibition effects to the biomass integral and to the growth rate between 36.1 and 130.3 mg form./L. The biomass integral also was significantly inhibited at 19 mg form./L. Stimulating effects were observed at 10 mg form./L.

Table B.9.3.1-20: Areas under the curve and growth rates

Nominal concentrations of formulation [mg/L]	Nominal concentrations of captan [mg/L]	% inhibition Area under growth curve (0-72 h)	% inhibition Growth rate (0-72 h)
Control	-	-	-
10.0	7.96	-13.4	-3.9
19.0	15.1	8.3	1.3
36.1	28.7	41.2	12.6
68.6	54.6	52.3	16.3
130.3	103.7	70.4	25.9

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

Conclusion:

72 h E_rC_{50} = 341.4 mg form./L corresponding to 271 mg ai/L

72 h E_rC_{10} = 42.4 mg form./L corresponding to 33.8 mg ai/L

NOEC = 19 mg form./L corresponding to 15.1 mg ai/L (growth rate)

72 h E_bC_{50} = 63.7 mg form./L corresponding to 50.7 mg ai/L

72 h E_bC_{10} = 14.8 mg form./L corresponding to 11.8 mg ai/L

NOEC = 10 mg form./L corresponding to 8.0 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 1.78 per day, which corresponds to an increase of biomass by a factor of 209.
- 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 27.4%.
- 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 0.4%, which is below the required 7% for *Desmodesmus subspicatus*.

Taking into account the validity criteria the study is considered valid.

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

- In the study, analytical measurements were conducted for three test concentrations only. According to the OECD test guideline analytical measurements should have been conducted for all test concentrations as the measured concentrations were below 80% at the end of the test.
- The calculation of the mean cell numbers per treatment does not correspond with the mean cell numbers given in the study report, e.g. the 48 h mean cell numbers of the 36.1 mg form./L treatment. The 48 hour cell numbers are exactly the same in the 19.0 and 36.1 mg form./L treatment group. This might be a copy and paste error. However, the actual cell numbers cannot be checked.
- The initial cell concentration for *Desmodesmus subspicatus* is higher (1 x 10^4 cells/mL) than recommended in the OECD guideline (2-5 x 10^3 cells/ml). According to the OECD guideline, the initial biomass in the test cultures should be sufficiently low to allow exponential growth throughout the incubation period without risk of nutrition depletion.
- 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: Based on the results of the analytical measurements no reliable endpoint could be determined. After 24, 48 and 72 hours the measured concentrations were below 80% of the nominal concentrations. The analytical measurements were only performed in three test concentrations. The choice of the test concentrations which were analysed were not according to the recommendations given in the OECD test guideline. In addition, no analytical measurements were performed in the control.

The RMS conducted a statistical re-evaluation assuming a mean measured concentration of 0.8% of nominal for the 15.1 mg ai/L treatment group and 38.2% of nominal for the 54.6 mg ai/L treatment group.

nominal (a.s.)	0 h	24 h	48 h	72 h	Mean	% of nominal	
7.96	7.66	0.015	0.0025	0.002	0.028	0.3	
15.12	-	-	-	-	0.121	0.8	mean of 0.3 and 1.2%
28.74	24.6	3.71	0.054	0.003	0.349	1.2	
54.61	-	-	-	-	20.86	38.2	mean of 1.2 and 75.1%
103.72	107.2	85	71.1	56.9	77.92	75.1	

Taking into account geometric mean measured concentrations the following endpoints for growth rate and yield were determined using the software ToxRat.

No reliable endpoints could be determined using non-linear regression. Hence, the following statistical evaluation was conducted based on Probit analysis using max. likelihood linear regression. The NOEC was determined using Multiple Sequentially-rejective Welsh-t-Test after Bonferroni-Holm.

Growth rate:

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,780	0,0070	6	
0,028	1,853	0,0291	3	-4,1
0,121	1,760	0,0132	3	1,1
0,349	1,558	0,0291	3	12,5
20,860	1,492	0,0134	3	16,2
77,920	1,321	0,0806	3	25,8

72 h E_rC_{50} > 77.92 mg ai/L

72 h E_rC_{20} = 28.79mg ai/L (95 % C.I, 13.159-70.030 mg ai/L)

72 h E_rC_{10} = 1.764 mg ai/L (95 % C.I. 0.302-4.574 mg ai/L)

NOE_rC = 0.121 mg ai/L

Yield:

Treatm.[mg/L]	Mean	Std. Dev.	n	%Decrease
Control	207,7	4,32	6	
0,028	258,9	22,10	3	-24,6
0,121	195,4	7,86	3	5,9
0,349	106,6	9,39	3	48,7
20,860	87,0	3,52	3	58,1
77,920	52,6	13,45	3	74,7

72 h E_yC_{50} = 4.96 mg ai/L (95% C.I. 1.639-17.982 mg ai/L)

72 h E_yC_{20} = 0.152 mg ai/L (95% C.I. 0.015-0.539 mg ai/L)

72 h E_yC_{10} = 0.025 mg ai/L (95% C.I. 0.001-0.125 mg ai/L)

NOE_yC = 0.121 mg ai/L

It has to be highlighted that there might be some errors in the study report considering the counted cell numbers (see above, deficiencies of the study). The statistical evaluation by the RMS was conducted based on the raw data given in the study report. However, considering the identified deficiencies the reliability of the endpoints is questionable.

Conclusion of the RMS: Based on the evaluation of the study the algae toxicity test is considered valid. However, due to the identified deficiencies (analytical measurements, incorrect raw data) the endpoint is not considered reliable.

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

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 (study summary refer to Vol.3, B.9 (AS)). In addition, a 28 -day prolonged toxicity test according to OECD 204 was conducted with the formulation Merpan 83 WP.
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 to be 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). 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 verified.
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).

Merpan 83 WP: Prolonged toxicity test with rainbow trout (Oncorhynchus mykiss) under static renewal conditions. (1999; IIA, 8.2.2.1/02)

The 28-day toxicity of 'Merpan' 83 WP, a wettable powder formulation containing 830 g captan/kg (purity 81.7% captan (w/w)) to the rainbow trout (Oncorhynchus mykiss) was determined in a semi-static test system. Groups of ten fish in 19.6 L glass aquaria containing 17 L test medium (13 ± 2°C, 16:8 hour light/dark regime) were exposed to nominal concentrations of 'Merpan' 83 WP, prepared by direct addition to EC reconstituted water of 15, 30, 60, 120 and 240 µg/L in comparison with a dilution water only (total hardness 244 to 270 mg CaCO3/L). On three occasions each week the test media were replaced. On each day of the test the fish were fed an amount equivalent to 2% of their body weight. On each day of test medium renewal the stock solution (1.5 g 'Merpan' 83 WP/L) was analysed for captan by HPLC. Measurements of pH, dissolved oxygen and temperature were taken from each freshly prepared medium and each spent medium three times each week. Temperature was also monitored continuously. Observations for mortality and abnormal behaviour of fish were made daily. At the end of the test all surviving fish were anaesthetised and body weights and wet weights measured.

The study met the essential criteria of OECD 204. It was conducted according to Good Laboratory Practice.

Mean measured concentrations of captan in the stock solutions ranged from 89.5% to 114.99% of the nominal concentration. Based on these results the outcome of the toxicity test was based on nominal concentrations.

Measured concentrations of captan in 'Merpan' 83 WP stock solutions prepared during a 28-day toxicity test with rainbow trout

Day	*Stock solution (% recovery of captan)* [a]
0	*102.86*
2	*102.31*
5	*114.68*
7	*114.99*
9	*63.57 (94.54)* [b]
12	*100.53*
14	*89.48*
16	*105.44*
19	*113.18*
21	*111.59*
23	*87.84*
26	*93.16*
Mean	*99.97*

[a] *Recovery with respect to the nominal 1.5 g/L.*

[b] *Measurement repeated due to instrument failure, new value in parenthesis.*

The physical and chemical parameters of the test solutions remained at expected values during the study with dissolved oxygen ranging from 62.4 to 121.9% ASV, pH from 7.05 to 8.5 and continuous temperature measurements of 12.2 to 13.7°C.

Other than one accidental death during a transfer procedure there were no fish mortalities in any treatment observed during the test. The only sublethal effects observed were at the 240 µg/L treatment in which 30% of the fish showed rapid respiration, but only at the four hour interval after the start of the test. At the end of the test, the mean wet weight of fish in all treatments (1.53 to 1.66 g) was greater than at the start of the test (0.93 g). There were no treatment-related effects on wet weight or fork length.

Summary of effects of 'Merpan' 83 WP on rainbow trout following 28-day exposure under semi-static test conditions

Nominal concentration of 'Merpan' 83 WP (µg/L)	*Cumulative mortality [%]*	*Mean fish weight [g]*	*Mean fish length [mm]*
	28 days	*28 days*	*28 days*
Water only	*0*	*1.57*	*53*
15	*0*	*1.62*	*53*
30	*0*	*1.57*	*53*
60	*10*[a]	*1.66*	*54*
120	*0*	*1.53*	*52*
240	*0*	*1.63*	*53*

[a] *Inadvertently killed during transfer procedure.*

The 28-day LC_{50} of 'Merpan' 83 WP to the rainbow trout under semi-static test conditions was greater than 240 µg/L. The 28-day NOEC was greater than or equal to 240 µg/L the highest concentration of 'Merpan' 83 WP tested. The 28-day LC_{50} and NOEC is equivalent to 199.2 µg captan/L, based on a nominal formulation content of 83% captan.

The 96-hour LC_{50} of captan in acute toxicity studies in rainbow trout and mirror carp were 0.05 and 0.24 mg/L, respectively. The 96-hour LC_{50} in mirror carp was greater than 0.1 mg/L which indicates that an early life stage test is not required. Although the 96-hour LC_{50} in rainbow trout was less than 0.1 mg/L, the LC_{50} in this species following 21 days exposure in a chronic study on juvenile fish was 0.075 mg/L, i.e. higher than the acute value. Captan is not expected to persist in surface waters (DT_{50} < 24 hours, see Section 5) and so prolonged exposure to fish is unlikely. The equilibrium bioconcentration factors for bluegill sunfish in two studies with captan were 113 and 140, respectively, with a rapid depuration of residues in both. Thus, significant accumulation of captan residues in fish tissues is unlikely. Although captan is acutely toxic to rainbow trout, the lack of prolonged toxicity, the very short persistence in water and the low tendency to accumulate in fish tissues means that an early life toxicity study is not necessary.

A fish life cycle test with captan is not required because the bioconcentration factors for whole fish in two bioaccumulation studies was less than 150 and captan residues were rapidly eliminated from fish tissues during depuration in both studies. Furthermore, captan is not stable in water: sediment systems. The DT_{50} value in two studies was less than 24 hours. Therefore, prolonged exposure to captan and accumulation of captan residues is very unlikely.

The newly submitted fish early life stage test (ELS-test) was conducted according to the OECD guideline 2010 (2013).

The study used a "semi-static" design producing repeated exposure-events at 7-day intervals. The exposure regime is intended to represent a worst-case in terms of potential for multiple-exposure of individual fish in the field.

For the ELS-test, rainbow trout was selected as the test species out of the four species named in the OECD guideline. Captan has an acute toxic mode-of-action in fish (irritation of gill membranes). Both brown trout and rainbow trout are salmonids with relatively high demand for dissolved oxygen. Hence, optimum gill-function is clearly important for their survival.

Brown trout which was identified to be the most sensitive species is not a listed species in the OECD test guideline 2010 (2013). As such, in this experimental context, for brown trout the conditions and methods conductive to egg-fertilisation, egg-survival, hatching-success, and long-term survival of hatchlings (sac-fry) and feeding-fry, are unknown. Hence, rainbow trout has been selected as the test species in the new study. Any potential differences in sensitivity to brown trout would in any case by covered by the use of an uncertainty factor when using the NOEC in the risk assessment.

Reference:	**Captan (tested as 80 WDG formulation): Early life-stage toxicity test on the rainbow trout, *Oncorhynchus mykiss*, with repeated static exposure**
Author(s), year:	2016
Report/Doc. number:	82420, R-36002
Guideline(s):	OECD test guideline 210 (2013), US EPAOPPTS 850.1400
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Captan, tested as 80 WDG formulation, batch no. : 94131283, purity : 81.2%
Test species:	Rainbow trout (*Oncorhynchus mykiss*) The unfertilised eggs and milt of rainbow trout were acclimated in an approximately 10.5 ºC water bath prior to fertilisation. Following temperature acclimation, the unfertilized eggs were transferred to a dry bowl, milt immediately added, and the eggs gently stirred by hand for approximately 5 seconds. Isothermal blended freshwater was added until the eggs were covered and the mixture gently stirred again by hand. After stirring, the eggs were left undisturbed for approximately 15 minutes. The excess fertilisation water and sperm were then poured off and the newly fertilized eggs rinsed several times with isothermal freshwater. Freshwater was again added until the eggs were well covered and allowed to water harden for at least one hour prior to distribution to the test chambers.
Number of organisms:	4 replicates per test concentration and control, 50 eggs per replicate, 20 eggs per replicate (after thinning)
Age:	Eggs, around 2 hours after fertilisation
Type of test:	Static, 95 days (61 days post-hatch)
Applied concentrations:	
Nominal:	0 (control), 0.0125, 0.025, 0.05, 0.1 and 0.2 mg ai/L
Measured (mean):	- (control), 0.0155, 0.0243, 0.041, 0.0796 and 0.202 mg ai/L
Solvent:	None
Exposure:	At each test level there were 12 dosing events, each separated by 7 days with the exception of the 10th and 11th dosing events which were separated by 6 days.
Test conditions:	
Water quality:	Laboratory freshwater (blending naturally hard well water with well water demineralised by reverse osmosis), total hardness: 140 - 146 mg/L as $CaCO_3$
Temperature:	9.7 - 11.1 °C
pH:	7.9 - 8.6
O_2 content:	8.9 - 11.7 mg/L (dissolved oxygen > 60% of air saturation)
Conductivity:	217 - 259 µS

Light regime:	Light/dark cycle of 16/8, light intensity 208 - 344 lux Photoperiod started on study day 39; darkness prior to this day.
Methods:	There were 4 replicate tanks (each holding 9.9 L of water) for the control and each treatment level. To maintain water quality and dissolved oxygen levels, a flow-through system was used to deliver dilution water to the test chambers. On the first day of dosing the test systems (Day 0), 50 newly-fertilized eggs were placed into an incubation cup (one per replicate) before the first dosing. Hence, 200 eggs per treatment were used. In addition, 50 newly-fertilized eggs were also placed into an extra incubation cup in one replicate of the control for early-determination of baseline egg-viability (i.e. fertilisation-success). Whilst the flow-through was turned off, an aqueous dosing-solution of test substance was administered manually, directly to the dilution water within each test chamber followed by gentle stirring, and sampling of water for chemical analysis. The flow-through system was turned off for 24 h after each dosing event. The test item was administered on 12 occasions, once every 7 days (Day 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 and 77). This was to cover the maximum number of 10 applications in the GAP and also to ensure that the feeding-fry (post swim-up fry which were free-feeding and with functioning gills) were repeatedly exposed to the test item. On Day 26 when embryonic development was observed, in accordance with the protocol the eggs were impartially reduced (thinned) to 20 successfully-fertilised eggs per replicate. This thinning step was necessary because fertilisation-success in controls and treatments turned out to be close to 100%. During the study each developing life-stage (egg → sac-fry → feeding-fry) was itself exposed to Captan on multiple occasions.

	Development Stage - Fertilized Eggs																											
Study Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Dosing Event (*)	*							*							*							*							*
Notes:	D1							D2							D3							D4					DT		D5; HS

	Development Stage - Fertilized Eggs & Sac Fry										Development Stage - Sac Fry & Fry Swim up																	
Study Day	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Days Post-Hatch	NA	NA	NA	NA	NA	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Dosing Event (*)							*							*							*							*
Notes:							D6				HC; R			D7		SS					D8							D9; SC

	Development Stage - Fry																											
Study Day	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
Days Post-Hatch	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Dosing Event (*)							*						*							*								
Notes:							D10						D11							D12								

	Development Stage - Fry										
Study Day	85	86	87	88	89	90	91	92	93	94	95
Days Post-Hatch	51	52	53	54	55	56	57	58	59	60	61
Dosing Event (*)	none										
Notes:											ST

Key to Abbreviations: D# = dosing event number; DT = day eyed-embryos were thinned to a constant number of 20 per replicate; HS = hatch started; NA = not applicable; HC = hatch completed; R = all live fry released from egg cups into the appropriate test chamber; SS = start of swim-up; SC = swim-up completed.

Figure 1: Dosing events and development stage during an early-life stage exposure of rainbow trout to Captan 80 WDF

Test parameters: Each day, the embryos were observed and dead embryos removed and discarded. At Day 11, the eggs placed in one control replicate for early-determination of baseline viability were removed, cleared in a 10% acetic acid solution and assessed to determine fertilisation-success (a destructive assessment). For the eggs in the main experiment, when embryonic development was visible (eyed-embryo stage; Day 26) surviving embryos were impartially reduced ('thinned') to 20 per replicate. All non-viable embryos observed at the time of thinning were removed. Out of 20 viable eggs per replicate, the length of time to 95% hatch, and % hatchability was then recorded. The date of 0 days-post-hatch (dph) was based on time of ≥ 95% hatch in the controls.

When hatching was complete, all live sac-fry (from the 20 eggs per replicate) were counted. The sac-fry were released into their respective test chambers prior to swim-up (i.e. the transition to feeding-fry). Survival was monitored daily and any behavioural changes were recorded. On dosing days, these observations were performed approximately one hour after dosing. At Day 95 (61 dph), the surviving feeding-fry were sacrificed, weighed and measured.

Temperature, pH, and dissolved oxygen concentration were measured in all replicates of the test substance treatments and control group at test initiation, at least weekly throughout the test, and at termination of the definitive test. Total hardness (as CaCO3) was measured in water samples collected from each test replicate at test initiation and at termination.

Analytical measurements: During the definitive test, the concentrations of captan were measured in test solution samples collected on day 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 69, and 76. Additionally, on day 21, samples were collected approximately 1, 2, 4, and 10 hours after dosing. Test solutions were analysed for the concentration of captan using a gas chromatography with mass selective detector (GC-MSD) system.

In addition, the DT_{50} and DT_{90} values of captan were determined. The rate captan concentrations declined immediately after a dosing event was established on Day 21. Analytical samples were collected 1, 2, 4, and 10 hours after the Day 21 dosing event at all treatment levels. Measured Captan concentrations in the two highest test substance treatments were plotted against time and fitted to single first-order kinetics.

Statistics: The NOEC and LOEC for egg viability, egg hatchability, and fry survival data were determined by using a Fisher's exact test. A Hochberg adjustment was used to control the experiment-wise error rate for the Fisher's test at the same alpha level. In addition, the NOEC for these parameters was estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test with the alternate hypothesis being the mean proportion hatched/surviving was reduced in comparison to the control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test (p = 0.01) and

a Levene's test (p = 0.01) were conducted to test for normality and homogeneity of variance, respectively, of the raw and transformed values over treatments.
The time to start of hatch and fry swim-up, and time to complete hatch and fry swim-up were evaluated using an ANOVA procedure and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter was greater for the test substance treatments compared to the control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, of the raw and transformed values over treatments.
The NOEC values for total length and blotted wet weight were determined using a nested ANOVA procedure, where the treatment means are weighted for the number of individually weighed and measured fish in each chamber, and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter for the test substance treatments was reduced in comparison to the control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, of the raw and transformed values over treatments.

Biological data (hatching success/embryo survival, fry survival and growth data (standard length and wet weight)) for the replicate chambers of each concentration were grouped together for analysis. Replicate means were used for statistical analysis since each test chamber (aquarium) was an experimental unit based on the design of the test system. Data in percent were arcsine transformed before analysis.
For each parameter analysed the following statistical tests were conducted:
- Shapiro Wilk-test procedure in order to test the correspondence with normal distribution
- Levene's-test to check homogeneity of variances
- One-sided William's test on multiple pair-wise comparisons was used subsequently to determine a significant difference between the treatment groups and the control with conclusions of statistical significance based on a 95 % confidence level ($\alpha = 0.05$).

<u>Regression Estimates:</u>
ECx-values were estimated by Probit analysis using linear max. likelihood regression. The observations used were replicate means (length and weight) or replicate proportions (hatching success/embryo survival, fry survival).

<u>Findings:</u>

Analytical data: The mean measured concentrations were between 80 and 124% of the nominal concentrations. The measured concentrations from four replicates at each exposure day were between 69 and 173%.

In addition, the captan exposure-profile was fully characterized for all treatment levels at the fourth dosing event which was on Day 21. This was to provide an exposure-profile relevant for the 12 dosing events. At 0.10 mg a.i./L, the DT_{50} was 2.48 hours and the DT_{90} was 8.25 hours. At 0.20 mg a.i./L the DT_{50} was 1.69 hours and the DT_{90} was 5.63 hours. See Figures 13 and 14.

Table B. 9.3.2-1: Analytical measurements

Nominal concentrations [mg ai/L]	Average measured concentrations [mg ai/L] (% of nominal)					
	Control	**0.0125**	**0.025**	**0.05**	**0.1**	**0.2**
0	< MQL	0.00758	0.0178	0.052	0.0816	0.190
7	< MQL	0.0115	0.0209	0.0393	0.0747	0.209
14	< MQL	0.00164	0.0274	0.0391	0.0765	0.217
21	< MQL	0.0164	0.0223	0.0382	0.0801	0.205
28	< MQL	0.0145	0.0252	0.0419	0.0806	0.237
35	< MQL	0.0148	0.0253	0.0437	0.0747	0.212
42	< MQL	0.0195	0.0280	0.0390	0.0739	0.180
49	< MQL	0.0216	0.0252	0.0251	0.0891	0.189
56	< MQL	0.0151	0.0280	0.0424	0.0897	0.211
63	< MQL	0.0112	0.0172	0.0393	0.0809	0.183
69	< MQL	0.0144	0.0278	0.0428	0.0774	0.210
76	< MQL	0.0225	0.0268	0.0483	0.0762	0.186
Mean (SD)	< MQL	0.0155 (0.00436)	0.0243 (0.00389)	0.0410 (0.00641)	0.0796 (0.00527)	0.202 (0.0170)

MQL…Minimum Quantifiable Limit (0.000224 – 0.000226 mg ai/L)
SD…Standard deviation

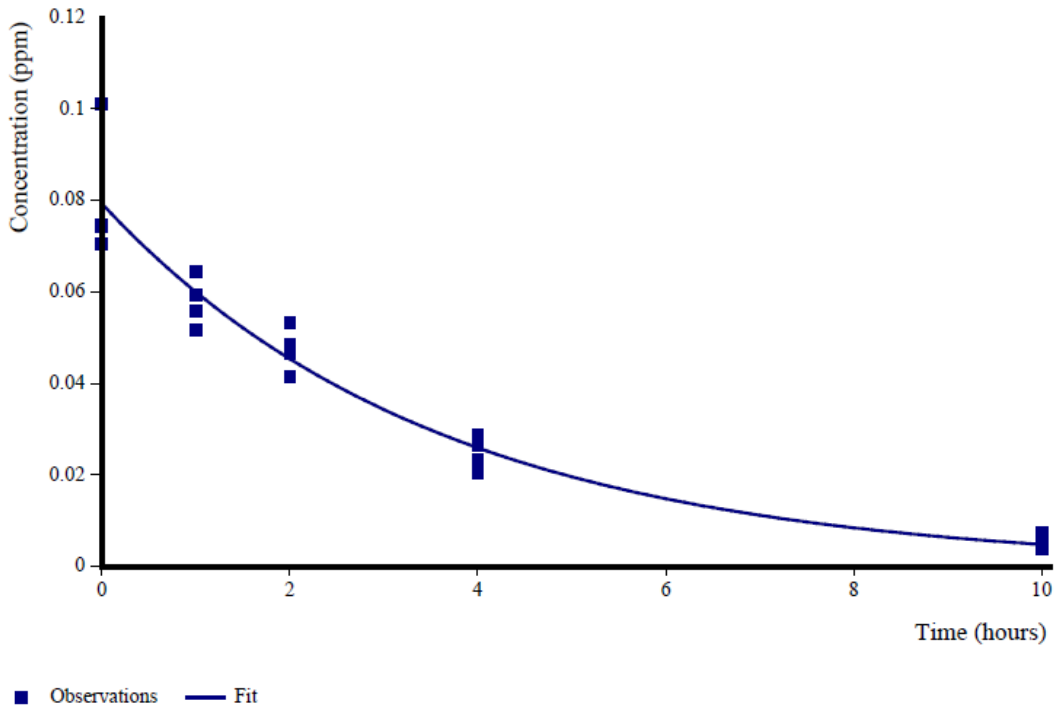


Figure 2: Decline of Captan in the 0.1 mg ai/L treatment level on study day 21

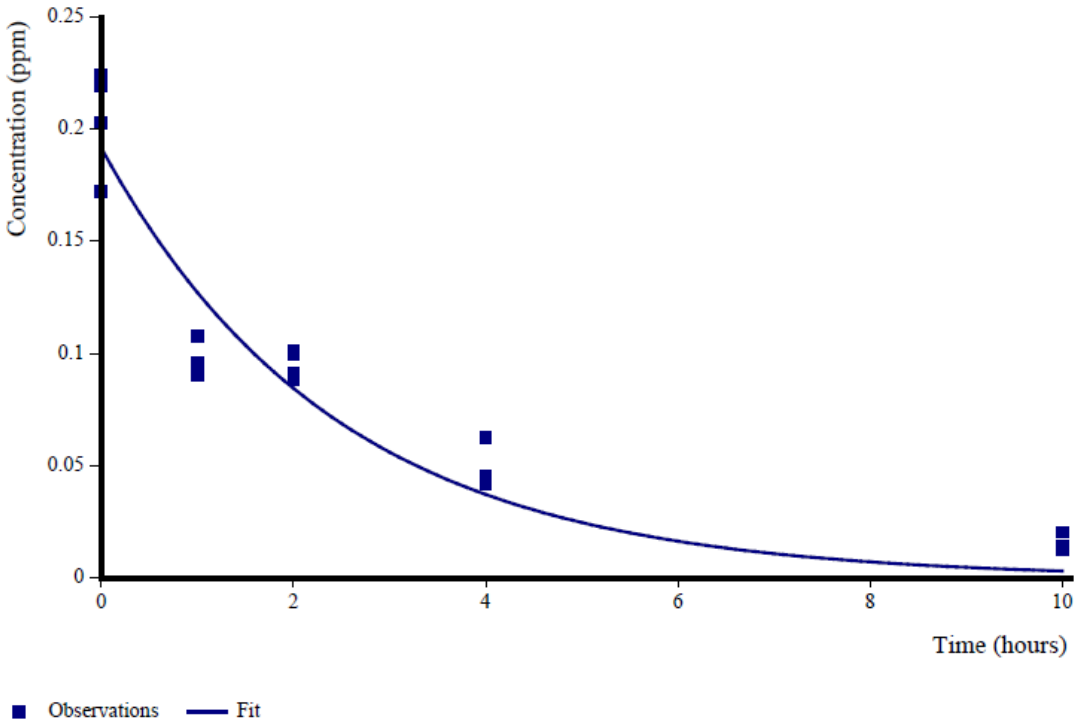


Figure 3: Decline of Captan in the 0.2 mg ai/L treatment level on study day 21

Reproduction: The mean percent of viable eggs prior to thinning was between 97 and 99%. No statistically significant effects compared to the control were observed.

The hatchability of rainbow trout embryos was between 98 and 100%. For detailed results see Table B. 9.3.2-2.

The swim-up stared between day 43 and 50. At the test concentration 0.025 mg ai/L a statistically significant effect (Dunnett's, $p \leq 0.05$) compared to the control was observed. However, this difference was not considered to be treatment-related because there was no statistically significant difference at all higher test substance concentrations including the highest nominal concentration tested (i.e., 0.200 mg a.i./L). There was no statistically significant difference in average time to completion of swim-up for any of the test substance treatments as compared to the control.

The day swim-up started was later (day 50) compared to the control (day 45). The day swim-up was nearly completed was at day 55. The number of days between swim-up start and completion was between 4 days and 12 days.

Table B. 9.3.2-2: Hatchability of rainbow trout embryos

Nominal concentration [mg ai/L]	Initial number of eggs per replicate	Mean days to hatch start	Mean days to hatch completion	Mean % egg hatchability
Control	20	32	34	100
0.0125	20	31	34	99
0.025	20	32	34	99
0.05	20	32	35	98
0.1	20	32	36	99
0.2	20	32	35	100

Survival: The mean percent post-hatch survival of rainbow trout was between 92 and 96%. No statistically significant effects on the survival were observed.

There were no statistically significant differences in mean growth as compared to the control mean.

Table B. 9.3.2-3: Post-hatch survival of rainbow trout and growth parameters

Nominal concentration [mg ai/L]	Mean % survival at termination	Mean total length [mm] (SD)	Mean wet weight [g] (SD)
Control	96	43.6 (2.8)	0.748 (0.176)
0.0125	96	44.0 (2.4)	0.768 (0.152)
0.025	92	43.2 (3.0)	0.736 (0.183)
0.05	93	43.7 (3.0)	0.776 (0.213)
0.1	95	43.3 (3.4)	0.758 (0.213)
0.2	94	43.1 (3.3)	0.734 (0.210)

SD…Standard deviation

Behaviour: Effects on the behaviours of fry and juveniles were observed at all test concentrations, except at the lowest test concentration (0.0125 mg ai/L) and the control group. The behavioural effects were loss of equilibrium, irregular respiration and fish were observed to be on the bottom of the test chamber.

Overall, these few occurrences were not indicative of a concentration-dependent response. The few occurrences of behavioural signs which were observed were generally similar before and immediately after a dosing event. At study day 95 (test termination), the surviving fish displayed no morphological or behavioural abnormalities.

Table B. 9.3.2-4: Behavioural effects

Nominal test concentration [mg a.s./L]	Rep. tank	Arbitrary Fry ID	First study day sign observed	Type of sign	Fry found dead [a]	Recovered from clinical signs
0.025	A	Fry 1	Day 53	B + LE + I	Day 89 to 94	-
0.050	B	Fry 2	Day 53	B + LE + I	Day 60	-
	A	Fry 3	Day 56-57	B + I	Day 71	-
	A	Fry 4	Day 56-57	B + I	Day 74-76	-
0.100	D	Fry 5	Day 53	B + LE	Survived	Day 58
0.200	B	Fry 6	Day 51	B + LE	Day 54	-
	B	Fry 7	Day 77	I	Day 78-81	-
	C	Fry 8	Day 52	B + LE	Day 54	-
	D	Fry 9	Day 53	LE	Survived	Day 82-88

[a] assuming is same fry as the one with sign
B…fish on bottom of test chamber, LE…loss of equilibrium, I…irregular respiration

Table B. 9.3.2-5: Summary of all endpoints

Endpoint	NOEC	LOEC
	[mg a.s./L]	
Time to hatch	0.2	> 0.2
Time to hatch completed	0.2	> 0.2
Egg hatchability	0.2	> 0.2
Time to fry swim-up start	0.2	> 0.2
Time to fry swim-up completed	0.2	> 0.2
Fry survival	0.2	> 0.2
Total length	0.2	> 0.2
Total wet weight	0.2	> 0.2

Conclusion: Based on the nominal peak concentrations of captan (as confirmed by chemical analysis), the NOEC and LOEC for rainbow trout egg viability, time to hatch start, time to hatch completion, egg hatchability, start of swim-up, time to completion of swim-up, total length, blotted wet weight and fry survival were 0.200 and >0.200

mg ai/L, respectively. Hence, the overall NOEC was 0.200 mg ai/L for 12 peaks of exposure.

Comment RMS:

Validity: The test was evaluated according to the OECD test guideline 210 (2013) and the US EPA test guideline OPPTS 850.1400.

According to the OECD test guideline (2013) the study is considered valid if

- the dissolved oxygen concentration is > 60% of the air saturation value throughout the test.
- the water temperature does not differ by more than ± 1.5 °C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species (*Oncorhynchus mykiss*: 10 ± 1.5 °C).
- the analytical measure of the test concentrations is compulsory.
- the overall survival of fertilised eggs and post-hatch success in the controls are greater than or equal to the limits defined in Annex 2 (*Oncorhynchus mykiss*: 75% minimum hatching success and post-hatch success).

According to the current valid US EPA test guideline OCSPP 850.1400 (2016) the test is considered valid if

- all test vessels and compartments were identical.
- the treatments were randomly or indiscriminately assigned to individual test vessel locations, or individual test organisms were randomly or indiscriminately assigned to test vessels or compartments.
- a dilution water control was included in the test.
- the test was begun with embryos before first cleavage of the blastodisc (see paragraph (e)(6)(ii) of this guideline).
- the test was terminated according to the appropriate duration given for the test species in Tables 3 and 4. (*Oncorhynchus mykiss*: 2 weeks after control are free-feeding or 60 days post-hatch)
- the control survival and hatching success did meet the performance standards in Tables 3 and 4. (*Oncorhynchus mykiss*: 66% minimum hatching success and 70% minimum post-hatch success)

According to the validity criteria, the study is considered acceptable. The environmental conditions (dissolved oxygen, temperature) are within the recommended ranges. The biological criteria, i.e. hatching success and post-hatch success in the control are 100% and 96%, respectively.

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

Endpoints: The RMS agrees on the determined NOEC of 200 µg ai/L 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, EC_x values should be determined for lethal and sub-lethal effects. In the study the percent reduction hatchability, fry survival, length and growth 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 fish toxicity test (ELS) is considered valid.

B.9.3.3. Further testing on aquatic organisms

Not required.

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
KCP 10.2.1		2018	Captan: Acute toxicity to fish, derivation of RACSSD, and identification of 'safe uses' for Annex 1 renewal ADAMA Makhteshim Ltd and RidgewayEco 000099346 Not GLP Not Published	N	N		ADM/ARY
KCP 10.2.1		2018	Captan – Acute toxicity to fish: Tier 2B-RACs based on Species Sensitivity Distributions Eurofins Regulatory AG ERA1781-A Not GLP Not Published	N	N		ADM/ARY
KCP 10.2.1		2018	ADDENDUM to the document: "R-37610_Captan - Tier 1 endpoint for fish and RAC for fish 24-5-2017_sub AGES", dated 24 May 2017 (Ref: Norman & Ortiz, 2017). ERA-1697-AB Not GLP Not Published	N	N		ADM/ARY
KCP 10.2.1		1993a	Captan: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) of an 83% WP formulation. Zeneca, Report No. BL5005/B (Company file: TMN-0021). GLP, Unpublished.	Y	N	N/A	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
KCP 10.2.1		1995	Acute toxicity of Merpan (Captan) 83 WP in rainbow trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae) under flow-through conditions GAB, Report No. 94131/02-AsOm (Company file: R-8249). GLP, Unpublished	Y	N	N/A	ADM/ARY
KCP 10.2.1		1996	Assessment of toxic effects of Merpan 80 WDG on *Daphnia magna* using the 48 h acute immobilisation test. GAB, Report No. 95194/01-AADm (Company file: R-8857). GLP, Unpublished.	N	N	N/A	ADM/ARY
KCP 10.2.1		1993b	Captan: Acute toxicity to *Daphnia magna* of an 83% WP formulation. Zeneca, Report No. BL4962/B (Company file: TMN-0007). GLP, Unpublished.	N	N	N/A	ADM/ARY
KCP 10.2.1		1996	Testing of toxic effects of Merpan 80 WDG on the single cell green alga *Scenedesmus subspicatus*. GAB, Report No. 95194/01-AASs (Company file: R-8855). GLP, Unpublished.	N	N	N/A	ADM/ARY
KCP 10.2.1		1993	Captan: Toxicity to the green algal *Selenastrum capricornutum* of an 83% WP formulation. Zeneca, Report No. BL5001/B (Company file: TMN-0015). GLP, Unpublished.	N	N	N/A	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
KCP 10.2.1		2016	CAPTAN 80 WDG Alga, Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 hours, including recovery phase ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., SPO16613 DR.U.NOACK-LABORATORIEN, Sarstedt, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	ADM/ARY
KCP 10.2.2.1		2016	Captan (tested as 80 WDG formulation): Early life-stage toxicity test on the rainbow trout, *Oncorhynchus mykiss*, with repeated static exposure ADAMA Makhteshim Ltd / Arysta LifeScience S.A.S., 82420, R-36002 ABC Laboratories, Columbia, Missouri, USA GLP: yes Published: no	Y	Y	New data for active ingredient, not previously submitted nor evaluated	ADM/ARY
KCP 10.2.4		2018	Captan: Acute toxicity to aquatic invertebrates and derivation of refined RAC value ADAMA Makhteshim Ltd and RidgewayEco Not GLP Not Published	N	N		ADM/ARY