Name: OECD / Short-term toxicity to aquatic invertebrates / Short-term toxicity to aquatic invertebrates.p-cymene_2018_Key / para-cymene / 1-isopropyl-4-methylbenzene / 99-87-6

Legal entity owner:

Printing date: 2019-01-09T08:54:30.379Z

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ENDPOINT_STUDY_RECORD: Short-term toxicity to aquatic invertebrates.p-cymene_2018_Key

UUID: 220af7c6-fa90-4269-8020-0f7b82cbb94b
Dossier UUID:
Author: Kristi Tatsi
Date: 2018-04-05T16:00:21.127Z
Remarks:

Administrative data

EU: REACH

Endpoint
short-term toxicity to aquatic invertebrates

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
false

Used for SDS
false

Study period
March - April 2018

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

p-Cymene: Determination of acute toxicity to Daphnia magna / D Hill / study report

Data access
data submitter is data owner

Data protection claimed
yes, but willing to share

Materials and methods

Test guideline

Qualifier
according to

Guideline
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)

GLP compliance
yes (incl. certificate)

Test material

Test material information
p-Cymene

Specific details on test material used for the study
Identity: p-Cymene
Batch/Lot Number: 8010011
CAS Number: 99-87-6
EC Number: 202-796-7
Scymaris Reference Number: 1027.006
Purity: 99.9%
Water Solubility: Immiscible
Appearance: Clear colourless liquid
Expiry Date: 31 October 2019
Storage Conditions: Room temperature

Sampling and analysis

Analytical monitoring
yes

Details on sampling
The concentrations of p-Cymene in the test solutions were measured at 0 and 48 hours for each test c oncentration using GCMS. Samples for analysis at 0 hours were taken from the excess test solutions of the dilution water control and each test concentration and at 48 hours from a single test replicate solution or pooled replicates of the dilution water control and each test concentration. If there was e vidence that the concentration of the substance being tested had been maintained within ± 20% of the nominal or measured initial concentration throughout the test, analysis of the results were based on nominal or measured initial values. If the deviation from the nominal or measured initial conce ntration was not within ± 20%, analysis of the results was based on the geometric mean concentration during exposure or models describing the decline of the concentration of the test substance.

Details on analytical methods
Samples were analysed by GC-MS and quantified by comparison against known standards of p-Cy mene.

Materials and reagents: A sample of test substance p-Cymene,,n-butylbenzene, Hexane, Acetone, Water.
Preparation of stocks and standards: Stock solutions were prepared in acetone and standard solutio ns were prepared in hexane containing 0.05 mg/L n-butylbenzene.

Sample Introduction and Chromatographic Separation

The following conditions were found to be suitable for the analysis of the p-Cymene:
Column 15 m x 0.25 mm
Stationary Phase TR-5MS (df = 0.25 µ)
Carrier Gas Helium @ 1.5 mL/min
Injection mode Splitless (split @ 1.0 min)
Injector Temperature 220°C
Injection Volume 2.0 µL
Column temperature 40°C hold for 2 min
Ramp to 120°C @30°C/min
Ramp to 220°C @80°C/min

Mass Spectrometer
Transfer line Temperature 300°C
Scan Type SIM +ve ion
MS Source Temperature 230°C
MS Quad Temperature 150°C

Group 1 p-Cymene
Time 2.00 Minutes
Plot Ion 119,134 m/z
Dwell 100, 100

Group 2 N-butylbenzene
Time 3.80 Minutes
Plot Ion 91, 134 m/z
Dwell 100, 100

Sample preparation
The following procedure was found to be suitable for the analysis of samples produced from Daphnia studies. Samples were extracted into hexane (containing 1.0 mg/L n-butylbenzene). 100ml sample was added to a 250ml volumetric flask. 2ml 1.0 mg/L n-butylbenzene in hexane was added, the flask was stoppered and shaken thoroughly for 2 minutes. The sample was left to settle for >15 minutes and RO water was added to the mark. An aliquot of the hexane layer was transferred to a 2ml glass sample vial, for analysis by GCMS.

Test solutions

Vehicle
yes Dimethylformamide (DMF)

Details on test solutions
The dilution water used for testing (and maintenance of stock cultures) was Elendt M4 medium. Physical and chemical analyses was performed on the dilution water used for testing. pH of the medium was within the range 6-9. Hardness of the medium was between 140 and 250 mg/L (as CaCO3).

Range-finding study
The range-finding study was run with a dilution water control and solvent control together with nominal concentrations of 0.0625, 1.25, 2.5, 5.0 and 10 mg/L. The test was performed under static conditions. The 4 replicate test vessels received approximately 200 mL of test solution and the remaining solution was retained for physical and chemical analysis.

Definitive test
This study was run with a dilution water control and solvent control together with nominal concentrations of 0.0625, 1.25, 2.5, 5.0 and 10 mg/L. The test was performed under semi-static conditions, test solutions were renewed after 24 hours. The 4 replicate test vessels received approximately 200 mL of test solution and the remaining solution was retained for physical and chemical analysis.

Test organisms

Test organisms (species)
Daphnia magna

Details on test organisms
The test organism used was the freshwater crustacean, D. magna, <24 hours old at the start of the test, and not first brood progeny, derived from continuous laboratory cultures. They were derived fro m a healthy culture (i.e. showing no signs of stress such as high mortality, presence of males and ephippia, delay in the production of the first brood, discoloured animals, etc.). This species has been selected because it is representative of a freshwater crustacean and is that recommended by OECD TG 202.
Culture methods and culture records will be documented in the current DC series record book. The D. magna used for testing will be cultured in the dilution water and under the same photoperiod an d temperature conditions used for the study. Test organisms were fed (with a dual algal diet of Pse udokirchneriella subcapitata and Chlorella vulgaris) prior to the start of the test in order to minimise potential starvation effects during the pre-test holding period.

Study design

Test type
semi-static

Water media type
freshwater Elendt M4 medium

Total exposure duration

48	h

Test conditions

Hardness
140 and 250 mg/L (as CaCO3)

Test temperature
18 - 22°C kept constant to within ± 1°C

pH
6-9

Nominal and measured concentrations
Nominal concentrations of 0.0625, 1.25, 2.5, 5.0 and 10 mg/l.

Details on test conditions
Materials in contact with the dilution water, test solutions, test substance and D. magna were, where practical, restricted to glass and unplasticised plastics. The test vessels were glass beakers of 250 mL nominal capacity (to contain 200 mL of test solution), with four replicates per test concentration. The beakers were covered with loose fitting glass panes and positions of the treatments were rando mised within the test area.

The test was performed under semi-static conditions. The test was initiated by the addition of 5 randomly selected D. magna in <2.0 mL of dilution water to each test vessel. D. magna were transf erred to each test vessel using a disposable plastic pipette. At 24 hours, D. magna were transferre d to ‘fresh’ test solutions using the same method. Each treatment will contain a total of 20 D. mag na. At least five test concentrations were used and these were arranged in a geometric series with

a separation factor not exceeding 2.2. The test solutions were maintained in the range 18 - 22°C ke pt constant to within ± 1°C, and a photoperiod of 16 hours light: 8 hours dark, with a 20 minute da wn:dusk transition period was provided. Test solutions were not aerated and D. magna were not fed during the course of this study.

Results and discussion

Effect concentrations

Key result
false

Duration

48 h

Dose descriptor
NOEC Immobilisation

Effect conc.

2.3 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
mobility

Key result
false

Duration

48 h

Dose descriptor
LOEC Immobilisation

Effect conc.

4.9 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
mobility

Key result
true

Duration

48 h

Dose descriptor
EC50 Immobilisation

Effect conc.

3.7 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
mobility

Details on results
Range-finding study
In the range-finding study there was no effect on mobility up to the nominal 10 mg/l concentration with only minor observations of daphnids on the surface. The chemistry showed that at 0 hours 52-82% of nominal was achieved and at 48 hours this had dropped to 5-9% of nominal. As such, a semi-sta tic study was selected for the definitive test with a renewal at 24 hours using the same nominal co ncentrations.

Definitive study
In the definitive study at a nominal concentration of 5.0 mg/l daphnids were considerably less active t han controls, at a nominal concentration of 10 mg/l the reamining mobile daphnids were less active than controls. The chemistry showed that at 0 hours 75-86% of nominal was achieved, at 24 hours 16-27% of the nominal was achieved and at 48 hours 19-30% of nominal wa achieved. This result ed in mean measured concentrations of 0, 0.30, 0.56, 1.30, 2.30, 4.90 mg/l (equivalent to nominal c oncentrations of 0, 0.625, 1.25, 2.5, 5.0, and 10 mg/l, respectively). The results showed a NOEC of 2.3 mg/l (immobilisation, mean measured concentration) and a LOEC of 4.9 mg/l (immobilisation, mean measured concentration), with the EC50 (immobilisation) of 2.3-4.9 mg/l (mean measured concentration.

Any other information on results incl. tables

Table 1. Range finding study animal data.

Nominal concentration (mg/L)	Number of dead/ immobile at 48 hours	Total dead/ immobile	Total tested	Percentage immobility
Control	0	0	10	0
Solvent Control	0	0	10	0
0.625	0	0	10	0
1.25	0	0	10	0
2.5	0	0	10	0
5	0	0	10	0
10	0	0	10	0

Test observations:

Shortly after test set up, ~90% of daphnids in the 10 mg/L concentration mobile but appear stuck on surface.

24 hours:

0.625 mg/L: 10% of daphnids on surface

2.5 mg/L: 20% of daphnids on surface

5.0 mg/L: 40% of daphnids on surface

10 mg/L: 80% of daphnids on surface

All daphnids on surface resubmerged with test solution in glass Pasteur pipette.

48 hours:

No symptoms of toxicity recorded

Table 2. Range finding study analytical data.

Nominal concentration (mg/L)	**0 hour measured concentration (mg/L)**	**0 hour measured conc. (% of nominal)**	**48 hour measured concentration (mg/L)**	**48 hour measured conc. (% of nominal)**	**Mean measured concentration (Geometric mean) (mg/L)**	**Mean measured concentration (Geometric mean) (% of nominal)**
Control	<LOQ	-	<LOQ	-	-	-
Solvent Control	<LOQ	-	<LOQ	-	-	-
0.625	0.47	76	0.053	9	0.16	26
1.25	0.65	52	0.057	5	0.19	15
2.5	1.3	53	0.14	6	0.43	17
5.0	3.0	60	0.30	6	0.95	19
10	8.2	82	0.66	7	2.3	23

Table 3. Definitive study animal data.

Nominal concentration (mg/L)	**Number of dead/ immobile at 24 hours**	**Number of dead/ immobile at 48 hours**	**Total dead/ immobile**	**Total tested**	**Percentage immobility (48 hours)**
Control	0	0	0	20	0
Solvent Control	0	0	0	19*	0
0.625	0	1	1	20	5
1.25	0	0	0	20	0
2.5	0	0	0	20	0
5.0	0	0	0	20	0
10	0	18	18	20	90

*One daphnid was damaged by accident during the 24h transfer to new test solutions.

Table 3. Definitive study analytical data.

Nominal concentration (mg/L)	0 hour measured conc. (mg/L)	0 hour measured conc. (% of nominal)	24 hour measured conc. (aged) (mg/L)	24 hour measured conc. (aged) (% of nominal)	24 hour measured conc. (fresh) (mg/L)	24 hour measured conc. (fresh) (% of nominal)	48 hour measured conc. (mg/L)	48 hour measured conc. (% of nominal)	Time-weighted mean conc. (mg/L)
Control	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	-
Solvent Control	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	-
0.625	0.52	83	0.13	20	0.71	114	0.12	19	0.30
1.25	1.0	81	0.25	20	1.2	93	0.24	19	0.56
2.5	2.1	84	0.51	20	3.2	130	0.60	24	1.3
5.0	4.3	86	0.81	16	4.4	89	1.3	26	2.3
10	7.5	75	2.7	27	7.8	78	3.0	30	4.9

Overall remarks, attachments

Overall remarks

In a 48 -hour study, Daphnia magna were exposed to para-cymene in dimethylformaide at nominal contrations of 0, 0.625, 1.25, 2.5, 5.0 and 10 mg/l (mean measured concentrations of 0, 0.30, 0.56, 1.30, 2.30, 4.90 mg/l, respectively). At the top two doses daphnids were significantly less mobile than controls and at the top dose 90% of the daphnids present were immobile. A NOEC of 2.3 mg/l (immobilisation, mean measured concentration) and a LOEC of 4.9 mg/l (immobilisation, mean measured concentration) were reported, and the EC50 (immobilisation) is 3.7 mg/l (mean measured concentration).

Applicant's summary and conclusion

Validity criteria fulfilled
yes

Conclusions
A NOEC of 2.3 mg/l (immobilisation, mean measured concentration) and a LOEC of 4.9 mg/l (im mobilisation, mean measured concentration) were reported, and the 48 h EC50 (immobilisation) is 3.7 mg/L (mean measured concentration).

Executive summary

In a 48-hour study (OECD TG 202), Daphnia magna were exposed to para-cymene in dimethylformaide at nominal contrations of 0, 0.625, 1.25, 2.5, 5.0 and 10 mg/l (mean measured concentrations of 0, 0.30, 0.56, 1.30, 2.30, 4.90 mg/l, respectively). At the top two doses daphnids were significantly less mobile than controls and at the top dose 90% of the daphnids present were immobile. A NOEC of 2.3 mg/l (immobilisation, mean measured concentration) and a LOEC of 4.9 mg/l (immobilisation, mean measured concentration) were reported, and the EC50 (immobilisation) is 3.7 mg/l (mean measured concentration).

References

TEST_MATERIAL_INFORMATION: p-Cymene

UUID: c716091d-e4ca-42c2-848d-3156c2a096bb
Dossier UUID:
Author: Kamila Solak
Date: 2018-03-16T13:49:40.606Z
Remarks:

Name
p-Cymene

Composition

Type
Constituent

Reference substance
para-cymene / 1-isopropyl-4-methylbenzene / 99-87-6 / 202-796-7

EC number	EC name
202-796-7	EC Inventory
CAS number	**CAS name**
99-87-6	
IUPAC name	
1-isopropyl-4-methylbenzene	

Composition / purity: other information
other: 99.6%

Other characteristics

Test material form
liquid

LITERATURE: p-Cymene: Determination of acute toxicity to Daphnia magna

UUID: 64f14a22-5300-4f83-9304-3170a263ac3b
Dossier UUID:
Author: Victoria Benson
Date: 2018-04-04T11:15:10.008Z
Remarks:

General information

Reference Type
study report

Title
p-Cymene: Determination of acute toxicity to Daphnia magna

Author
D Hill

Year
2018

Testing facility
Scymaris Ltd.

Report no.
1027.00602

Study sponsor
Symrise AG

Study no.
1027.00602