Annex I 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:

3-aminomethyl-3,5,5-trimethylcyclohexylamine; isophorene diamine [IPD]

EC Number: 220-666-8

CAS Number: 2855-13-2

Index Number: 612-067-00-9

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1 PHYSICAL HAZARDS

Not evaluated in this dossier

2 HEALTH HAZARDS

Acute toxicity

2.1 Acute toxicity - oral route

2.1.1 Animal data

[Study 1]

Administrative Data

Purpose flag	key study; robust study summary; used for classification		
Study result type	experimental result	**Study period**	Between 1965-03-04 and 1965-06-10
Reliability	3 (not reliable)		
Rationale for reliability incl. deficiencies	Comparable to guideline study with significant deviations: no data on mortality / dose group and how LD_{50} was calculated, only male animals used. Evidence from repeated dose studies indicates that there is no significant difference in sensitivity between males and females and that the acute oral toxicity is not higher by an order of magnitude or more (chapter 7.5.1 entry # 1: 13 week LOAEL ca. 150 mg/kg bw/day for males and females).		

Reference

Reference Type: study report, unpublished

Title: Toxikologische Prüfung von IPD und TMD

Year: 1965

Report Date: 1965-06-10

Materials and methods

Test type

standard acute method

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 401 (Acute Oral Toxicity)	Only male animals were used, deficiencies in reporting

Principles of method if other than guideline

see Test Conditions

GLP compliance

no

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
CAS number	2855-13-2
common name	Isophorone diamine
EC name	3-aminomethyl-3,5,5-trimethylcyclohexylamine

Details on test material

Isophorone diamine, no data on purity

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Details on test animals and environmental conditions

- Weight at study initiation: 110-130 g
- Fasting period before study: since day before

Administration / exposure

Route of administration

oral: gavage

Vehicle

water

Details on oral exposure

- Concentration in vehicle: 50 % (v/v)
- Amount of preparation: 0.5, 1.0, 1.5, 2.0 and 2.5 ml per kg b.w.

Doses

50 % v/v solution in water, 0.5, 1.0, 1.5, 2.0 and 2.5 ml per kg b.w.

No. of animals per sex per dose

5

Control animals

no

Details on study design

Post dose observation period: 14 days
Urine control for proteine

EXAMINATIONS: organs not listed

Statistics

no data

Any other information on materials and methods incl. tables

no further information

Results and discussions

Preliminary study (if fixed dose study)

not applicable

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male	LD_{50}	kg bw			

Mortality

Time of death: after 12 to 18 hours in lateral position, no data on mortality / dose group

Clinical signs

1 hour after dosing, animals showed restlessness, thirst, rough fur and tiredness.

Body weight

no data

Gross pathology

Irritation of the intestinal mucosa, with a few animals showing a slight increase in kidney weight and protein in the urine

POTENTIAL TARGET ORGANS: kidney

Other findings

no other findings

Any other information on results incl. tables

no further remarks

Overall remarks, attachments

Remarks on results including tables and figures

no further remarks

Applicant's summary and conclusion

Conclusions

The LD_{50} value of acute oral toxicity in male rats of the test substance isophorone diamine was determined to be 1030 mg/kg bw.

Executive summary

The acute oral toxicity LD_{50} value in male Sprague-Dawley rats was determined to be 1030 mg/kg b.w.. Doses of 0.5, 1.0, 1.5, 2.0, or 2.5 ml/kg bw of a 50 % v/v solution in water were applied by gavage followed by a post dose observation period of 14 days.Clinical signs observed from 1 hour after dosing were restlessness, thirst, rough fur and tiredness. At necropsy, irritation of the intestinal mucosa was observed. A few animals (no further data) showed a slight increase in kidney weight and protein in the urine, which may indicate that the kidney is a target organ.

2.2 Acute toxicity - dermal route

2.2.1 Animal data

[Study 1]

Purpose flag	key study		
Study result type	experimental result	**Study period**	2010-06-08 to 2010-08-04
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	Guideline study		

Study reference:

Reference Type: study report

Title: Unnamed

Year: 2010

Materials and methods

Test type

fixed dose procedure

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 402 (Acute Dermal Toxicity)	no

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material

Lot No. 10021006
clear liquid, odor of amine,
purity: > 99%

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

- Strain: Sprague-Dawley (Crl:CD(SD)), SPF
- Sex, number, age and body weight range (at receipt)
12 males, 7 weeks old, 197.9 - 210.7 g
12 females, 8 weeks old, 198.8 - 212.1 g
- Sex, number, age and body weight range (on administration)
10 males, 8 weeks old, 263.5 - 288.9 g
10 females, 9 weeks old, 215.6 - 235.9 g
- All animals were observed for general condition and clinical signs daily and body weights were on Day 7 after receipt. All animals were quarantined for 3 days, and acclimated for 4 days.
- Animal husbandry:

Type & size of a cage: stainless wire mesh cages, 260Wx350Dx210H (mm)
Number of animals per cage: one animal/cage (during the study)
Temperature: 21.0 - 23.8 °C
Relative humidity: 40.3 - 53.4%
Air changes: 10 - 15 clean, fresh, filtered air changes per hour
Lighting: 12 hours light/dark
Intensity of illumination: 150 - 300 Lux
- Feed:
Type: Pelleted rodent chow
Method: The diet was placed in feeders and provided ad libitum
- Water:
Type /method: Public tap water was filtered and irradiated; provided ad libitum

Administration / exposure

Type of coverage

occlusive

Vehicle

unchanged (no vehicle)

Details on dermal exposure

- Method of administration:
The subscapular dorsa surface (approx. 5 cm x 6 cm) of each animal`s back was clipped with an electric clipper approx. 24 hours prior to dosing. 4 cm x 5 cm of these shaved areas were designated as the treated sites. After the treatment of the test substance to lint tape, the treated sites were covered with lint tape and plastic film. Each animals back was over-wrapped with Soft Cloth Tape with Liner. At the end of a 24-hour exposure period, lint tape, plastic film and Soft Cloth Tape with Liner were removed and any residual test substance was removed using adsorbent cotton moistened with tepid water. The shaved treated sites of the control animals were dressed in the same manner as the treated animals.

Duration of exposure

24 hours

Doses

The dose level of 2,000 mg/kg was selected for this study, which was expected to show low toxicity.

No. of animals per sex per dose

5

Control animals

yes, concurrent no treatment

Details on study design

Parameters Evaluated:
- Clinical signs: All animals were observed for mortality, general condition and clinical signs

(time onset, severity and recovery) for 30 minutes after dosing and at 1, 2, 4 and 6 hours after dosing on Day 0 and once saily thereafter for 14 days (Days 1 to 14).
- Body weights: Body weights were recovered once on Day 0 prior to treatment on Day 3 and 7 and 0n the day of necropsy, day 14.
- Necropsy: On Day 14, all surviving animals were anesthetised with CO2 and exsanguinated from the abdorminal aorta. Complete gross postmortem examinations were performed on all animals in the study.
- Histopathology: In necropsy findings, crust was observed on the treated sites of all animls in the 2,000 mg/kg dosing group. Therefore, histopathological examinations were performed.

Statistics

Statistical analysis: using SAS Programm;
Body weights: Folded-F test for homogeneity of variance (significant level: 0.05);
Student t-test was employed on homogeneous data (significance level: 0.05);
Aspin-Welch t-test was employed for heterogeneous data (significant level: 0.01)

Any other information on materials and methods incl. tables

no further information

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD50	> 2000 mg/kg bw	test mat.		no mortalities

Mortality

No mortality was observed at 2,000 mg/kg treatment throughout the course of the study.

Clinical signs

On the treated sites at 2,000 mg/kg treatment, discolouration of skin (black) in all males and females and crust formation in two males and five females were observed on Days 1 and/or 2. In addition, discolourations of skin and crust formations were observed in all males and females from Days 3 to 14 after dosing. Scar was observed on the treated sites of three males and females at 2,000 mg/kg treatment on Days 11 to 14.

Body weight

Normal body weight gains were observed in all animals at 2,000 mg/kg treatment.

Gross pathology

Necropsy and histopathological findings:
Crust were observed on the treated sites of all animals at 2,000 mg/kg treatment. In histopathological findings, scar as mild to moderate was observed on the treated sites of all animals.

Other findings:

No other findings

Any other information on results incl. tables

No further information

Overall remarks, attachments

Remarks on results including tables and figures

No overall remarks

Applicant's summary and conclusion

Interpretation of results

not classified

Criteria used for interpretation of results

OECD GHS

Conclusions

Based on the results of this study, the acute toxicity of isophorone diamine after dermal application to male and female rats is low: the LD50 value was determined to be > 2000 mg/kg bw.

Executive summary

This study was conducted to assess the potential toxicity of the test substance isophorone diamine, following a single dermal treatment to Sprague-Dawley rats.

All animals at 2,000 mg/kg treatment survived the duration of the study.

Discolouration of skin and crust formation from Days 1 to 14 after dosing and scar from Days 11 to 14 were observed on the treated sites of all animals at 2,000 mg/kg treatment. These were considered to be test substance-related effects. No test substance-realted affected on body weights were observed.

In necropsy findings, crust was observed on the treated sites of all animals at 2,000 mg/kg treatment. In histopatholocical findings, scar as mild to moderate was observed on the treated sites of all animals. These were considered to be skin wounds caused by the test substance.

Based on the results of this study, Acute toxicity of isophorone diamine, the dermal LD50 was > 2,000 mg/kg in male and female rats.

2.3 Skin corrosion/irritation

Not evaluated in this dossier

2.4 Serious eye damage/eye irritation

2.4.1 Animal data

[Study 1]

Purpose flag	key study; robust study summary; used for classification		
Study result type	experimental result	**Study period**	1983-11-24 - 1983-11-25
Reliability	2 (reliable with restrictions): Original study report was not available, only IUCLID summary		
Rationale for reliability incl. deficiencies	Guideline study		

Study reference:

Reference Type: study report

Title: Unnamed

Year: 1983

Report Date: 1983

Materials and methods

Type of method
in vivo
Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 405 (Acute Eye Irritation / Corrosion) (1981)	no

GLP compliance
no
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes

Test material identity

Identifier	Identity
CAS number	2855-13-2
EC name	3-aminomethyl-3,5,5-trimethylcyclohexylamine
common name	Isophorone diamine

Test animals
Species
rabbit
Strain
other: Small white Russian
Details on test animals and environmental conditions

- Sex: female
- Source: Dr. Karl Thomae, Biberach
- Weight at study initiation: 2.3 kg
- Sex: female
- Weight at study initiation: 2.8 kg
- Controls: untreated eye
- Housing: single housing
- Diet: ad libitum, special diet for rabbits, SSniff K4
- Water: ad libitum, tab water
- Acclimation period: 14 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20.0 +/- 1 °C
- Humidity (%): 60 +/- 5%
- Air changes (per hr): 15 times per hour
- Photoperiod (hrs dark / hrs light): 12 hours artificial light, 12 hours dark

Test system
Vehicle
unchanged (no vehicle)
Amount/concentration applied
undiluted
Amount applied: 0.1 ml
Duration of treatment / exposure
24 hour(s)
Observation period
Postexposure period: 24 hours
Number of animals: 1

Control animals
other: Controls: right eye
Details on study design
Comment: not rinsed
Any other information on materials and methods incl. tables
no further information

Results and discussions
Overall irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
overall irritation score	animal #1	24 hours	110	110	not reversible	Due to the strong corrosive effects of the test substance Isophorone diamine a scoring was not possible and therefore not reported (details see in section "Remarks on results including tables and figures")

Irritant/corrosive response data
The undiluted substance produced serious injury almost immediately after application (corrosive effects, opalescence). 24 hours after treatment

conjunctiva showed necrosis. Due to the corrosive effect of the test material, only 1 animal was used and the experiment terminated after 24 hours.

Other effects

no other effects

Any other information on results incl. tables

Due to the strong corrosive effects of the test substance Isophorone diamine a scoring was not possible and therefore not reported. The undiluted substance produced serious injury almost immediately after application (corrosive effects, opalescence). 24 hours after treatment conjunctiva showed necrosis. Due to the corrosive effect of the test material, only 1 animal was used and the experiment terminated after 24 hours.

Conclusions

Isophorone diamine showed a strongly irritant and corrosive effect on the eye and on the mucosa of the rabbit under the conditions of the study.

Executive summary

Isophorone diamine was investigated for irritant effects on the eye and associated mucous membranes of 1 female rabbit according to OECD TG 405 (1981). The test substance was applied in a single dose of 0.1 ml into the conjuntival sac of the animal. The undiluted substance produced serious injury almost immediately after application (corrosive effects, opalescence). 24 hours after treatment conjunctiva showed necrosis. Due to the corrosive effect of the test material, only 1 animal was used and the experiment terminated after 24 hours.The lesions were not reversible. Therefore Isophorone diamine has to be considered to be corrosive.

2.5 Skin sensitisation

2.5.1 Animal data

[Study 1]

Administrative Data

Purpose flag	key study		
Study result type	experimental result	**Study period**	1983-09-19 - 1983-10-14
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Guideline study with acceptable restrictions: no positive control group (not required by 1981 version of guideline)		

Study reference:

Reference Type: study report

Title: Isophorondiamin. Prüfung der Hautsensibilisierung nach Magnusson-Kligman

Year: 1983

Report Date: 1983-11-11

- Materials and methods
- Type of method

in vivo

- Type of study

Guinea pig maximisation test

- Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 406 (Skin Sensitisation) (1981)	no

- GLP compliance

no

- Test materials
- Identity of test material same as for substance defined in section 1 (if not read-across)

yes

- Test material identity

Identifier	Identity
CAS number	2855-13-2
common name	Isophorone diamine
EC name	3-aminomethyl-3,5,5-trimethylcyclohexylamine

- Details on test material

Isophorone diamine of Hüls AG, purity >= 99.7%

- Test animals
- Species

guinea pig

- Strain

Dunkin-Hartley

- Sex

male

- Details on test animals and environmental conditions

TEST ANIMALS:
- Strain: Dunkin-Hartley (Bor: DHPW)
- Source: Winkelmann, Borchen (Germany)
- Weight at study initiation: 363 g (mean)
- Diet: ad libitum, special diet for guinea pigs, SSniff G 4 (Ssniff, Soest, Germany)
- Water: ad libitum, tab water
- Acclimation period: 5-8 days
ENVIRONMENTAL CONDITIONS
- Temperature: 20 +/- 1 °C
- Humidity: 60 +/- 5%
- Photoperiod: 12 hours artificial light, 12 hours dark

- Test system
- Traditional sensitisation test
- Route of induction exposure

other: intracutaneous (1st) and occlusive epicutaneous (2nd)

- Route of challenge exposure

epicutaneous, occlusive

- Vehicle

other: 10% ethanol

- Concentration

1st application: Induction 0.1% intracutaneous
2nd application: Induction 7.5% occlusive epicutaneous
3rd application: Challenge occlusive epicutaneous

- No. of animals per dose

9 (10) control group, 1 animal died after 1 week, cause of death is unkwon
20 test group

- Details on study design (Traditional tests)

- Induction schedule: injection followed 1 week later by patch treatment (0.3 ml) for 48 hours;
- Injection details: 0.1 ml each at 6 positions on shoulders:
2 x Freund's Complete Adjuvant
2 x test substance in 10% ethanol
2 x Freund's Complete Adjuvant / 0.2% test substance (1:1)
simultaneous and symmetrical application of each solution
controls: 10% ethanol instead of test substance
- Challenge schedule: 2 weeks after end of induction patch treatment for 24 hours
- Concentrations used for challenge: 2.5 and 5%; readings 24, 48, and 72 hours after removal of patch
- Rechallenge: no

EXAMINATIONS
- Grading system: possible scores 0 / 1 / 2 / 3
- Pilot study: dose range finding study

- Challenge controls

9 (10) animals, 1 animal died after 1 week, cause of death is unkwon
vehicle treatment

- Positive control substance(s)

no

Results and discussion

- Positive control results

not applicable

- Traditional sensitisation test
- Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
1st reading	24	test group	2.5%	7	20	see section "Remarks on results including tables and figures"
2nd reading	48	test group	2.5%	5	20	see section "Remarks on results including tables and figures"
other: 3rd reading	72	test group	2.5%	2	20	see section "Remarks on results including tables and figures"
1st reading	24	negative control	2.5% test substance used for challenge	0	9	see section "Remarks on results including tables and figures"

2nd reading	48	negative control	2.5% test substance used for challenge	0	9	see section "Remarks on results including tables and figures"
other: 3rd reading	72	negative control	2.5% test substance used for challenge	0	9	see section "Remarks on results including tables and figures"
1st reading	24	test group	5%	18	20	see section "Remarks on results including tables and figures"
2nd reading	48	test group	5%	15	20	see section "Remarks on results including tables and figures"
other: 3rd reading	72	test group	5%	10	20	see section "Remarks on results including tables and figures"
1st reading	24	negative control	5% test substance used for challenge	0	9	see section "Remarks on results including tables and figures"
2nd reading	48	negative control	5% test substance used for challenge	0	9	see section "Remarks on results including tables and figures"
other: 3rd reading	72	negative control	5% test substance used for challenge	0	9	see section "Remarks on results including tables and figures"

Clinical signs:

Local effects:
- 24 hours after intracutaneous applications, animals mainly from the test substance treated group displayed poorly healing necroticinflammations.- After the induction patch treatment for 48 hours, the animals treated with the test substance had bleeding and matter dischargeinflammations at the places of injections leading to thick crusts.- In general after removal of the patch, severe inflammation and itching were observed,causing particularly the animals treated withtest substance to scratch their skins open in the area of injection.- after challenge scale formation and desiccation of skin was observed during the observation period on some animals.

Conclusions

Isophorone diamine has to be considered to be sensitising to the skin of guinea pigs under the conditions of the study.

Executive summary

The skin sensitising properties of isophorone diamine were determined in a guinea pig maximation test according to OECD TG 406 (positive controls not required by 1981 guideline version). Twenty female guinea pigs were intradermally injected with isophorone diamine at 0.1% in 10% ethanol and one week later epidermally exposed to a 7.5% concentration of test substance for 48 hours (occlusive). Ten control animals were similary treated, but with vehicle alone. Two weeks after the epidermal application all animals were challegend with 2.5% and 5%

test substance and with vehicle (24 hours occlusive). At challenge concentration of 2.5% 7/20 animals showed a sensitisation 24 hours after the patch test, 5/20 animals 48 hours after the test and 2/20 72 hours after the test. At challenge concentration 5% 18/20 animals showed a sensitisation 24 hours after the patch test, 15/20 animals 48 hours after the patch test and still 10/20 72 hours after the test. No animal of the control group showed any positive reaction. Therefore Isophorone diamine has to be considered to be sensitising to the skin of guinea pigs under the conditions of the study.

[Study 2]

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	**Study period**	1981-01-14 - 1981-02-07
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Comparable to guideline study with acceptable restrictions: no positive control group (not required by 1981 version of guideline)		

Study reference:
Reference Type: study report
Title: Isophorondiamin: Sensitisation potential in guinea pigs
Year: 1981
Report Date: 1981-03

Materials and methods
Type of method
in vivo
Type of study
Guinea pig maximisation test
Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 406 (Skin Sensitisation) (1981)	

GLP compliance
no
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2
common name	Isophorone diamine
EC name	3-aminomethyl-3,5,5-trimethylcyclohexylamine

Details on test material
Isophorone diamine, Hüls AG, no data on purity
Test animals
Species

guinea pig
Strain
Dunkin-Hartley
Sex
female

Details on test animals and environmental conditions
- Source: Porcellus Animals Limited (UK)
- Weight at study initiation: 300-350 g
- Dose group: 20 animals
- Controls: 10 animals, Freund's Complete Adjuvant
- Diet: ad libitum, special diet for guinea pigs, BP Nutrition FD1, supplemented with hay
- Water: ad libitum, tab water
- Acclimation period: 5-8 days
ENVIRONMENTAL CONDITIONS
- Temperature: 20 °C (17.5 - 22°C)
- Humidity: 50% (40% - 59%)

Test system
Traditional sensitisation test
Route of induction exposure
other: 1st intracutaneous, 2nd occlusive epicutaneous
Route of challenge exposure
epicutaneous, occlusive
Vehicle
water
Concentration
1st application: Induction 1% intracutaneous
2nd application: Induction 1% occlusive epicutaneous
3rd application: Challenge 5% and 10% occlusive epicutaneous
No. of animals per dose
20 test group
10 control group
Details on study design (Traditional tests)
- Induction schedule: intradermal injection followed after one week by occlusive patch treatment for a further 48 hours
- Injection details: 0.1 ml each at 6 positions in scapular region:
2 x Freund's Complete Adjuvant (FCA)
2 x test material (1% in dist. water)
2 x 1:1 emulsion of FCA / test material (1% in dist. water)
simultaneous and symmetrical application of each solution;
control and dose finding animals: 2 x 0.1 ml FCA only each
- Challenge schedule: 3 weeks after injection occlusive patch treatment for 24 hours, reading 24 hours after removal of patch
- Concentrations used for challenge: 10% and 5%
- Rechallenge: no
Challenge controls
Vehicle treatment
Positive control substance(s)
no

Results and discussion
Positive control results
not applicable
Traditional sensitisation test
Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
1st reading	24	test group	5%	0	20	See section "Remarks on results including tables and figures"
1st reading	24	negative control	5%	0	10	See section "Remarks on results including tables and figures"
1st reading	24	test group	10%	12	20	See section "Remarks on results including tables and figures"
1st reading	24	negative control	10%	0	10	See section "Remarks on results including tables and figures"

LLNA
Any other information on results incl. tables

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RESULTS OF PILOT STUDY: no responses regarding skin irritation were observed
at any concentration used in the pre-test (10%, 5%, 2%, 1% test substance)

RESULTS OF TEST

- Sensitisation reaction:

-12/20 animals positive (i.e. erythema) at 10% challenge concentration

-no animals positive at 5% challenge concentration

-no erythema in control group
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Overall remarks, attachments

Remarks on results including tables and figures

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no overall remarks
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Applicant's summary and conclusion

Conclusions

It is concluded that the results of the study are indicative of skin sensitisation and that Isophorone diamine is considered to be a dermal sensitiser in guinea pigs under the conditions of the study.

Executive summary

The skin sensitising properties of isophorone diamine were determined in a guinea pig maximation test according to OECD TG 406 (positive controls not required by 1981 guideline version). Twenty female guinea pigs were intradermally injected with isophorone diamine at 1% in dist. water and one week later epidermally exposed to a 1% concentration of test substance for 48 hours (occlusive). Ten control animals were similary treated, but with vehicle

alone. Two weeks after the epidermal application all animals were challegend with 5% and 10% test substance and with vehicle (24 hours occlusive). No control group animal showed erythema at either 10 or 5% challenge concentration, no erythema was noted in test group animals after challenge with 5% test item, in test group challenged with 10% isophorone diamine 12/20 animals showed erythema. Therefore isophorone diamine is considered to be a dermal sensitiser in guinea pigs under the conditions of the study.

[Study 3]

Administrative Data

Purpose flag	supporting study;		
Study result type	experimental result	**Study period**	approx. 1977 / 1978
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Study well documented, meets generally accepted scientific principles, acceptable for assessment		

Study reference:

Reference Type: publication

Title: Sensitisation capacity of epoxy resin hardeners in the guinea pig

Author: Thorgeirsson A

Year: 1978

Bibliographic source: Acta Derm. Venereol. 58, 332-336

Report Date: 1977

Materials and methods

Type of method
in vivo
Type of study
Guinea pig maximisation test
Principles of method if other than guideline
According to Magnusson B, Kligman AM (1969). J. Invest. Dermatol. 52, 268.
GLP compliance
no data
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2
common name	isophorone diamine
EC name	3-aminomethyl-3,5,5-trimethylcyclohexylamine

Details on test material
commercial isophorone diamine supplied by the Swedish Plastics Federation, no data on purity
Test animals
Species

guinea pig
Strain
no data
Sex
no data
Test system
Traditional sensitisation test
Route of induction exposure
other: 1st intracutaneous, 2nd epicutaneous, occlusion not reported
Route of challenge exposure
epicutaneous, occlusive
Vehicle
other: acetone, CAS No. 67-64-1
Concentration
1st application: Induction 0.5% intracutaneous
2nd application: Induction 0.5% other: epicutaneous, occlusion not reported
3rd application: Challenge 2% occlusive epicutaneous
No. of animals per dose
no information
Details on study design (Traditional tests)
TEST ANIMALS:
- Controls: vehicle and Freunds Complete Adjuvant
ADMINISTRATION/EXPOSURE
- Induction schedule: not reported
- Challenge schedule: two weeks after the second stage of sensitisation, 24-hour patch test, evaluation 24 hours after removal of patch
EXAMINATIONS
- Grading system: obvious redness and swelling judged by two persons independently
Challenge controls
described
Positive control substance(s)
no
Results and discussion
Positive control results
not applicable
LLNA
Any other information on results incl. tables
RESULTS OF TEST

- Sensitisation reaction: 100% of the animals positive
Overall remarks, attachments
Remarks on results including tables and figures
73% of the test animals were positive in another test after induction with an adduct of isophorone diamine and a low molecular weight epoxy resin (5% in both induction steps) and challenge with isophorone diamine (2%).

Applicant's summary and conclusion

Conclusions

Isophorone diamine is considered to be a dermal sensitiser for guinea pigs under the conditions of the study.

Executive summary

The skin sensitising properties of isophorone diamine were determined in a guinea pig maximation test. Guinea pigs were intradermally injected with isophorone diamine 0.5% in acetone and later epidermally exposed to a 0.5% concentration of test substance occlusive. Control animals were similary treated, but with vehicle alone regarding to the induction. Two weeks after the epidermal application all animals were challegend with 2% test substance (24 hours occlusive). All test animals showed positive reactions. Therefore isophorone diamine is considered to be a dermal sensitiser for guinea pigs under the conditions of the study.

3 ENVIRONMENTAL HAZARDS

3.1 Degradation

3.1.1 Ready biodegradability (screening studies)

[Study 1]

Administrative Data

Purpose flag	key study		
Study result type	experimental result	**Study period**	1992-07-07 to 1992-08-07
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	Guideline study		

Study reference:

Reference Type: study report

Title: Unnamed

Year: 1993

Report Date: 1993

Materials and methods

Test type
ready biodegradability
Test guideline

Qualifier	Guideline	Deviations
according to	EU Method C.4-A (Determination of the "Ready" Biodegradability - Dissolved Organic Carbon (DOC) Die-Away Test) (Cited as Directive 92/69/EEC, C.4-A)	

GLP compliance
yes
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
Test sustance: Isophorone diamine of Hüls AG, purity 99.9% (area), produced 23 March 1992, ID No. 3630/81404
Study design
Oxygen conditions
aerobic
Inoculum or test system
activated sludge (adaptation not specified)

Details on inoculum
- Sampling site: Municipal WWTP Marl-Ost, sampled 27 Oct 1992
- Preparation of inoculum: sampling, centrifugation (1100 g / 10 min), discard supernatant and resuspend with mineral medium, repeat centrifugation as above, resuspension of sludge (5.17 g dry weight/l), aeration through frit
- Initial cell concentration: 25.9 mg/l

Duration of test (contact time)
28 d

Initial test substance concentration

Initial conc.	Based on
6.9 mg/L	DOC

Parameter followed for biodegradation estimation

DOC removal

Details on analytical methods

ANALYTICAL PARAMETER: DOC (Carbon analyzer, Shimadzu), determination with and without removal of inorganic carbon on day 0, 7, 14, 21, 27 and 28

Details on study design

TEST SYSTEM
- Culturing apparatus: 2000 ml Erlenmeyer flask covered loosely with aluminum sheet, filled with 900 ml test soln.
- Number of culture flasks per concentration: 2 with test substance (10.5 mg DOC/l) and inoculum; 2 with control substance (9.7 mg DOC/l) and inoculum; 1 with inoculum only
- Aeration device: shaking for 28 days in the dark
- Test temperature: 21.8-22.1 degree C

METHOD OF PREPARATION OF TEST SOLUTION:
- Stock solution: 431 mg DOC/l

SAMPLING: Days 0, 7, 14, 21, 27, 28

TEST CONDITIONS
- Composition of medium: a 8.5 g KH_2PO_4/l 21.75 g K_2HPO_4/l 33.3 g Na_2HPO_4/l x 2 H_2O 20.0 g $(NH_4)Cl$/l b 22.5 g $MgSO_4$ x 7 H_2O/l c 27.5 g $CaCl_2$/l d 0.25 g $FeCl_3$ x 6 H_2O/l

Reference substance
benzoic acid, sodium salt

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
8		DOC removal	28 d	

Details on results

Kinetic of test substance (in %):
= 4 after 7 day(s)
= 4 after 14 day(s)
< 0 after 21 day(s)
= 3 after 27 day(s)
= 8 after 28 day(s)

Kinetic of control substance (in %):
= 99 after 7 day(s)
= 99 after 14 day(s)
Degradation products: not measured
BOD5 / COD results
Results with reference substance
REFERENCE SUBSTANCE: purity 97%, theoretical conc. 9.7 mg DOC/l, stock solution 570 mg DOC/l

99% degradation within 10 days
Applicant's summary and conclusion
Validity criteria fulfilled
no data
Interpretation of results
under test conditions no biodegradation observed

Conclusions
The test substance is considered as not readily biodegradable.

Executive summary
Isophorone diamine proved to be not readily biodegradable in a study conducted according to EU method C4 A (8% degradation after 28 d).

3.2 Acute toxicity

3.2.1 Short-term toxicity to fish

[Study 1]

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Guideline study

Study reference:

Reference Type: study report
Title: Unnamed
Year: 1993
Report Date: 1993

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method C.1 (Acute Toxicity for Fish) Cited as Directive 84/449/EEC, C.1 ("Acute toxicity for fish")	

GLP compliance
yes
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
Test substance: Hüls AG, purity 99.9% (area), produced 23 March 1992, ID No. 3630/81404
Analytical monitoring
yes
Details on sampling
The concentration of the test item was measured 0, 24 48 and 72 hours after the test item application
Details on analytical methods
MONITORING OF TEST SUBSTANCE CONCENTRATION: gas chromatography
Vehicle
no

Details on test solutions
Stock solution: 56.0005 g of the test item were dissolved in 2 L tap water

Test organisms
Test organisms (species)
Leuciscus idus

Details on test organisms
- Strain: Leuciscus idus melanotus HECKEL
- Supplier: Eggers, Hohenwestedt
- Wild caught: no
- Age/size/weight/loading: 6 +/- 2 cm, 1.5 g average weight
- Feeding: TetraMin, approximately 3% of body weight / day
- Pretreatment: single treatment with Zephirol 1:50,000 for 1 hour followed by 14 days under quarantine
- Feeding during test: no

Study design
Test type
semi-static
Water media type
freshwater
Limit test
no
Total exposure duration
96 h
Test conditions
Hardness
12.7 degree dH (monthly mean Jan 1993)
Test temperature
20 +/-1 °C
pH
Control: 7.6 - 8.3
Treatments: 8.4 - 9.9
Dissolved oxygen
8.1 - 9.3 mg/l
Nominal and measured concentrations
Nominal: 70 / 100 / 140 / 200 / 280 mg/l
Measured: 70.3 / 101.8 / 142.3 / 202.0 / 281.0 mg/l (mean values)
- nominal concentrations were used for evaluation

Details on test conditions
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Concentration of vehicle/ solvent: 56.0005 g test substance in 2 l demineralised water, no additional solvent

DILUTION WATER
- Source: dechlorinated drinking water (Gelsenwasser AG)
- Aeration: continuous

TEST SYSTEM
- Exposure vessel type: 20 l aquarium
- Number of replicates, fish per replicate: 1, 10
- Photoperiod: 16 hours bright / 8 hours dark

Any other information on materials and methods incl. tables

```
pH values:  after 0 / 24 / 48 / 72 / 96 h:

control:  7.6 / 7.6 / 7.6 / 7.6 / 8.3

70 mg/l: 9.0 / 9.1 / 9.2 / 9.2 / 8.4

100 mg/l: 9.2 / 9.4 / 9.3 / 9.4 / 8.8

140 mg/l: 9.4 / 9.6 / 9.6 / 8.8 / -

200 mg/l: 9.7 / 9.8 / -

280 mg/l: 9.9 / 9.6 / -
```

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC100	140 mg/L	nominal	test mat.	mortality	
96 h	LC50	110 mg/L	nominal	test mat.	mortality	
96 h	LC0	70 mg/L	nominal	test mat.	mortality	

Details on results

LC50 after
24hs: 170.4 mg/L;
48h: 130.3 mg/L;
72h: 113.2 mg/L;
96h: 110.0 mg/L

Reported statistics and error estimates

The EC50 was derived from a graphical presentation instead of using a suitable statistical tool (e.g. Probit analysis). Therefore, no error estimates were reported.

Any other information on results incl. tables

```
- Concentration / response curve:

concn., mg/l:  70   100   140   200   280   control

% mortality     0    30   100   100   100     0 (96 hours)

% mortality     0    20   100   100   100     0 (72 hours)

% mortality     0    10    60   100   100     0 (48 hours)

% mortality     0     0     0    90   100     0 (24 hours)
```

Overall remarks, attachments

Remarks on results including tables and figures

```
The basic properties / high pH may have contributed to the observed  effects,
but  this is not discussed by the authors.
```

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The 96h-LC50 was determined as 110 mg/L indicating that the test substance does not pose a significant hazard towards fish.

Executive summary

In a 96-h acute toxicity study, *Leucisus idus* were exposed to Isophoronediamine at nominal concentrations of 0 (control), 70, 100, 140, 200 and 280 mg/l under semi-static conditions. The 96-h $LC_{50}$was determined as 110 mg/L.

Test organism length: 6 +/- 2 cm

Test type: semi-static

LC_{50}: 110 mg/L

Endpoint(s) monitored: mortality, sublethal effects
This study is classified as "reliable without restrictions".

3.2.2 Short-term toxicity to aquatic invertebrates

[Study 1]

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Guideline study

Study reference:

Reference Type: study report
Title: Unnamed
Year: 2002
Report Date: 2002

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) (1984)	no data
according to	EU Method C.2 (Acute Toxicity for Daphnia) (1992)	no data

GLP compliance
Yes

Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
Test substance from Degussa AG,
Batch No. LIMS-Nr. 02006502, manufactured 03 May 2002 - 0730
Sample No. 1903/020516, ID No. 0649/82219
purity 99.75%
Analytical monitoring
yes

Details on sampling
The analytical verification of the test item concentrations was carried out after 0 and 48 hours.
For stability control an additional series of the same concentrations was prepared in separate vessels without test organisms
Details on analytical methods
Derivatisation of 1 mL of the aqueous sample with dansy chloride (solution of dansyl chloride in acetone), thus a UV-active derivative of isophorone diamine was formed.
Analysis via Gradient HPLC-system HP 1100
Vehicle
no
Details on test solutions
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Concentration: 1.04 g/l; 0.52 g of the test item were equilibrated directly in 500 ml synthetic fresh water.

DILUTION WATER
- Source: Synthetic:
$CaCl_2 \times 2 H_2O$: 294 mg/l
$MgSO_4 \times 6 H_2O$: 114 mg/l
$NaHCO_3$: 65 mg/l
KCl: 6 mg/l
- Ca/Mg ratio: 4:1
- Na/K ratio: 10:1
Test organisms
Test organisms (species)
Daphnia magna
Details on test organisms
TEST ORGANISMS
- Strain: Daphnia magna Straus, clone 5
- Source/supplier: received from Bayer AG in 1991, further bred inhouse
- Breeding method: in 1 l beakers with M4 medium, water renewal each 2-3 days, isolation of juveniles for further breeding each ca. 4 weeks
- Age: < 24 hours
- Feeding: Desmodesmus subspicatus, as much as consumed
- Pretreatment: Filtration of adults 24 h prior to testing
- Feeding during test: no
Study design
Test type
static
Water media type

freshwater
Limit test
no
Total exposure duration
48 h
Test conditions
Hardness
14 degree German hardness = 250 mg CaCO3/l
Test temperature
20.22 - 20.28 (mean 20.25) degree C
pH
Study start: 7.6 (control); 7.9; 8.2; 8.5; 8.9; 9.2; 9.6 (highest conc.)
Dissolved oxygen
48 hours: 7.8 (control); 7.9; 7.9; 7.9; 8.1; 8.0; 8.2 (highest conc.) mg O2/l
Nominal and measured concentrations
Nominal: 0.0; 2.1; 4.2; 8.3; 16.6; 33.2; 66.4 mg/l
Measured: - ; 1.9; 3.9; 7.7; 15.5; 32.1; 66.3 mg/l (48 hours)
Details on test conditions
- Exposure vessel type: 10 ml round-bottom test tubes
- Number of replicates, individuals per replicate: 4 replicates with 5 individuals each
- Intensity of irradiation: dark
- Photoperiod: no
- Aeration: no
- Control group: 1 blank control simultaneously and 2 reference substance control

TEST PARAMETER: immobilisation
Reference substance (positive control)
yes (Potassium dichromate, CAS RN 7778-50-9)
Results and discussions
Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC100	66.4 mg/L	nominal	test mat.	mobility	
48 h	EC50	23 mg/L	nominal	test mat.	mobility	17-31 mg/L
48 h	NOEC	8.3 mg/L	nominal	test mat.	mobility	
24 h	EC50	27 mg/L	nominal	test mat.	mobility	18-40 mg/L

Details on results
The EC50-values were calculated by probit analysis according toe Cavalli-Sforza (1972)
Results with reference substance
- Concentrations: 1.0; 2.0 mg/l
- Results: 40; 100% immobilisation
- tested every 3 months
Reported statistics and error estimates
- 95% confidence interval of EC50:
18 - 40 mg/l (24 hours); EC50 = 27 mg/l
17 - 31 mg/l (48 hours); EC50 = 23 mg/l
Any other information on results incl. tables
RESULTS: EXPOSED

- Concentration / response table:

```
0.0; 2.1; 4.2; 8.3; 16.6; 33.2; 66.4 mg/l nominal

0;   0;   0;  15;   30;   40;  100% immobile (24 hours)

0;   0;   0;  10;   30;   65;  100% immobile (48 hours)
```

Overall remarks, attachments
Remarks on results including tables and figures

```
The basic properties / high pH may have contributed to the observed  effects,
but this is not discussed by the authors.
```

Applicant's summary and conclusion
Validity criteria fulfilled
no data

Conclusions
The EC50 (48 hours) was determined as 23 mg/L indicating that the test substance may be harmful to aquatic invertebrates.

Executive summary
The acute toxicity of 3 -aminomethyl-3,5,5 -trimethylcyclohexylamine to*Daphnia magna*was studied under static conditions over a period of 48 hours according to OECD TGD 201 and EU-method C.2. Six concentrations ranging from 2.1 to 66.4 mg/L were tested. Immobilisation was observed. The following endpoint was derived:
EC_{50}(48 h): 23 mg/L; CL: 17-31 mg/L
Thestudy was classified as "reliable without restrictions".

[Study 2]

Administrative Data

Purpose flag	key study		
Study result type	experimental result	**Study period**	no data
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	Test procedure in accordance with generally accepted scientific standards and described in sufficient detail		

Reference

Reference Type: publication
Title: Unnamed
Year: 1982
Report Date: 1982

Materials and methods

Principles of method if other than guideline
Method: other: see Test Conditions
GLP compliance
no data
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes

Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
Test substance: from ICN Pharm. Inc.; no further information
Analytical monitoring
no
Vehicle
no data
Details on test solutions
The test solutions were prepared from concentrated stock solutions and naural seawater. Where necessary, an organic solvent (preferably Dimethylsulfoxide) was used to prepare the stock solution (as 24 compounds were tested not clear, if used for this particular test).

DILUTION WATER (=seawater)
- Source: Eastern Scheldt (NL), prepared by sand filtration, filtration over activated charcoal and 0.2 um millipore filter
- pH: about 8
- Control group: natural seawater

Test organisms
Test organisms (species)
other aquatic crustacea: Chaetogammarus marinus
Details on test organisms
TEST ORGANISMS
- Breeding method: Readily grown in seawater aquarium systems with shelter for concealment
- Age: Young gammarids, about 5 mm long
- Feeding: Fucus spec. - Feeding during test: yes (Fucus or Tetramin); reason: to prevent cannibalism

Study design
Test type
semi-static
Water media type
saltwater
Limit test
no
Total exposure duration
96 h
Test conditions
Test temperature

15 +/- 1 degree C
pH
8.0 (control); 8.5; 8.8; 9.1; 9.5; 10.0; 10.2; 10.2 (highest conc.)
Dissolved oxygen
almost saturated for the whole test
Salinity
28 o/oo
Nominal and measured concentrations
Nominal: 0; 32; 56; 100; 180; 320; 560; 1000 mg/l

Details on test conditions

TEST CONDITIONS
- Renewal of test solution: once a day
- Aeration: no
- Exposure vessel type: 1 l glass beakers with 1 l test solution, covered with a watch glass
- Number of replicates, individuals per replicate: 2 beakers with 10 animals each for each concentration
- Adjustment of pH: no
- solvent control, if solvent is used

TEST PARAMETER: mortality; counting and removal of dead animals daily accompanied by visual inspection of survivors
Reference substance (positive control)
no data
Results and discussions
Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/ Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	NOEC	100 mg/L	nominal	test mat.	other: mortality and behaviour compared to the control	
24 h	LC50	572 mg/L	nominal	test mat.	mortality	505-648 mg/L
48 h	LC50	388 mg/L	nominal	test mat.	mortality	229-444 mg/L
72 h	LC50	362 mg/L	nominal	test mat.	mortality	318-412 mg/L
96 h	LC50	324 mg/L	nominal	test mat.	mortality	286-366 mg/L

Reported statistics and error estimates
LC50-values and error estimates (confidence limits)
24 hours: 572 (505-648) mg/l
48 hours: 388 (339-444) mg/l
72 hours: 362 (318-412) mg/l
96 hours: 324 (286-366) mg/l
Overall remarks, attachments
Remarks on results including tables and figures
The basic properties / high pH may have contributed to the observed effects, but this is not discussed by the authors.

Applicant's summary and conclusion

Validity criteria fulfilled
no data

Executive summary
The aquatic toxicity of 3-aminomethyl-3,5,5-trimethylcyclohexylamine was tested in the marine invertebrate Chaetogammarus
marinus under semistatic conditions. Five concentrations ranging from 32 to 1000 mg/L were tested. The test organisms were observerd daily for mortality and abnormal behaviour / symptoms. The 96 hour-LC50 was determined as 324 mg/l.
The study was assessed as "valid without restrictions".

[Study 3]

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Scientifically acceptable publication

Reference

Reference Type: study report
Title: Unnamed
Year: 2000

Materials and methods
Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	

GLP compliance
no
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
3-aminomethyl-3,5,5-trimethylcyclohexylamine
CAS No. 2855-13-2
- Analytical purity: 99%
Analytical monitoring

no data
Vehicle
no
Details on test solutions
PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances)
- Method: An appropriate amount of test substance was weighed into the test medium establishing a stock solution of a nominal concentration of 500 mg/l. In order to facilitate the solution, the mixture was ultrasonicated for 5 min. followed by magnetic stirring for 22 hours. After separation of test medium and possible remaining particles or droplets for 2 hours, aliquots were sampled from the mid-fraction of the stock solution for preparation of the test concentrations.

Test organisms

Test organisms (species)
Daphnia magna

Details on test organisms
TEST ORGANISM
- Source: isolated from Lake Langedam, Birkerod, Denmark in 1979 and has been cultured at the VKI (Water Quality Institute) since then.
- Age at study initiation (mean and range, SD): < 24 h
- Method of breeding: The animals are cultured in water from Lake Bradebaek (Denmark) at 20 °C fed three times every day with Raphidocelis subcapitata and a supply of yeast cells once or twice every week.

Study design
Test type
static
Water media type
freshwater
Limit test
no
Total exposure duration
48 h
Test conditions
Hardness
250 ±25 mg/l CaCO3
Test temperature
20 °C +/- 1 °C
pH
7.8 +/- 0.1
Dissolved oxygen
% Saturation after
24 h
99 - 100%

48 h
96 - 97%
Nominal and measured concentrations
Nominal concentrations (mg/l)
control
5
10
20
40
80
160

Details on test conditions

TEST SYSTEM
- Test vessel: glass vessels (capacity 250 ml)
- Type (delete if not applicable): covered with plastic sheets
- Fill volume: 125 ml
- Renewal rate of test solution (frequency/flow rate): After 24 hours, the test animals were transferred to new test vessels prepared as specified
under "Details on test solutions".
- No. of organisms per vessel: 5
- No. of vessels per concentration (replicates): 4
- No. of vessels per control (replicates): 6

TEST MEDIUM / WATER PARAMETERS
- Source/preparation of dilution water: Freshly produced 150-medium was used in the tests. The medium was prepared from deionised water, and salts were added to the water according to the standard procedure (IS0 International Standard 6341)

OTHER TEST CONDITIONS
- Adjustment of pH: to 7.8 +/- 0.1
- Photoperiod: 16:8 day-night regime

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) :
Mobility

Reference substance (positive control)
yes (potassium dichromate)
Results and discussions
Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
24 h	EC10	20.7 mg/L	nominal		mobility	
24 h	EC50	37.4 mg/L	nominal		mobility	
24 h	other: EC90	54 mg/L	nominal		mobility	

48 h	EC10	8.33 mg/L	nominal		mobility	
48 h	EC50	17.4 mg/L	nominal		mobility	
48 h	other: EC90	26.4 mg/L	nominal		mobility	

[Study 4]

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Test procedure generally in accordance with national standard methods with acceptable restrictions (e.g. no controls)

Reference

Reference Type: study report
Title: Unnamed
Year: 1996
Report Date: 1996

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: DIN 38412, part 11	

GLP compliance
no
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
Test substance: Hüls AG, commercial sample
Analytical monitoring
no
Vehicle
no
Details on test solutions
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Concentration: 1 g/l
Test organisms
Test organisms (species)
Daphnia magna
Details on test organisms

- Strain: Daphnia magna, Huels
- Source/supplier: Huels AG (inhouse)
- Breeding method: in 1 l jars with dechlorinated drinking water, water renewal every 2-3 days, isolation of juveniles for further breeding every ca. 4 weeks
- Age: < 24 hours
- Feeding: Chlorella vulgaris, as much as consumed
- Pretreatment: Filtration of adults 24 h prior to testing
- Feeding during test: no

Study design

Test type

static

Water media type

freshwater

Limit test

no

Total exposure duration

24 h

Test conditions

Test temperature

20 +/- 1 degree C

Nominal and measured concentrations

Nominal concentrations: 12; 18; 25; 35; 50; 70; 100 mg/l

Details on test conditions

- Exposure vessel type: 25 ml graduated cylinder
- 7 test concentrations + 2 reference substance concentrations (no blank)
- Number of replicates, individuals per replicate: 4 replicates with 5 individuals each
- Intensity of irradiation: dark
- Photoperiod: no (dark)
- Aeration: no

Reference substance (positive control)

yes (potassium dichromate, CAS RN 7778-50-9)

Any other information on materials and methods incl. tables

```
- Control group: 2 reference substance controls; no blank


DILUTION WATER

- Source: Synthetic:

CaCl2 x 2 H2O: 294 mg/l

MgSO4 x 7 H2O: 123 mg/l

NaHCO3: 63 mg/l

KCl: 5.5 mg/l

- Ca/Mg ratio: 4:1
```

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
24 h	NOEC	25 mg/L	nominal	test mat.	mobility	
24 h	EC100	70 mg/L	nominal	test mat.	mobility	
24 h	EC50	44 mg/L	nominal	test mat.	mortality	35-50 mg/L

Details on results
- Concentration / response table:
12; 18; 25; 35; 50; 70; 100 mg/l
0; 0; 0; 35; 60; 100; 100% immobile

Results with reference substance
- Concentrations: 0.9; 1.9 mg/l
- Results: 10; 65% immobilisation

Reported statistics and error estimates
- 95% confidence interval of LC50: 35-50 mg/l

Overall remarks, attachments

Remarks on results including tables and figures

```
The basic properties / high pH may have contributed to the observed  effects,
but this is not discussed by the authors.
```

Applicant's summary and conclusion

Validity criteria fulfilled
no data

Conclusions

The 24h-EC50 was determined as 44 mg/L indicating that the test substance may be harmful to aquatic invertebrates.

Executive summary

The acute toxicity of 3-aminomethyl-3,5,5-trimethylcyclohexylamine to*Daphnia magna*was studied under static conditions over a period of 24 hours according to DIN 38412, part 11. Seven concentrations ranging from 12 to 100 mg/L plus control were tested. Immobilisation was observed. The following endpoint was derived:
EC_{50}(24 h): 44 mg/L; CL: 35-50 mg/L
Thestudy was classified as "reliable with restrictions".

3.2.3 Algal growth inhibition tests

[Study 1]

Administrative Data

Purpose flag	key study		
Study result type	experimental result	**Study period**	1992-08-04 to 1992-08-07
Reliability	2 (reliable with restrictions)		

Rationale for reliability incl. deficiencies	Guideline study with acceptable restrictions: no analytical monitoring

Study reference:

Reference Type: study report
Title: Unnamed
Year: 1993
Report Date: 1993

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method C.3 (Algal Inhibition test) Cited as Directive 87/302/EEC, part C, p. 89 (Algal inhibition test)	

GLP compliance
yes
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
Test substance: Hüls AG, purity 99.9% (area), produced 23 March 1992, ID No. 3630/81404
Analytical monitoring
no
Vehicle
no
Details on test solutions
Stock solution: 1 g test substance/l (deionised water), no vehicle or solvent

Test organisms
Test organisms (species)
Scenedesmus subspicatus (new name: Desmodesmus subspicatus)
Details on test organisms
- Strain: CHODAT (86.81 SAG)
- Source/supplier: Origin: Institut fuer Wasser-, Boden- und Lufthygiene, Berlin, further bred inhouse
- Laboratory culture: From a stock culture, a preculture is seeded three days before begin of test. Test cultures are seeded from the latter.
- Method of cultivation: Erlenmeyer flasks on tables exposed to light

- Controls: yes
- Initial cell concentration: ca. 20,000 cells/ml

Study design

Test type

static

Water media type

freshwater

Limit test

no

Total exposure duration

72 h

Test conditions

Test temperature

24 +/- 2 degree C

pH

7.9-8.9 at start, 8.4-9.0 at end of test

Nominal and measured concentrations

Nominal: control; 0.75; 1.5; 3.0; 6.0; 12.5; 25; 50 mg/l

Details on test conditions

- Number of replicates: 5 (exposed) or 8 (control)
- Method of cultivation: Erlenmeyer flasks on tables exposed to light
- Intensity of irradiation: ca. 8000 lux white
- Monitoring of algae: photometric at 685 nm, calibration with standard curve
- Renewal of test solution: no

Reference substance (positive control)

no data

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
72 h	EC50	37 mg/L	nominal	test mat.	cell number	
72 h	EC10	3.1 mg/L	nominal	test mat.	cell number	
72 h	EC50	> 50 mg/L	nominal	test mat.	growth rate	
72 h	EC10	11.2 mg/L	nominal	test mat.	growth rate	
72 h	NOEC	1.5 mg/L	nominal	test mat.	cell number	

Details on results

Probit analysis according to Cavalli-Sforza (1972) was applied to the areas under the growth curves.

Any other information on results incl. tables

RESULTS

- Cell density data: (x 1.0E+04 cells/ml)

concentration	0 hours	24 hours	48 hours	72 hours
control	2	6	25	98
0.75 mg/l	2	5	24	97
1.5 mg/l	2	6	24	92

3.0 mg/l	2	6	23	82
6.0 mg/l	2	6	24	78
12.5 mg/l	2	7	25	69
25 mg/l	2	5	20	54
50 mg/l	2	4	10	31

Overall remarks, attachments
Remarks on results including tables and figures
An increase of pH observed with some test solutions was not considered to affect the growth.

Applicant's summary and conclusion

Validity criteria fulfilled
no data
Conclusions
The EC50 values (72 hours) were determined as >50 mg/L (growth rate) and 37 mg/L (cell number) indicating that the test substance may be harmful to algae.

Executive summary
In a 72 hour toxicity study, cultures of*Scenedesmus subspicatus*were exposed to3-aminomethyl-3,5,5-trimethylcyclohexylamine
at nominal concentrations of 0.75; 1.5; 3.0; 6.0; 12.5; 25; 50 mg/l under static conditions in accordance with EU Method C3. The NOEC and EC_{50}values based on biomass were 1.5 mg/L and 37 mg/L, respectively. The 72 h-EC50 value based on growth rate was >50 mg/L. There were no compound related phytotoxic effects.

Growth rate:
72 hr E_rC_{50}: >50 mg/L 95% CL: n.a.

72 hr NOEC: not available

Biomass:
72 hr E_bC_{50}: 37 mg/L 95% CL: n.a.
72 hr NOEC: 1.5 mg/L
The study was assessed as "reliable without restriction".

3.3 Chronic toxicity

3.3.1 Chronic toxicity to aquatic invertebrates

[Study 1]

Administrative Data

Purpose flag	key study; robust study summary; used for classification
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Guideline study

Study reference:

Reference Type: study report
Title: Unnamed
Year: 1993
Report Date: 1993

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD 202, part 2 (1984)	no data

GLP compliance
yes
Test materials
Identity of test material same as for substance defined in section 1 (if not read-across)
yes
Test material identity

Identifier	Identity
CAS number	2855-13-2

Details on test material
Test substance: Hüls AG, purity 99.9% (area), produced 23 March 1992, ID No. 3630/81404
Analytical monitoring
yes
Details on sampling
MONITORING OF TEST SUBSTANCE CONCENTRATION:
only at >= 1 mg/l; 0.1 and 0.3 mg/l below detection limit
Vehicle
no
Details on test solutions
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Dilution watert: M4 medium (Elendt)
- Concentration of vehicle/ solvent: 0.2 g/l

DILUTION WATER
- Synthetic freshwater according to Elendt (1990)
Test organisms
Test organisms (species)
Daphnia magna
Details on test organisms

- Strain: Daphnia magna Straus, clone 5
- Supplier: inhouse
- Breeding method: in synthetic medium according to Elendt (1990), water renewal every 2-3 days, isolation of juveniles for further breeding every ca. 4 weeks
- Age/size/weight/loading: < 24 hours
- Feeding: Scenedesmus subspicatus, as much as consumed
- Pretreatment: Filtration of adults 24 h prior to testing
- Feeding during test: Scenedesmus subspicatus, days 0-6: 4.0E+06 cells/(individual x day) days 7-21: 2.0E+07 cells/(individual x day)

Study design

Test type

semi-static

Water media type

freshwater

Limit test

no

Total exposure duration

21 d

Test conditions

Test temperature

20 +/- 1 degree C

pH

Range of 9 determinations:
control: 7.5-8.0; 0.1 mg/l: 7.1-7.9; 0.3 mg/l: 7.5-7.9; 1.0 mg/l: 7.3-7.9; 3.0 mg/l: 7.6-7.9 ; 10.0 mg/l: 7.6-8.2; 30.0 mg/l: 8.3/8.2 (interrupted after 2 determinations)

Dissolved oxygen

Range of 9 determinations:
Control: 94-107%; 0.1 mg/l: 94-105% ; 0.3 mg/l: 93-100% ; 1.0 mg/l: 93-105%; 3.0 mg/l: 95-106%; 10.0 mg/l: 96-110%; 30.0 mg/l: 103/113% (interrupted after 2 determinations)

Nominal and measured concentrations

Nominal: 0.1; 0.3; 1.0; 3.0; 10.0; 30.0 mg/l
Measured (mean of initical conc.): <LOD (lowest conc.); <LOD; 1.3; 3.1; 10.1; 30.3 (highest conc.)

Details on test conditions

- Exposure vessel type: beakers, 250 ml filled with 200 ml test solution (days 0-6), 150 ml filled with 80 ml (days 7-21)
- Number of replicates, individuals per replicate: days 0-6: 4 replicates, 5 individuals each; days 7-21: <= 10 replicates, 1 individual each
- Renewal of test solution: each Monday, Wednesday and Friday
- Intensity of irradiation: 58 W = ca. 1000 Lux
- Photoperiod: 20 hours bright / 4 hours dark

Reference substance (positive control)

no data

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	NOEC	3 mg/L	nominal	test mat.	reproduction	
21 d	LOEC	10 mg/L	nominal	test mat.	reproduction	

Any other information on results incl. tables
- Concentration / response curve:

concentration: % mortality / % reproduction / % inhibition

control: 0 / 51.3+/-13.2 / -

0.1 mg/l: 10 / 44.4+/-5.1 / 13.5 (not significant)

0.3 mg/l: 0 / 53.1+/-16.4 / -3.5

1.0 mg/l: 0 / 48.0+/-4.7 / 6.4 (not significant)

3.0 mg/l: 0 / 55.7+/-19.6 / -8.6

10.0 mg/l: 20 / 42.1+/-14.8 / 17.9 (not significant)

30.0 mg/l:100 / 0.0+/-0.0 / 100

Overall remarks, attachments

Remarks on results including tables and figures

With reference to the development of new EC methods for the 21 day daphnia test, the test conditions deviate from the OECD test guideline. Reporting is also done according to EU protocols. An effect on the test results is, however, not expected.

Evaluation was based on nominal concentrations. A slight increase of pH was considered to have no effect on the results.

10% mortality, which was observed at the lowest test concentration but not at the next three concentration levels, is considered insignificant because this mortality rate is allowed in the controls according to the OECD test guideline.

Applicant's summary and conclusion

Validity criteria fulfilled

no data

Conclusions

The long-term NOEC for reproduction (21 days) was determined as 3 mg/L indicating that the test item may have chronic effects on aquatic invertebrates.

Executive summary

The long-term toxicity of 3-aminomethyl-3,5,5-trimethylcyclohexylamine on Daphnia magna was investigated over a test period of 21 days according to OECD 202, part 2 (1984). The reproduction rate and the mortality of the parent animals were monitored at nominal concentrations ranging from 0.1 to 30.0 mg/L. The long-term NOEC for reproduction (21 days) was determined as 3 mg/L and the LOEC (reproduction) as 10 mg/L.

The study was assessed as "reliable without restrictions".