

FOREWORD

INTRODUCTION

MALONIC ACID DIESTERS

Dimethylmalonate, 108-59-8

Diethylmalonate, 105-53-3

SIDS Initial Assessment Report

For

SIAM 20

Paris, France, 19 – 22 April 2005

1. **Chemical Name:** Category of malonic acid diesters: Dimethylmalonate and Diethylmalonate
2. **CAS Number:** 108-59-8
105-53-3
3. **Sponsor Country:** Germany
Contact Point:
BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit)
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4. **Shared Partnership with:** Degussa AG, Germany
5. **Roles/Responsibilities of the Partners:** -
 - Name of industry sponsor /consortium: Degussa AG Germany
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 - Process used: See next page
6. **Sponsorship History**
 - How was the chemical or category brought into the OECD HPV Chemicals Programme? By ICCA HPV initiative
7. **Review Process Prior to the SIAM:** last literature search (update):
18 November 2004 (Human Health): databases medline, topline; search profile CAS-No. and special search terms
12 November 2004 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms OECD/ICCA
8. **Quality check process:** IUCLID was used as a basis for the SIDS dossier. All data were checked and validated by BUA. A final evaluation of the human health part has been performed by the Federal Institute for Risk Assessment (BfR) and of the ecotoxicological part by the Federal Environment Agency (UBA).
9. **Date of Submission:** Deadline for circulation: 21 January 2005
10. **Date of last Update:** Last literature search (update) of sponsor company: CAS-No. and special search terms
DMM: August 17, 2004: CIS, DIMDI, STN, Dialog
DEM: April 24 and May 14, 2003, DIMDI, CIS, Datastar, Dialog, STN, Beilstein.

1*1. Comments:

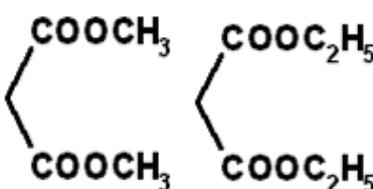
OECD/ICCA – The BUA* Peer Review Process

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according to robust summary requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability 4, i.e. reliability not assignable)
- Review of validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing.

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS Nos.	108-59-8, 105-53-3
Chemical Names	Category of malonic acid diesters: Dimethylmalonate (DMM) and Diethylmalonate (DEM)
Structural Formulas	

SUMMARY CONCLUSIONS OF THE SIAR**Category Justification**

The production and use pattern of Diethylmalonate (DEM) and Dimethylmalonate (DMM) are comparable. The two chemicals have very similar physico-chemical properties and both esters are hydrolyzed via a two step reaction to malonic acid and the corresponding alcohol, methanol or ethanol. It is likely that unspecific esterases in the body catalyze the hydrolysis. The alcohols and malonic acid are physiological substances that are metabolized via physiological pathways. Ethanol (CAS No. 64-17-5) and methanol (CAS No. 67-56-1) were assessed at SIAM 19. For ethanol it was concluded that the chemical is currently of low priority for further work, because the hazardous properties of ethanol are manifest only at doses associated with consumption of alcoholic beverages. As it is impossible to reach these exposure levels as a consequence of the manufacture and use of malonates, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. For methanol, SIAM 19 decided that this chemical is a candidate for further work. Methanol exhibits potential hazardous properties for human health (neurological effects, CNS depression, ocular effects, reproductive and developmental effects, and other organ toxicity). The effects of methanol on the CNS and retina in humans only occur at doses at which formate accumulates due to a rate-limiting conversion to carbon dioxide. In primates, formate accumulation was observed at methanol doses greater than 500 mg/kg bw (which would require a DMM dose of more than 1000 mg/kg bw). As there were no indications of a methanol associated toxicity from a well performed repeated dose toxicity study with DMM in rodents (which are, however, known to be less sensitive to methanol toxicity than humans), and because methanol toxicity would not be expected up to doses as high as 1000 mg DMM/kg bw/day, it was concluded that methanol does not make a relevant contribution to the toxicity profile of DMM. A possible mode of action for systemic toxicity of DMM and DEM can only be deduced from the repeated dose study with DMM, indicating a reversible liver hypertrophy at the cellular level at high doses of 1000 mg/kg bw/day. This effect can be an indication of an induction of metabolism in the liver rather than a clear systemic toxicity.

Human Health

From the physical chemical properties of both substances it can be assumed that they are readily absorbed through mucous membranes and distributed into the water compartments. Absorption through skin in *in vitro* experiments in different species varied widely depending on the experimental conditions. *In vivo* skin absorption of undiluted [2-¹⁴C]-DEM was highest in nude mice with 15 % absorption and lowest in pigs (2.5 %). In human skin grafted on nude mice and in hairless dogs absorption was 4 %. These experiments indicate relatively low skin absorption under non-occluded conditions. Both DMM and DEM are likely to be metabolized by esterases under cleavage of one or two ester bonds yielding the corresponding alcohols and malonic acid monoesters or malonic acid.

No acute inhalation study is available for DMM. In the dermal toxicity study in rats following OECD guideline TG 402 and GLP (limit test) the LD₅₀ was > 2000 mg/kg bw. An acute oral toxicity study in rats revealed an LD₅₀ > 2000 mg/kg bw. In both studies no test substance related effects were observed.

For DEM only limited literature data are available. No toxicity was observed after 8 h inhalation of concentrated vapors in rats. The dermal LD₅₀ in rabbits was reported to be > 16 960 mg/kg bw, the oral LD₅₀ in rats 15 794 mg/kg bw. Taken together the studies for both substances suggest that they are of low acute toxicity via the oral and dermal route and likely to be also of low toxicity after inhalation exposure.

DMM was not irritating to rabbit skin in a guideline study according to OECD TG 404 and GLP. For DEM no guideline study is available on skin irritation, but a slightly irritating effect was reported in the literature after 24 hours of occlusive exposure. Both substances showed slight to moderate eye irritating effects in rabbits that were completely reversible within the observation period. The studies were conducted according or similar to OECD TG 405 and under GLP.

DMM did not reveal any skin sensitizing effect in a Bühler test according to OECD TG 406 and GLP. Reports of maximization tests in human volunteers with both, DMM and DEM did not indicate any skin sensitizing properties.

One repeated dose study in rats by the oral route (gavage) according to OECD TG 422 and GLP is available for DMM. The only effect observed was a reversible hepatocellular hypertrophy in animals of the high dose group (1000 mg/kg bw/day). The NOAEL was 300 mg/kg bw per day. Only a limited dietary 90 day study in rats is available with DEM, which indicated no treatment related effect at dose levels of 36 and 41 mg/kg bw per day for male and female animals, respectively (only one dose level was tested). Although the information available for DEM is limited, it is considered sufficient because DEM is not likely to be more toxic than DMM. Overall, the toxicity of DMM and DEM after repeated dosing is considered to be low.

Both DMM and DEM were not mutagenic in the standard Ames assay in bacteria with and without metabolic activation. DMM did not show any clastogenic activity in the *in vitro* cytogenetic assay with peripheral human lymphocytes in the presence and absence of a metabolic activation system. All tests were conducted according to OECD or EC guidelines and GLP. For both substances, there is no structural alert for genotoxicity. In conclusion, from the available information there is no indication of a mutagenic potential of the substances, both for gene mutations and chromosomal aberrations.

Based on the findings in a combined oral (gavage) repeated dose reproduction/developmental toxicity study in rats according to OECD TG 422 and GLP with DMM a NOAEL for parental toxicity of 300 mg/kg bw/day for males and females and a NOAEL for reproductive and developmental toxicity of 1000 mg/kg bw/day, the highest dose tested, can be derived. No reproductive/developmental toxicity study was available for DEM. Because it is impossible to reach blood levels of ethanol which are associated with reproductive/developmental toxicity as a consequence of the manufacture and normal use of DEM, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. Taking also into account that DEM is not likely to be more toxic than DMM, which has shown no potential for reproductive and developmental toxicity, it is overall concluded that there is no indication for a relevant reproductive and/or developmental toxicity of DMM and DEM.

Environment

Both, dimethyl- and diethylmalonate are colorless organic liquids with an ester like odor. DMM has a melting point of -62 °C, a boiling point of 181.4 °C, a water solubility of about 142 g/l at 20 °C, a vapor pressure of 0.48 - 0.5 hPa at 20 °C and a measured log Kow of -0.05. DEM has a melting point of -48.7 to -51.1 °C, a boiling point of 199.3 °C, a water solubility of 20 g/l at 20 °C, a vapor pressure of 0.36 hPa at 25 °C and a measured log Kow of 0.96. Both substances are readily biodegradable (100 % (DMM) and 98 % (DEM) in a DOC-die away test) and undergo a two-step hydrolytic degradation in a first step to the monoester and in a second step to malonic acid and the corresponding alcohol, methanol or ethanol respectively. The half lives were shortest at pH 9, < 2.4 h (50 °C) for both substances, and increased to 5.7 h (50 °C) and 15.9 h (50 °C) for DMM and DEM respectively at pH 7 and 859 h (50 °C) for DMM at pH 4. At pH 4 and 50 °C DEM showed less than 10 % degradation within 5 days. For photodegradation via oxidation by OH-radicals half lives of about 31 days for DMM and 4.7 days for DEM in air were estimated. For DEM a 100% photolytic ozonisation after 40 min under UV-irradiation in water was reported. The generic fugacity model I indicates that both substances are preferably distributed in the water phase (98 % for DMM and 90 % for DEM) with a low amount distributing potentially into air (1.5 and 9.9 % respectively). The fugacity model III however, indicates that a considerable amount may be distributed to the soil if the substances are primarily released into air (36 % for DMM and DEM) or soil (38 % for DMM and 44.5 % for DEM). The measured octanol-water partition coefficients (log Kow -0.05 for DMM and 0.96 for DEM) indicate a low potential for bio- or geoaccumulation.

Acute toxicity data for 3 trophic levels of the aquatic environment are available for both substances.

Acute toxicity in mg/l:

	DMM	DEM
LC ₅₀ fish: 96 h, <i>Danio rerio</i>	21	-
LC ₅₀ fish: 96 h, <i>Pimephales promelas</i>	-	12 - 17
EC ₅₀ Daphnia: 48 h, <i>Daphnia magna</i>	> 728	179
Algae EC ₅₀ : 72h, <i>Desmodesmus subspicatus</i> ; growth rate (biomass)	240(92)	> 667(424)

Based on the lowest LC₅₀-value for fish of 21 mg/l for DMM and 12 mg/l for DEM and an assessment factor of 1000 PNEC-values of 21 and 12 µg/l can be derived for DMM and DEM, respectively.

No growth inhibition of DEM to terrestrial plants in soil was observed up to concentrations of > 100 mg/kg soil and no toxicity to *Eisenia fetida* was observed at concentrations of DEM of 1000 mg/kg bw after 14 days of exposure.

Exposure

The worldwide production capacity of malonates with DMM and DEM as the most important products was estimated to be more than 20 000 t/a. The breakdown by country in 2000 was estimated as follows: Europe: 8000 t/a (Sponsor country: 8000 t/a by 1 Producer), Japan 4000 t/a, China 12 000 t/a, Korea 2000 t/a, and India 600 t/a. DMM and DEM are widely used in the chemical industry as intermediates for the synthesis of a variety of organic chemicals, for example to introduce an acetic moiety or a hydroxyester group into molecules. The end products of the different processes in which malonates are used as intermediates include pharmaceuticals, agrochemicals, vitamins, fragrances and dyes. It was estimated that about one third each of the volume of DMM is used in the production of agrochemicals, pharmaceuticals and industrial chemicals. For DEM the estimated breakdown is 30 % as an intermediate for the production of agrochemicals, 50 % pharma-intermediate, 20 % as intermediate for industrial chemicals. Because of the predominant production and use in chemical industry under controlled conditions, environmental exposure from production and use is considered low. DMM is a naturally occurring substance and has been detected in a number of fruits as a volatile aroma compound for example in pineapples, bananas and blackberries.

In production from the process description very low occupational exposure is anticipated. No data are available for the uses. As the majority of the products are used as intermediates in the chemical industry a controlled exposure situation is anticipated.

With regard to consumer exposure WHO (2000) evaluated the combined daily intake of 47 flavoring substances including DEM in Europe and the US. The annual production volume of these 47 substances was 200 metric tons in Europe and 1700 metric tons in the US. From this an estimate per capita daily intake of 28 mg in Europe and 300 mg in the US was derived. This intake was considered of no concern.

DMM is contained in the Swedish and Swiss Product Registers, but not in the SPIN Database. DEM is contained in the Swedish and Swiss Product Registers and in the SPIN Database.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemicals of this category are currently of low priority for further work due to their low hazard profile.

Environment: The chemicals of this category possess properties indicating a hazard for the environment. Although these hazards do not warrant further work (as they are related to acute toxicity which may become evident only at high exposure levels) they should nevertheless be noted by chemical safety professionals and users. The chemicals are currently of low priority for further work.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substances

Substance	Dimethylmalonate	Diethylmalonate
CAS Number:	108-59-8	105-53-3
IUPAC Name:	Dimethyl malonate	Diethyl malonate
Molecular Formula:	C ₅ H ₈ O ₄	C ₇ H ₁₂ O ₄
Structural Formula:		
Molecular Weight:	132.12 Dalton	160.2 Dalton
Synonyms:	Propanedioic acid, dimethylester, dimethyl malonate, malonic acid, dimethylester, DMM	Propanedioic acid, diethylester, diethyl malonate, malonic acid, diethylester, DEM

1.2 Purity/Impurities/Additives

Both dimethyl- and diethylmalonate are colorless organic liquids with an ester like odor. The purity is typically > 99 %. Impurities from the production process include methanol (ca. 0.3 % w/w) and dimethyl methylmalonate (ca. 0.2 % w/w) for DMM and ethanol (ca. 0.1 % w/w), ethyl acetate (ca. 0.05 % w/w), and ethyl methyl malonate (ca. 0.05 % w/w) for DEM. (Degussa, 2005).

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Substance	Dimethylmalonate	Diethylmalonate	Reference (DMM / DEM)
Property	Value	Value	
Physical state/color/odor	liquid/colorless/ester-like	Liquid/colorless/ esterlike	Degussa, 2004a / Degussa, 2004b
Melting point	-62 to -61.9 °C	-51.5 to -48.7 °C	Kendall and Booge, 1916; Palomaa and Mikkilä, 1942 / Jäger, 1917; Timmermans and Delcourt, 1934
Boiling point (1013 hPa)	181.4 °C	199.3 °C	Lecat, 1928 / Timmermans and Delcourt, 1934
Relative density (20 °C)	1.153 g/cm ³	1.055 g/cm ³	Vogel, 1934; Palomaa and Mikkilä, 1942 / Mumford and Phillips, 1950
Vapor pressure	0.48 - 0.5 hPa (20 °C)	0.36 hPa (25 °C)	Derived from D'Ans- Lax, 1967; Degussa, 2004a / Daubert and Danner, 1989
Water solubility (20 °C)	99 g/l	20 g/l	Meylan et al., 1996 / O'Neil et al., 2001
Partition coefficient n- octanol/water (log value) (measured)	-0.05	0.96	Hansch et al., 1995
Henry's law constant (25 °C)	0.0422 Pa m ³ mol ⁻¹ (calculated)	0.0746 Pa m ³ mol ⁻¹ (calculated)	Degussa, 2003a / Degussa, 2003b
Flash point (closed cup)	90 °C	93 °C	BIA, 2001; Hawley, 1981 / Lide, 2004
Autoflammability, self ignition temperature	440 °C	435 °C	Degussa, 2004a / Degussa, 2004b

1.4 Category Justification

The production and use pattern of Diethylmalonate (DEM) and Dimethylmalonate (DMM) are comparable. The two chemicals have very similar physico-chemical properties and both esters are hydrolyzed via a two step reaction to malonic acid and the corresponding alcohol, methanol or ethanol. It is likely that unspecific esterases in the body catalyze the hydrolysis. The alcohols and malonic acid are physiological substances that are metabolized via physiological pathways. Ethanol and methanol were assessed at SIAM 19 (OECD, 2004a,b). For ethanol it was concluded that the chemical is currently of low priority for further work, because the hazardous properties of ethanol are manifest only at doses associated with consumption of alcoholic beverages. As it is impossible to reach these exposure levels as a consequence of the manufacture and use of malonates, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. For methanol, SIAM 19 decided that this chemical is a candidate for further work. Methanol exhibits potential hazardous properties for human health (neurological effects, CNS depression, ocular effects, reproductive and developmental effects, and other organ toxicity). The

effects of methanol on the CNS and retina in humans only occur at doses at which formate accumulates due to a rate-limiting conversion to carbon dioxide. In primates, formate accumulation was observed at methanol doses greater than 500 mg/kg bw (which would require a DMM dose of more than 1,000 mg/kg bw). As there were no indications of a methanol associated toxicity from a well performed repeated dose toxicity study with DMM in rodents (which are, however, known to be less sensitive to methanol toxicity than humans), and because methanol toxicity would not be expected up to doses as high as 1,000 mg DMM/kg bw/day, it was concluded that methanol does not make a relevant contribution to the toxicity profile of DMM. A possible mode of action for systemic toxicity of DMM and DEM can only be deduced from the repeated dose study with DMM, indicating a reversible liver hypertrophy at the cellular level at high doses of 1000 mg/kg bw/day. This effect can be an indication of an induction of metabolism in the liver rather than a clear systemic toxicity.

Data Availability for Dimethyl and diethylmalonate

Acute toxicity and ecotoxicity data are available for both substances. In order to gain information on repeated dose toxicity, reproductive toxicity endpoints and chromosomal aberration studies for the methyl ester have been performed according to current guidelines that are used as surrogate data for the ethyl ester as well.

The available data for both members of the category are summarized in the following table.

Table 2 Data availability

OECD SIDS Endpoint	Dimethylmalonate 108-59-8	Diethylmalonate 105-53-3
Physicochemical Properties		
Melting point	✓	✓
Boiling point	✓	✓
Density	✓	✓
Vapor pressure	✓	✓
Partition Coefficient	✓	✓
Water solubility	✓	✓
Fate		
Biodegradation	✓	✓
Photodegradation	✓ calculated	✓ calculated
Hydrolysis	✓	✓
Fugacity	✓ calculated	✓ calculated
Ecotoxicological data		
Acute Fish Toxicity	✓	✓
Acute Daphnia Toxicity	✓	✓
Algae Toxicity	✓	✓
Terrestrial plants	-	✓
Toxicological data		
Acute Toxicity	Oral: ✓ Dermal: ✓ Inhalation: -	Oral: ✓ Dermal: ✓ Inhalation ✓
Repeated Dose Toxicity	✓ OECD 422	✓ (limited validity)
Genotoxicity, <i>in vitro</i> Ames	✓	✓
Genotoxicity, <i>in vitro</i> Cytogenetic test	- ✓	-
Reproductive Toxicity	✓ OECD 422	-
Developmental Toxicity	✓ OECD 422	-
Additional data		
Toxicity to soil organisms		✓
Skin irritation	✓	✓
Eye irritation	✓	✓
Skin sensitization	✓	-

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

2.1.1 Production

Malonates are produced either by cobalt-catalyzed alkoxy-carbonylation of chloroacetates with carbon monoxide in the presence of alcohol (Carbon monoxide process) or by hydrolysis of cyanoacetic acid followed by esterification with the respective alcohol (hydrogen cyanide process). (Hildbrand and Pollak, 2002).

In the carbon monoxide process DMM and DEM are produced by a di-cobalt octacarbonyl catalyzed reaction of methyl- or ethyl chloroacetate with carbon monoxide in the presence of the respective alcohol. For DEM the reaction takes place at 100 °C and 18 bar at pH 5.7. Ethylacetate is formed as a major by product. After completion of the reaction sodium chloride and the catalyst are separated, and the ester is subsequently purified by several distillation steps. (Hildbrand and Pollak, 2002).

The German producer uses the carbon monoxide process for the production of DMM. In the German producer company the reaction takes place in a closed, discontinuous process. Loading and de-loading operations are performed in a closed system using a vapor recovery device. Sampling is performed in a closed system through a sampling valve with a special syringe. From production there are no emissions into water or air. Exhausts are incinerated and waste water is treated in a biological sewage treatment plant. Product containing waste waters (e.g. from maintenance operations) are incinerated. No waste containing DMM is produced. The substance is transported in road tankers, tank containers and drums (Degussa, 2004c).

The hydrogen cyanide process normally takes place in a closed plant. In the first step sodium cyanide is reacted with sodium chloroacetate in an aqueous solution at elevated temperatures (90 °C) yielding sodium cyanoacetate. Sodium cyanoacetate is concentrated by evaporation under vacuum and then reacted with an alcohol/mineral acid mixture at temperatures between 60 and 80 °C via the non-isolated intermediate malonic acid monamide to the dialkylmalonate. Purification steps include solvent extraction and distillation under vacuum. (Hildbrand and Pollak, 2002).

DEM can also be produced by transesterification of DMM with ethanol. This process is used by the German producer company in a closed continuous process. Loading and de-loading operations are performed in a closed system using a vapor recovery device. Sampling is performed in a closed system through a sampling valve with a special syringe. From production there are no emissions into water or air. Exhausts are incinerated and waste water is treated in a biological sewage treatment plant. Product containing waste waters (e.g. from maintenance operations) are incinerated. No waste containing DMM is produced. The substance is transported in road tankers, tank containers, drums and IBC's (Degussa, 2004d).

The worldwide production capacity of malonates with DMM and DEM as the most important products was estimated to be more than 20,000 metric t per year. The breakdown by country was estimated as follows: Europe: 8000 t/year (Sponsor country: 8000 t/year by one producer), Japan 4000 t/year, China 12,000 t/year, Korea 2000 t/yr, and India 600 t/yr. (Hildbrand and Pollak, 2002).

2.1.2 Processing and Use

DMM and DEM are widely used in the chemical industry as intermediates for the synthesis of a variety of organic chemicals, for example to introduce an acetic moiety or a hydroxyester group into molecules. Reaction of malonates with hydrazines can be used for the synthesis of nitrogen heterocycles. The end products of the different processes in which malonates are used as intermediates include pharmaceuticals, agrochemicals, vitamins, fragrances and dyes (Hildbrand and Pollak, 2002). It was estimated that about one third each of the volume of DMM is used in the production of agrochemicals, pharmaceuticals and industrial chemicals. For DEM the estimated breakdown is 30 % as an intermediate for the production of agrochemicals, 50 % as a pharmaceutical, 20 % as intermediate for industrial chemicals (Degussa, 2004c, d). An additional use of DEM in low amounts is as fragrance and artificial flavoring substance in foods (WHO, 2000).

The Swedish Product Register (2004) contains confidential data on DMM on the whole and the note that there are no consumer products containing DMM. One entry on DMM is contained in the Swiss Product Register (2004): 1 commercial product with a DMM-content of 100 %, i.e. the pure chemical. The SPIN database (2004) does not contain any entries on DMM.

The Swedish product Register (2005) contains data on DEM: 13 products containing 0-2% DEM, 2 of which are consumer products, with a tonnage of 0.0 t/a, and 4 products containing 2-20% DEM, 2 of which are consumer products, with a tonnage of 2.0 t/a. Information on uses of consumer products is confidential. Most frequent industrial uses are adhesives, hardeners for adhesive and industrial use, the most common industry category is sales and repair establishments for motor vehicles and motorcycles. DEM is contained in the SPIN database (2004): For 2002, 79 preparations with an overall amount of 0.3 tonnes are noted for Denmark, 7 preparations with an overall amount of 0.1 tonnes are noted for Norway, and data on 12 preparations including consumer products are listed as confidential for Sweden.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Exposure

From production in the sponsor country there are no emissions into water or air. Exhausts are incinerated and waste water is treated in a biological sewage treatment plant. Product containing waste waters (e.g. from maintenance operations) are incinerated. No waste containing DMM or DEM is produced (Degussa, 2004 c, d)

From use as flavoring agent no emission data are known.

Natural occurrence

Dimethylmalonate has been detected in a number of fruits as a volatile aroma compound, e.g. in pineapple by gas chromatography (GLC) and infrared spectroscopy (IR) (Creveling et al., 1968), by capillary GC and mass spectrometry (MS) of vacuum stream distillates and head space analysis of the blended pulp at concentrations of 19 ppb (Takeoka et al., 1989). Umano et al. (1992) found a higher content in green pineapples (18 µg/kg) than in ripe fruits (17 µg/kg) by GC-MS analysis and comparison of peak areas. DMM was also identified in liquid-liquid extracts of fresh blackberries by GC-MS (Georgilopoulos and Gallois, 1987), or in banana (< 5 µg/100 g extract) (Berger et al., 1986). Miyazawa and Kameoka (1987) identified DMM as a volatile flavor component of astragal roots (*Astragalus membranaceus* Bunge) by GC/MS and IR analysis.

2.2.2 Photodegradation

In air for DMM a photochemical reaction rate constant of $5.25 \cdot 10^{-13} \text{ cm}^3/(\text{molecule} \cdot \text{s})$ and a half-life of 30.6 days (Degussa, 2003c; Meylan and Howard, 1993a), and for DEM a photochemical reaction rate constant of $3.41 \cdot 10^{-12} \text{ cm}^3/(\text{molecule} \cdot \text{s})$ and a half-life of 4.7 days (Degussa, 2003d; Meylan and Howard, 1993b) were calculated using the APOWIN program version 1.90. For DEM a 100 % photochemical degradation in water within 35 to 40 minutes by photolytic ozonation (ozone dose rate 0.000013 mmol/l x min) at a concentration of 5 mg/l and 23 °C under UV light of ca. 254 nm was reported by Peyton et al. (1989).

2.2.3 Stability in Water

Standard studies on hydrolysis as function of pH performed according to OECD TG 111 and under GLP are available for both esters. The results are summarized in table 3.

Table 3: Summary of Hydrolysis data

Ester	Half-life at			Test Type	Reference
	pH 4	pH 7	pH 9		
DMM	859 h (50 °C) 351 d (25 °C)	5.7 h (50 °C) 52.5 h (25 °C)	< 2.4 h (50 °C)	OECD 111 (92/69/EEC, C.7)	Degussa, 2004e
DEM	- (hydrolysis < 10 % after 5 d, 50 °C)	15.9 h (50 °C) 137.5 h (25 °C)	< 2.4 h (50 °C)	OECD 111 (92/69/EEC, C.7)	Degussa, 2004f

As the substances have two hydrolysable groups, the hydrolysis reaction can be summarized as follows:



In the study the intermediate monoester could not be determined due to analytical reasons (decarboxylation in the GC-injector block) but by following the formation of the alcohols it was possible to estimate the formation of reaction products, monoester and malonic acid by performing a mass balance analysis.

At pH 9 hydrolysis is fast for both esters with half lives of < 2.4 h at 50 °C. For DMM hydrolysis was 95.9 %, for DEM 81.1 % after 2.4 h. The hydrolysis of one ester group occurs first, but the subsequent hydrolysis of the second ester bond also takes place within about 2 half-lives (Degussa, 2004g).

At pH 7 the hydrolysis was slower with half-lives of 5.7 h at 50°C and 52.5 h at 25 °C for DMM and 15.9 h at 50 °C and 137.5 h at 25 °C for DEM. The reaction is mainly due to the formation of monoester. However after longer reaction periods a cleavage of the monoester was also observed (Degussa, 2004g).

At pH 4 the esters are more stable and the half life at 50 °C was 859 h for DMM and DEM was stable (less than 10 % degradation) after 5 days at 50 °C. For DMM a half-life of 351 d at 25 °C was calculated from the data. Hydrolysis of the monoester was relatively quickly followed by further hydrolysis to malonic acid under acidic conditions (Degussa, 2004e, f, g).

It can be concluded that both esters hydrolyze rapidly under alkaline conditions, first under formation of the monoesters followed by formation of malonic acid. Under neutral conditions the reaction is still relatively rapid in particular the first step, formation of the monoester, while in acid conditions the half-lives are considerably longer and hydrolysis of both ester groups occurs almost simultaneously. The velocity of the hydrolysis is higher for DMM compared to DEM, and the difference increases with decreasing pH. Under alkaline conditions however, the half-lives are comparable.

2.2.4 Transport between Environmental Compartments

DMM and DEM have water solubilities of 142 and 20 g/l respectively at 20 °C. The vapor pressure is 0.48-0.5 hPa (20 °C) for DMM and 0.36 hPa (25 °C) for DEM indicating that volatilization from water is expected to be relatively low. This is corroborated by the relatively low calculates Henry's law constants of 0.0422 Pa m³ mol⁻¹ and 0.0746 Pa m³ mol⁻¹ respectively (Degussa, 2003a, b). The equilibrium partition characteristics in the environment were estimated using the Mackay level I model calculation (Degussa, 2004h, i).

Table 4 Mackay level I model calculation

Compartment	DMM Theoretical Distribution [%]	DEM Theoretical Distribution [%]
Air	1.55	9.86
Water	98.44	90.01
Soil	0.01	0.06
Sediment	< 0.01	0.07
Biota (as fish)	< 0.01	< 0.01

Based on this calculation the most likely target compartment for both compounds for theoretical environmental emissions is the hydrosphere with a small amount also distributing to the atmosphere, to a slightly higher extent for DEM.

The generic Mackay Level III calculation (estimated entry 3000 kg/h to air, water or soil) yielded the following distribution pattern (Degussa, 2004 j, k).

Table 5 Mackay level III model calculation

	Substance	Air	Water	Soil	Sediment
Release 100 % into air Distribution [%]	DMM	2.58	61.3	36.0	0.02
	DEM	15.2	48.8	36.0	0.02
Release 100 % into water Distribution [%]	DMM	0	99.9	0.01	0.04
	DEM	0.01	99.9	0.01	0.04
Release 100 % into soil Distribution [%]	DMM	0.02	61.7	38.3	0.02
	DEM	0.03	55.4	44.5	0.02

When released into air or soil the majority of the esters will distribute almost equally to water and soil, while when the substance is released into water it will stay in the water compartment. Distribution to sediment is negligible.

2.2.5 Biodegradation

Both DMM and DEM were readily biodegradable in a DOC-die away test conducted according to current OECD and EU guidelines and GLP. Degradation rates after 28 days were 100 % for DMM and 98 % for DEM and 86 - 87 % or 90 - 94 % respectively after 7 days. (Hüls, 1992a; Hüls, 1993a).

2.2.6 Bioaccumulation

The low octanol-water partition coefficients (DMM: $\log K_{OW} = -0.05$ (measured) (Hansch et al., 1995); DEM: $\log K_{OW} = 0.96$ (measured) (Hansch et al., 1995) indicate a low potential for bioaccumulation.

2.2.7 Geoaccumulation

Soil sorption coefficients (K_{OC}) of 1.74 for DMM and 10 for DEM were calculated by PCKOCWIN (v. 1.66, SRC, 2000) (Degussa, 2003g, h) and indicate a low potential for geoaccumulation.

2.2.8 Other Information on Environmental Fate

No information available.

2.3 Human Exposure

2.3.1 Occupational Exposure

The German producer uses closed systems including gas tight flushes for loading and de-loading operations and closed valve-syringe systems for sampling (Degussa, 2004c, d). From the process description very low occupational exposure is anticipated. No data are available for the uses. As the majority of the products are used as intermediates in the chemical industry a controlled exposure situation is anticipated.

2.3.2 Consumer Exposure

WHO (2000) evaluated the combined daily intake of 47 flavoring substances including DEM in Europe and the US. The annual production volume of these 47 substances was 200 metric tons in Europe and 1700 metric tons in the US. From this an estimated per capita daily intake of 28 mg in Europe and 300 mg in the US was derived (based on a body weight of 60 kg these intakes would correspond to 0.47 and 5 mg/kg bw/day in Europe and the US, respectively). This intake was considered of no concern.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

Diethylmalonate (DEM) and Dimethylmalonate (DMM) have very similar physico-chemical properties and both esters are hydrolyzed via a two step reaction to malonic acid and the corresponding alcohols, methanol or ethanol.

Ethanol and methanol were assessed at SIAM 19 (OECD, 2004a, b). For ethanol it was concluded that the chemical is currently of low priority for further work, because the hazardous properties of ethanol are manifest only at doses associated with consumption of alcoholic beverages. As it is impossible to reach these exposure levels as a consequence of the manufacture and use of DEM, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM.

For methanol, SIAM 19 decided that this chemical is a candidate for further work. Methanol exhibits potential hazardous properties for human health (neurological effects, CNS depression, ocular effects, reproductive and developmental effects, and other organ toxicity). The effects of methanol on the CNS and retina in humans only occur at doses at which formate accumulates due to a rate-limiting conversion to carbon dioxide. In primates, formate accumulation was observed at methanol doses greater than 500 mg/kg bw. (Such high methanol exposures could only be produced from DMM doses of more than 1000 mg/kg bw). As there were no indications of a methanol associated toxicity from a well performed repeated dose toxicity study with DMM in rodents (which are, however, known to be less sensitive to methanol toxicity than humans), and because methanol toxicity would not be expected up to doses as high as 1000 mg DMM/kg bw/day, it was concluded that methanol does not make a relevant contribution to the toxicity profile of DMM.

A possible mode of action for systemic toxicity of DMM and DEM can only be deduced from the repeated dose study with DMM indicating a reversible liver hypertrophy at the cellular level at high doses of 1000 mg/kg bw/day. This effect can be an indication of an induction of metabolism in the liver rather than a clear systemic toxicity.

3.1.1 Toxicokinetics, Metabolism and Distribution

From the physico-chemical properties of both substances it can be assumed that they are readily absorbed via mucous membranes. Distribution is likely to occur in the water compartments and accumulation in fat is unlikely due to the physical chemical properties. Both substances are likely to be metabolized by unspecific (serine-) esterases of different tissues, in particular in the liver to the mono esters and finally to malonic acid and the corresponding alcohols, methanol and ethanol, respectively. This is corroborated by the findings of the abiotic hydrolysis, in particular at alkaline pH that can be regarded as qualitatively similar to the hydrolysis catalyzed by unspecific esterases (Jacobi and Hoffmann, 1989). The hydrolysis products are likely to be metabolized via physiological pathways as the tricarboxylic acid cycle because they are part of the normal intermediate metabolism (WHO, 2000). Some data on *in vivo* and *in vitro* skin absorption and enzymatic cleavage of the ester bond are available for DEM.

Studies in Animals

In vitro Studies

Dermal absorption studies

The percutaneous absorption of radiolabeled diethylmalonate was studied in a flow through perfusion cell with freshly prepared skin of weanling Yorkshire pigs (1.8 cm diameter, 1.9 mm

thickness) and Tyrode's solution as receptor fluid. [2-¹⁴C]-DEM was applied either undiluted (100 µg/cm²) or diluted in ethanol (12.5 mg/ml) 100 µg/cm² or (0.5 mg/ml) 4 µg/cm². The donor cell had a temperature of 24 °C, the receptor cell temperature was 37 °C; the receptor cell flow rate was 5 ml/h, and the incubation period amounted to 50 h. The results are summarized in table 6 (Hawkins and Reifenrath, 1984).

Table 6 Percent of the skin penetration of [2-¹⁴C]-DEM in Yorkshire pig skin *in vitro* (50 h)

Applied dose/cm ²	Percent radioactivity in receptor fluid	Percent radioactivity in skin	Percent radioactivity evaporated
Undiluted, 100 µg	3 (± 1) %	8.8 (± 0.5) %	25-50 %
100 µg in ethanol	6 (± 3) %	13 (± 2) %	
4 µg in ethanol	10 (± 3) %	30 (± 10) %	

In a worst case assumption the amount penetrating and adsorbed to the skin can be regarded as potentially absorbed. This would amount to an absorption of 11.8 % for the undiluted substance, 19 % for an equal amount when dissolved in ethanol, and 40 % for a diluted solution in ethanol. However as parts of the stratum corneum will be sloughed off, it is likely that skin adsorption *in vivo* would be lower. In this experiment ethanol seemed to enhance absorption of the test substance.

In another experiment with freshly prepared skin of weanling Yorkshire pigs (1 mm thick, split thickness skin containing epidermis and a portion of the dermis, area: 0.8 cm²) the 24 h penetration rates of 1 mg/cm² of [2-¹⁴C]-DEM in 10 µl of acetone were determined. The experiment was performed under flow through conditions with a flow of the receptor fluid of 5 ml/h (temperature 37 °C). The receptor fluid was collected every hour in the first 12 hours and every 2 hours thereafter. At the same time hydrolysis of DEM in the living skin was studied by analyzing the receptor fluid for monoethyl malonate and malonic acid. After 24 h 0.2 to 1.6 % of DEM was found in the receptor fluid, 0.2 to 0.9 % were found in the skin and 0.6 to 0.7 % on the skin surface. The skin mediated hydrolysis of DEM amounted to 15 to 35 % of the applied dose. In the receptor fluid 20 to 21 % of the applied dose was present as hydrolysis products, in the skin hydrolysis products amounted to 3 to 5 %, and on the skin surface to 2 - 4 % of the applied dose. However, hydrolysis (1.2 to 16 %) also occurred when the receptor fluid was incubated with the test substance. The maximum penetration rate of the hydrolysis products was reached after 5 h and amounted to approximately 2 % of the applied dose/hour. Preincubation of the skin at 80 °C for 5 min to inactivate esterases considerably decreased the amount of hydrolysis products and increased the penetration of DEM. (Chellquist and Reifenrath, 1988).

Metabolism

Two limited studies are available that studied enzyme catalyzed ester hydrolysis of DEM. Incubation of 10 µmoles DEM with 2 µg of purified lipase of pork adipose tissue for 20 min at 37 °C and pH 7 yielded 1.9 µmoles of malonic acid. (Lynn and Perryman, 1960). When 29.5 mg of DEM/ml were incubated with 0.5 mg alpha-Chymotrypsin for 20 h at 25 °C and pH 7.2, 73 % of DEM was converted to monoethyl malonate. (Cohen and Crossley, 1964).

Malonic acid can be activated to malonyl-CoA and undergoes decarboxylation to acetyl-CoA by various mammalian tissues (Koeppen et al., 1978).

In vivo Studies

The percutaneous penetration of radiolabeled diethyl malonate was studied in different animal models, athymic nude mouse, human and pig skin grafted to athymic nude mice, weanling pigs, hairless dogs. [²⁻¹⁴C] radiolabeled DEM was applied at a dose of 0.1 mg/cm² for 24 h to mice skin

(area: 1.27 cm²) or for 48 h to pigs and hairless dogs (area 25 cm²) under a non-occlusive protective patch. After the application time the skin was washed with ethanol. The percutaneous penetration was estimated from the recovery of radioactivity in urine and faeces and corrected for the recovery observed after parenteral (s. c.) administration. Absorption was 15 % in nude mice, 4 % in human skin grafted to nude mice, 6 % in pig skin grafted to nude mice, 2.5 % in pigs and 4 % in dogs (Reifenrath et al., 1984). Overall, the *in vivo* data from pigs correspond well to the percentages detected in the receptor fluid in the *in vitro* studies. The apparent difference between *in vitro* and *in vivo* data is most probably only related to the very conservative approach of the *in vitro* studies to also consider the amount on the skin as “absorbed”.

Studies in Humans

In vitro Studies

An *in vitro* skin absorption study was performed with DEM. Human cadaver split thickness skin of male Chinese, 60 to 80 years old with a thickness of 600 µm comprising the epidermis and the uppermost layer of the dermis was used. The experiment was performed in flow through cells with a total diffusion volume of 0.32 cm³. The exposed surface area was 0.8 cm², the chamber temperature 32 °C. Perfusion was continuous with a steady flow of 8 ml/h of the receptor fluid, 0.9 % saline. A small constant air flow was maintained above the surface to mimic unoccluded conditions. 4 µl of DEM was applied to the skin samples and the outer chamber was sealed and covered with a tenax tube to collect evaporating test substance. The experiment was performed for 24 h. Every 2 h samples of the receptor fluid were taken. Amounts adsorbed to the skin surface were extracted from the skin samples with 10 ml of ethanol for 2 h. Analyses of the receptor fluid, skin and tenax tube extracts was performed by GC/FID. After 24 h 16 % of the applied dose had penetrated through the skin. The maximum flux rate was reached after 5 h and amounted to 280 µg/h (350 µg cm⁻²h⁻¹); the mean penetration rate was 99 µg/h (120 µg cm⁻²h⁻¹). The majority of the test substance, 45 to 50 % evaporated from the skin, and 34 to 39 % remained on the skin. (Loke et al., 1999).

The mammalian metabolism of methanol, a metabolite of DMM, occurs mainly in the liver, where methanol is converted to formaldehyde, which is in turn converted to formate. Formate is then finally converted to carbon dioxide and water. In humans, the conversion to formaldehyde is mediated by alcohol dehydrogenase. In rodents, the reaction occurs mainly via a catalase-peroxide pathway. In rodents, the first step is rate limiting and methanol in turn accumulates in the blood. In primates, the conversion of formate to carbon dioxide is rate-limiting, leading to a disproportionate increase of formate in the blood and sensitive target tissues (such as CNS and the retina) (OECD, 2004a).

The DEM metabolite ethanol is readily absorbed by the oral and inhalation routes and subsequently, metabolized and excreted in humans. At exposures relevant to occupational and consumer exposure during manufacture and use of ethanol containing products, the alcohol dehydrogenase metabolic route in the liver dominates and does not become saturated. This mechanism follows first order kinetics. The first step of the metabolic path is the rate-determining step; concentrations of the intermediate metabolite acetaldehyde are very low. Ethanol is not accumulated in the body. Dermal uptake of ethanol is very low (OECD, 2004b).

Conclusion

From the physical chemical properties of both substances it can be assumed that they are readily absorbed through mucous membranes and distributed into the water compartments. Absorption through skin in *in vitro* experiments in different species varied widely depending on the experimental conditions. *In vivo* skin absorption of undiluted [2-¹⁴C]-DEM was highest in nude

mice with 15 % absorption and lowest in pigs (2.5 %). In human skin grafted on nude mice and in hairless dogs absorption was 4 %. These experiments indicate relatively low skin absorption under non-occluded conditions *in vivo*. Both DMM and DEM are likely to be metabolized by esterases under cleavage of one or two ester bonds yielding the corresponding alcohols and malonic acid monoesters or malonic acid.

3.1.2 Acute Toxicity

Studies in Animals

Acute toxicity studies in rats or rabbits are available for both substances and indicate a low acute toxicity via the oral, dermal and inhalation route. The information on DEM is however limited.

Methanol and ethanol, the metabolites of DMM and DEM, were assessed at SIAM 19 (OECD 2004a, b). Typical symptoms of methanol intoxication in humans (i.e. acidosis and ophthalmologic changes) do not occur in rodents or rabbits, which are able to remove formate (i.e. the ultimate methanol toxicant in humans) more efficiently. In these animals, CNS depression is usually the cause of defects and finally death. Ethanol, as used in industrial processes (i.e. not considering its use as alcoholic beverage) has a low order of acute toxicity by all routes of exposure.

Inhalation

A limited inhalation study reported no deaths in rats that were exposed to concentrated vapors of DEM for 8 h. No details were reported. (Smyth et al., 1969).

No data on the acute inhalation toxicity of DMM are available.

With regard to the acute inhalation toxicity of methanol, SIAM 19 (OECD, 2004a) concluded that "...In rats, LC₅₀ values have been calculated to be 83.2 and 128.8 mg/l after 4 hours. In cats, the LC₅₀ was 85.4 mg/l after 4 hours. In monkeys, air concentrations of 52 mg/l after 1 - 4 hours and 13 mg/l after 18 hours led to an unspecified level of mortality."

The lowest robustly reported value for ethanol (OECD, 2004b) is an inhalation LC₅₀ of > 60 000 ppm (114 000 mg/m³; 1 hour, mouse).

Dermal

A limit study according to OECD TG 402 and GLP with DMM revealed no mortality, no clinical signs, no local irritation at the site of contact, no effects on body weight and no macroscopic organ changes attributable to the test substance at the limit dose of 2000 mg/kg bw. (Hüls, 1992b).

For DEM an acute dermal toxicity in rabbits of > 16 960 mg/kg bw with 24 h of exposure was reported. (Smyth et al., 1969). This study was not conducted according to modern guidelines, but corroborates the study with DMM.

With regard to acute dermal toxicity of methanol, SIAM 19 (OECD, 2004a) concluded that "Dermal LD₅₀s in rabbits range from 15 800 to 20 000 mg/kg bw. In rats, the dermal LD₅₀ is greater than 45 000 mg/kg. In monkeys, four daily dermal doses of 400 mg/kg bw eventually resulted in death." No robust LD₅₀ values were reported for ethanol (OECD, 2004b).

Oral

For DMM a GLP- limit study was conducted in rats and revealed no substance related mortality, clinical symptoms, body weight changes or macroscopic findings at the limit dose of 2000 mg/kg bw (Hüls, 1992c).

Smyth et al. (1969) report an acute oral toxicity in rats of DEM of 15 794 mg/kg bw. This study was not conducted according to modern guidelines, but corroborates the study with DMM.

With regard to the acute oral methanol toxicity, SIAM 19 (OECD, 2004a) concluded, that “Oral LD₅₀s in rats range from < 790 to 13 000 mg/kg bw, in mice, the values range from 7300 to 10 000 mg/kg bw; in rabbits, the LD₅₀ was approximately 14 200 to 14,400 mg/kg bw; and in monkeys, the values range from 7000 to 9000 mg/kg bw. Although most of the references for these values provided only limited details, the values are consistent within species and route of exposure.” The lowest robustly reported value for ethanol (OECD, 2004b) is an oral LD₅₀ of 8300 mg/kg bw (mouse).

Human Exposure Experience

The acute toxicity of methanol, a metabolite of DMM, was assessed at SIAM 19, and the following conclusions were reached (OECD, 2004a):

“...Formate is considered to be the ultimate toxicant in acute intoxication in humans. Acidosis and ophthalmologic changes are typical primary effects. A blood level of 500 mg methanol/l in acutely poisoned patients generally is regarded as requiring hemodialysis. This blood concentration can transiently be achieved in an adult person (70 kg) by ingestion of 0.4 ml methanol/kg bw. Generally, in humans, transient central nervous system (CNS) effects appear above blood methanol levels of 200 mg/l and serious ocular symptoms appear above 500 mg/l. The minimal acute methanol dose to humans that can result in death is considered to be 300 to 1000 mg/kg bw by ingestion, and fatalities have occurred in untreated patients with initial methanol blood levels in the range of 1500 - 2000 mg/l. However, such high blood methanol levels able to cause death are hardly achievable through inhalation exposure. For example, 2.6 or 6.5 mg/l resulted in methanol blood levels that barely exceed 100 and 200 mg/l, respectively, after an 8-hour working shift. Exposure to 0.26 mg methanol/l for 4 hours was without significant physiologic effects in human volunteers.”

For ethanol, the metabolite of DEM the following conclusions were reached at SIAM 19 (OECD, 2004b): “Oral consumption of ethanol containing beverages is known to produce symptoms of intoxication (e.g. drowsiness, loss of concentration). However, there is no evidence that such effects can be produced by inhalation or dermal routes of exposure.”

Conclusion

DMM

No acute inhalation study is available for DMM. In the dermal toxicity study in rats following OECD guideline TG 402 and GLP (limit test) the LD₅₀ was > 2000 mg/kg bw. An acute oral toxicity study in rats revealed an LD₅₀ > 2000 mg/kg bw. In both studies no test substance related effects were observed.

DEM

Only limited literature data are available. No toxicity was observed after 8 h inhalation of concentrated vapors in rats. The dermal LD₅₀ in rabbits was reported to be > 16 960 mg/kg bw, the oral LD₅₀ in rats 15 794 mg/kg bw.

Taken together the studies for both substances suggest that they are of low acute toxicity via the oral and dermal route and likely to be also of low toxicity after inhalation exposure.

3.1.3 Irritation

Skin Irritation

Studies in Animals

DMM was not irritating to rabbit skin in a study according to OECD TG 404 and GLP with 4 hours exposure under semi occlusive conditions. Slight erythema was only observed 30 to 60 minutes after the removal of the patch (Hüls, 1992d).

No guideline study is available for DEM, but it was reported to be slightly irritating to rabbit skin after 24 h occlusive exposure (Moreno, 1975).

As the studies for both substances give comparable results it can be concluded that DMM and DEM should not be regarded as skin irritants. Methanol and ethanol were not irritating to the skin (OECD 2004a, b).

Eye Irritation

Studies in Animals

When undiluted DMM was administered to rabbit eyes (0.1 ml) without rinsing, slight to moderate eye irritation was observed including conjunctival redness, chemosis, iriditis and slight corneal opacity. All effects were reversible within 8 days. The study was performed according to OECD TG 405 and GLP (Hüls, 1992e). For DEM a comparable study according to FIFRA, F 81-4 and GLP showed comparable slight to moderate irritating effects after administration of 0.1 ml of the undiluted test substance to rabbit eyes (Hüls, 1989). Similar effects were reported for methanol and ethanol (OECD 2004a, b).

Conclusion

DMM was not irritating to rabbit skin in a guideline study according to OECD TG 404 and GLP. For DEM no guideline study is available on skin irritation, but a slightly irritating effect was reported in the literature after 24 hours of occlusive exposure. Both substances showed slight to moderate eye irritating effects in rabbits that were completely reversible within the observation period. The studies were conducted according or similar to OECD TG 405 and under GLP.

3.1.4 Sensitization

Studies in Animals

Skin

DMM was not sensitizing in a Bühler Test in guinea pigs according to OECD TG 406 and GLP (Hüls, 1992f). Animal studies with DEM are not available.

Studies in Humans

Skin

In a maximization test with 25 volunteers DMM was not sensitizing when applied at a concentration of 8 % in petrolatum (Kligman, 1966; Kligman, 1976; Kligman and Epstein, 1975). DEM was reported not to have sensitizing properties in a maximization test in 23 human volunteers when applied at a concentration of 4 % in petrolatum (Epstein, 1975).

Conclusion

DMM did not reveal any skin sensitizing effect in a Bühler test according to OECD TG 406 and GLP. Reports of maximization tests in human volunteers with both DMM and DEM did not indicate any skin sensitizing properties.

3.1.5 Repeated Dose Toxicity

Only studies by the oral route are available.

Studies in Animals

Oral

In a subacute combined repeated dose reproduction/developmental screening test with DMM according to OECD TG 422 and GLP, groups of 10 male and female Wistar rats received doses of 100, 300 and 1000 mg/kg bw per day by gavage once daily, 7 d per week. A high dose recovery and recovery control group of 5 animals of each sex per group was also included in the study. Males received the test item 2 weeks prior to mating, during the mating period and approximately 2 weeks post mating, with a total of 39 treatment days. Females were treated 2 weeks prior to mating, during the mating period, throughout pregnancy and up to lactation day 4. Recovery animals were treated for 39 days followed by a post exposure observation period of 14 days. The animals were examined daily for clinical signs and a FOB was performed in randomly selected 5 males and females of each group at the end of the dosing period for males and during the lactation period for females. Body weights were recorded at the beginning of the study, at last weekly thereafter and at termination. All dams were weighed on gestation days 0, 7, 14, and 20 and lactation days 1 and 4. Food consumption was recorded weekly. Standard hematology and clinical chemistry parameters were determined in 5 randomly selected males and females of each group at the end of the pre-mating period and the recovery period respectively. Organ weights of liver, adrenals, kidneys, thymus, spleen, brain, and heart were determined of 5 males and females of each group. Testes and epididymis weights were determined of all adult males of each group. All adult animals and pups were examined for any structural abnormalities and pathological changes. Standard histopathology was performed on all major tissues of 5 males and 5 females of the control and high dose groups as well as all animals of the recovery and recovery control groups. Livers and testes of 5 males and females in the low and mid dose groups were also examined histopathologically. Stages of spermatogenesis and interstitial testicular structure were determined additionally.

No treatment related effects were observed on clinical symptoms, performance in the FOB, body weight and body weight gain, food consumption, clinical chemistry, hematology, organ weights or gross pathology. In the histopathological examination livers of animals of both sexes in the high dose group showed a significantly increased incidence of hepatocellular hypertrophy. Similar changes were not observed in the high dose recovery group indicating reversibility of the effect. At dose levels of 300 and 100 mg/kg bw per day no treatment related histopathological changes were observed. The NOAEL for repeated dose toxicity is therefore 300 mg/kg bw. There was no indication of a hazardous property associated with methanol toxicity. The observations concerning reproduction and development are reported in chapter 3.1.8. (Degussa, 2003e).

Methanol, a metabolite of DMM, was assessed at SIAM 19 (OECD, 2004a). Methanol exhibits potential hazardous properties for human health (neurological effects, CNS depression, ocular effects, reproductive and developmental effects, and other organ toxicity). The effects of methanol on the CNS and retina in humans only occur at doses at which formate accumulates due to a rate-limiting conversion to carbon dioxide. In primates, formate accumulation was observed at methanol doses greater than 500 mg/kg bw (which would require a DMM dose of more than 1,000 mg/kg

bw). As there were no indications of a methanol associated toxicity from the available repeated dose toxicity study with DMM in rodents (which are, however, known to be less sensitive to methanol toxicity than humans), and because methanol toxicity would also not be expected in humans up to doses as high as 1000 mg DMM/kg bw/day, it is concluded that methanol does not make a relevant contribution to the toxicity profile of DMM.

For DEM a limited subchronic toxicity study was reported in the literature. Ten to 16 male and female CD-rats received DEM in their diet for 90 days at dose levels of 36 mg/kg bw per day for males and 41 mg/kg bw per day for females. A comparable untreated group of rats served as control. Details of the study were not reported. The authors report that no treatment related differences were found between the two groups with regard to growth, food intake, hematological and clinical chemistry parameters, blood-urea levels, organ weights and organ pathology (Posternak, Lindner, and Vodoz, 1969). Although limited, the available repeated dose study for DEM did not show any effect associated with ethanol toxicity after 90 days at about 40 mg/kg bw/day (only tested dose). This would, however, also not be expected, because the lowest reported NOAEL for ethanol in repeated dose studies on rats was approximately 2400 mg/kg bw. At still higher ethanol doses, male rats showed minor changes to organ weights and hematology/biochemistry; female rats showed minor biochemistry changes and increased length of estrus cycle along with liver nodules; adverse liver effects were observed at concentrations of 3600mg ethanol/kg.bw/day and above (OECD, 2004b). As DEM would therefore not be expected to be more toxic than DMM (because ethanol is less toxic than methanol), the DMM study could be regarded as a “worst-case”. The available data are therefore considered to be consistent, with no indication for a relevant toxicity of DEM and DMM after repeated administration.

Conclusion

One repeated dose study in rats by the oral route (gavage) according to OECD TG 422 and GLP is available for DMM. The only effect observed was a reversible hepatocellular hypertrophy in animals of the high dose group (1000 mg/kg bw/day). The NOAEL was 300 mg/kg bw per day. Only a limited dietary 90 day study in rats is available with DEM, which indicated no treatment related effect at dose levels of 36 and 41 mg/kg bw per day for male and female animals, respectively (only one dose level was tested). Although the information available for DEM is limited, it is considered sufficient because DEM is not likely to be more toxic than DMM (as ethanol is less toxic than methanol). Overall, the toxicity of DMM and DEM after repeated dosing is considered to be low.

3.1.6 Mutagenicity

In vitro Studies

Both materials, DMM and DEM were not mutagenic in standard Ames assays with and without metabolic activation according to Dir. 84/449/ECC B 14 and GLP in *S. typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 up to concentrations of 5000 µg/plate. Cytotoxicity was observed for DMM at concentrations at and above 1000 µg/plate while for DEM no cytotoxicity was observed at the highest concentration of 5000 µg/plate (Hüls, 1992g; Hüls, 1993b).

DMM was not mutagenic in an *in vitro* cytogenetic assay in human peripheral lymphocytes with and without metabolic activation according to OECD TG 473 and GLP at concentrations of up to 5000 µg/ml. A reduction in mitotic index indicating cytotoxicity was observed at a concentration of 5000 µg/ml with and without metabolic activation (Degussa, 2003f). No cytogenetic assay was available for DEM. Based on the overall balance of evidence that ethanol is not genotoxic (see below), the data for chromosomal aberrations for DEM has been read-across from DMM.

With methanol, numerous *in vitro* assays (including seven Ames assays, four micronucleus/cytogenetic assays, a mammalian gene mutation assay, a yeast gene mutation assay, a mouse lymphoma test, three cell transformation assays, and a DNA damage and repair assay) were conducted. The majority of these assays are negative, with the exception of a positive result in the mouse lymphoma test, an ambiguous result in an Ames assay for strain TA102, and an ambiguous result in the DNA damage and repair assay. Only limited details were available for the mouse lymphoma test and for the DNA damage assay (OECD, 2004a). The balance of evidence is that ethanol is not genotoxic. Negative results from a number of bacterial mutation assays appear to be reliable. Of the mammalian cell mutation assays a weak mutagenic effect in mouse lymphoma cells occurred only at very high ethanol concentrations (OECD, 2004b).

In vivo Studies

No *in vivo* studies are available for DMM and DEM. Of the eleven *in vivo* assays performed with methanol (all micronucleus and cytogenicity assays plus a *Drosophila* SLRL assay), all are negative except one cytogenetic assay, which was positive for aneuploidy, sister chromatid exchange, and micronuclei. Limited information was available regarding this positive result (OECD 2004a).

In vivo tests with ethanol for chromosome aberrations in both rats and Chinese hamsters have given negative results. There is very little evidence to suggest that ethanol is genotoxic in somatic cells and it may have a very limited capacity to induce genetic changes *in vivo* but under very specific circumstances and at very high doses achievable in humans only by deliberate oral ingestion (OECD, 2004b).

Conclusion

Both DMM and DEM were not mutagenic in the standard Ames assay in bacteria with and without metabolic activation. DMM did not show any clastogenic activity in the *in vitro* cytogenetic assay with peripheral human lymphocytes in the presence and absence of a metabolic activation system. All tests were conducted according to OECD or EC guidelines and GLP. For both substances, there is no structural alert for genotoxicity. In conclusion, from the available information, there is no indication of a mutagenic potential of the substances, both for gene mutations and chromosomal aberrations.

3.1.7 Carcinogenicity

No data are available.

3.1.8 Toxicity for Reproduction

One study according to OECD TG 422 and GLP is available for DMM.

Studies in Animals

Effects on Fertility

In the combined repeated dose reproduction/developmental screening test with DMM in Wistar rats no treatment related changes in fertility index for males and females, gestation index, testes and epididymis weights were observed compared to the control. No treatment related histopathological changes of the sex organs or stages of spermatogenesis were reported (Degussa, 2003e). Details of the study methodology are described in chapter 3.1.5. The NOAEL for fertility corresponds therefore to the highest dose tested: 1000 mg/kg bw per day.

No reproductive toxicity study was available for DEM. For ethanol, the lowest reported NOAEL for fertility by the oral route was 2000 mg/kg bw in rats, equivalent to a blood alcohol concentration of 1320 mg/l, although this was based on a significant increase in the number of small pups rather than a direct effect on fertility; such direct effects are not seen until much higher doses (OECD, 2004b). Because it is impossible to reach blood levels of ethanol which are associated with reproductive toxicity as a consequence of the manufacture and normal use of DEM, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. Taking also into account that DEM is not likely to be more toxic than DMM, which has shown no potential for reproductive toxicity, it is overall concluded that there is no indication for a relevant reproductive toxicity of DMM and DEM.

Developmental Toxicity

The combined repeated dose reproduction/developmental screening test with DMM in Wistar rats revealed no treatment related changes in the duration of gestation, the gestation index, parturition and pre-implantation loss compared to controls. (For details of the study design see chapter 3.1.3). In the low dose group post-implantation loss was increased and consequently the percentage of live pups born was statistically significantly reduced compared to controls. These changes were considered incidental and not treatment related as the effects were not observed in the mid and high dose groups. No statistically significant differences between treated and control groups were observed for the number of pregnancies, number of dams that littered, number of live litters, mean litter size, mean viable litter size, sex ratio at birth, number of pups dead at first observation or day 2 to 4 post partum, number of live pups at day 0, 3, and 4 post partum and the associated survival indices. A significantly higher percentage of male pups and lower number of females on day 4 post partum in the low dose group was considered incidental as no comparable change was observed in the mid and high dose group or at other time intervals. The mean number and mean weights of male and female pups as well as both sexes combined were otherwise not statistically significantly different from controls. No statistically significant difference in the external abnormalities of live and dead pups compared to controls was observed at all dose levels. Maternal toxicity was restricted to a reversible hepatocellular hypertrophy (see chapter 3.1.5) at 1000 mg/kg bw, with a maternal NOAEL of 300 mg/kg bw. The NOAEL for developmental toxicity was 1000 mg/kg bw per day (Degussa, 2003e).

It is noted, that despite the known differences in methanol metabolism between rodents and humans, rodents are adequate models for human exposure to methanol at levels where formate does not accumulate, i.e. at methanol levels below 500 mg/kg bw (i.e. levels which would require DMM doses of more than 1000 mg/kg bw). Blood methanol concentrations associated with serious teratogenic effects and reproductive toxicity observed in rodent studies are in the range associated with formate accumulation, which is likely to result in metabolic acidosis and visual and clinical effects in humans. (OECD, 2004a)

No developmental toxicity study was available for DEM. Many studies exist examining the developmental end point for ethanol. However, most use very high doses and few are individually robust enough to allow a NOAEL to be established. The collective weight of evidence is that the NOAEL for developmental effects in animals is high, typically ≥ 6400 mg/kg bw, compared to maternally toxic effects at 3600 mg/kg bw (OECD, 2004b). The potential for reproductive and developmental toxicity exists in humans only from deliberate over-consumption of ethanol. Blood ethanol concentrations resulting from ethanol exposure by any other route are unlikely to produce reproductive or developmental effects. Because it is impossible to reach blood levels of ethanol which are associated with developmental toxicity as a consequence of the manufacture and normal use of DEM (cf. OECD, 2004b), it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. Taking also into account that DEM is not likely to be more toxic

than DMM, which has shown no potential for developmental toxicity, it is overall concluded that there is no indication for a relevant developmental toxicity of DMM and DEM.

Conclusion

Based on the findings in a combined oral (gavage) repeated dose reproduction/developmental toxicity study in rats according to OECD TG 422 and GLP with DMM a NOAEL for parental toxicity of 300 mg/kg bw/day for males and females and a NOAEL for reproductive and developmental toxicity of 1000 mg/kg bw/day, the highest dose tested, can be derived. No reproductive/developmental toxicity study was available for DEM. Because it is impossible to reach blood levels of ethanol which are associated with reproductive/developmental toxicity as a consequence of the manufacture and normal use of DEM, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. Taking also into account that DEM is not likely to be more toxic than DMM, which has shown no potential for reproductive and developmental toxicity, it is overall concluded that there is no indication for a relevant reproductive and/or developmental toxicity of DMM and DEM.

3.2 Initial Assessment for Human Health

From the physical chemical properties of both substances it can be assumed that they are readily absorbed through mucous membranes and distributed into the water compartments. Absorption through skin in *in vitro* experiments in different species varied widely depending on the experimental conditions. *In vivo* skin absorption of undiluted [2-¹⁴C]-DEM was highest in nude mice with 15 % absorption and lowest in pigs (2.5 %). In human skin grafted on nude mice and in hairless dogs absorption was 4 %. These experiments indicate relatively low skin absorption under non-occluded conditions. Both DMM and DEM are likely to be metabolized by esterases under cleavage of one or two ester bonds yielding the corresponding alcohols and malonic acid monoesters or malonic acid.

No acute inhalation study is available for DMM. In the dermal toxicity study in rats following OECD guideline TG 402 and GLP (limit test) the LD₅₀ was > 2000 mg/kg bw. An acute oral toxicity study in rats revealed an LD₅₀ > 2000 mg/kg bw. In both studies no test substance related effects were observed.

For DEM only limited literature data are available. No toxicity was observed after 8 h inhalation of concentrated vapors in rats. The dermal LD₅₀ in rabbits was reported to be > 16 960 mg/kg bw, the oral LD₅₀ in rats 15 794 mg/kg bw.

Taken together the studies for both substances suggest that they are of low acute toxicity via the oral and dermal route and likely to be also of low toxicity after inhalation exposure.

DMM was not irritating to rabbit skin in a guideline study according to OECD TG 404 and GLP. For DEM no guideline study is available on skin irritation, but a slightly irritating effect was reported in the literature after 24 hours of occlusive exposure. Both substances showed slight to moderate eye irritating effects in rabbits that were completely reversible within the observation period. The studies were conducted according or similar to OECD TG 405 and under GLP.

DMM did not reveal any skin sensitizing effect in a Bühler test according to OECD TG 406 and GLP. Reports of maximization tests in human volunteers with both, DMM and DEM did not indicate any skin sensitizing properties.

One repeated dose study in rats by the oral route (gavage) according to OECD TG 422 and GLP is available for DMM. The only effect observed was a reversible hepatocellular hypertrophy in animals of the high dose group (1000 mg/kg). The NOAEL was 300 mg/kg bw per day. Only a

limited dietary 90 day study in rats is available with DEM, which indicated no treatment related effect at dose levels of 36 and 41 mg/kg bw per day for male and female animals respectively (only one dose level was tested). Although the information available for DEM is limited, it is considered sufficient because DEM is not likely to be more toxic than DMM. Overall, the toxicity of DMM and DEM after repeated dosing is considered to be low.

Both DMM and DEM were not mutagenic in the Standard Ames assay in bacteria with and without metabolic activation. DMM did not show any clastogenic activity in the *in vitro* cytogenetic assay with peripheral human lymphocytes in the presence and absence of a metabolic activation system. All tests were conducted according to OECD or EC guidelines and GLP. For both substances, there is no structural alert for genotoxicity. In conclusion, from the available information, there is no indication of a mutagenic potential of the substances, both for gene mutations and chromosomal aberrations.

Based on the findings in a combined oral (gavage) repeated dose reproduction/developmental toxicity study in rats according to OECD TG 422 and GLP with DMM a NOAEL for parental toxicity of 300 mg/kg bw/day for males and females and a NOAEL for reproductive and developmental toxicity of 1000 mg/kg bw/day, the highest dose tested, can be derived. No reproductive/developmental toxicity study was available for DEM. Because it is impossible to reach blood levels of ethanol which are associated with reproductive/developmental toxicity as a consequence of the manufacture and normal use of DEM, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. Taking also into account that DEM is not likely to be more toxic than DMM, which has shown no potential for reproductive and developmental toxicity, it is overall concluded that there is no indication for a relevant reproductive and/or developmental toxicity of DMM and DEM.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

The acute toxicity test results are summarized in tables 7 to 9.

Effects on fish

Studies according to standard EC or OECD TG or comparable to those are available for both substances. Most studies were performed under flow through conditions and analytical monitoring revealed the stability of the test substance concentration for the study duration. The 96 h LC₅₀ was 21 mg/l (0.16 mmol/l) for DMM and 12 to 17 mg/l (0.07 to 0.1 mmol/l) for DEM. Both substances have thus comparable LC₅₀ values.

Table 7 Toxicity to fish

Substance	Study Type	Exposure time	Species	Endpoint	Value [mg/l]	Reference
DMM	84/449/EC C.1; GLP, flow through, analytical monitoring	96 h	<i>Danio rerio</i>	Mortality LC ₅₀	21	Hüls, 1993c
DEM	Comparable to OECD 203, flow through, no data on GLP, analytical monitoring	96 h	<i>Pimephales promelas</i>	Mortality LC ₅₀	12	Geiger et al., 1984a
DEM	Comparable to OECD 203, flow through, no data on GLP, analytical monitoring	96 h	<i>Pimephales promelas</i>	Mortality LC ₅₀	15	Brooke et al., 1984; Call et al., 1981
DEM	Comparable to OECD 203, flow through, no data on GLP, analytical monitoring	96 h	<i>Pimephales promelas</i>	Mortality LC ₅₀	17	Geiger et al., 1984b
DEM	DIN 38412 part 15, no GLP, no analytical monitoring, static	48 h	<i>Leuciscus idus</i>	Mortality LC ₅₀	73	Hüls, 1988

Effects on invertebrates

For both substances, DMM and DEM, studies according to EU guidelines and GLP without analytical monitoring are available. From the physical chemical data and the fish toxicity data it can be assumed that the test substances did not volatilize during the test period of 48 h. However it seems possible that hydrolysis may have occurred.

The 48 h EC₅₀ value for *Daphnia magna* was > 1000 mg/l for DMM and 200 mg/l for DEM based on nominal concentrations. When a correction for hydrolysis at pH 7 is introduced the EC₅₀ values would be 179 mg/l for DEM and > 728 mg/l for DMM. From these data it can be concluded that both substances are of relatively low toxicity to *Daphnia magna*, but DEM seems to be slightly more toxic.

Table 8 Acute toxicity to invertebrates

Substance	Study Type	Exposure time	Species	Endpoint	Value [mg/l]	Reference
DMM	84/499/EEC C.2, GLP, static, no analytical monitoring	48 h	<i>Daphnia magna</i>	Immobilization EC ₅₀	> 1000 ^a (> 728) ^b	Hüls, 1992h
DEM	84/499/EEC C.2, GLP, static, no analytical monitoring	48 h	<i>Daphnia magna</i>	Immobilization EC ₅₀	200 (179) ^b	Hüls, 1993d

^a highest concentration tested, ^b corrected for hydrolysis at pH 7.

Effects on aquatic plants / algae

For both substances, DMM and DEM, studies according to EU guidelines and GLP without analytical monitoring are available. From the physical chemical data and the fish toxicity data it can be assumed that the test substances did not volatilize during the test period of 96 h. However, regarding the test substance vials in the DMM study the pH was lowered during the study and compared to controls at concentrations between 40 and 320 mg/l which could be indicative of hydrolysis of the test substance. An additional effect of the lowered pH on algae cannot be excluded. This could also explain the lower EC₅₀ values of DMM (for growth rate: 386 mg/l) compared to DEM (> 800 mg/l). If the values are corrected for hydrolysis at pH 7 the EC₅₀ values for growth rate would correspond to 240 mg/l for DMM and > 667 mg/l for DEM. An increase in pH in the control and lowest test concentration (10 mg/l) during the duration of the test is a common phenomenon that can be explained by the CO₂ depletion caused by the algae.

Table 9 Toxicity to algae

Substance	Study Type	Exposure time	Species	Endpoint	Value [mg/l]	Reference
DMM	OECD 201, GLP, no analytical monitoring	72 h	<i>Desmodesmus subspicatus</i>	Cell growth (biomass) EC ₅₀	148 (92) ^b	Hüls, 1993e
				Growth rate EC ₅₀	386 (240) ^b	
DEM	88/302/EEC, GLP, no analytical monitoring	72 h	<i>Desmodesmus subspicatus</i>	Cell growth (biomass) EC ₅₀	508 (424) ^b	Hüls, 1993f
				Growth rate EC ₅₀	> 800 ^a (> 667) ^b	

^a highest concentration tested, ^b corrected for hydrolysis at pH 7.

Toxicity to Microorganisms

For DMM toxicity towards *Pseudomonas putida* was determined in an assay according to DIN 38412 part 8. The 18 hour EC₅₀ was not reached at the maximum tested concentration of 12,500 mg/l, the EC₁₀ was 6154 mg/l (Hüls, 1993g)

For DEM a 16 hour EC₅₀ of 3097 mg/l and an EC₁₀ of 1092 mg/l in a similar study with *Pseudomonas putida* according to DIN 38412 part 8 was reported (Hüls, 1993h).

For the aquatic protozoa *Tetrahymena pyriformis* IC₅₀-values for growth inhibition of 20 mmol/l for DMM (2640 mg/l) and 10 mmol/l (1600 mg/l) for DEM were reported in a 2-dimensional static assay (Jaworska et al., 1997).

PNEC derivation

Based on the lowest LC₅₀ for fish of 21 mg/l for DMM and 12 mg/l for DEM a PNEC of 21 µg/l for DMM and 12 µg/l for DEM can be derived using an assessment factor of 1000 according to the EU technical guidance document.

4.2 Terrestrial Effects

Acute Toxicity Test Results

Terrestrial Plants

For DEM a study on growth inhibition for terrestrial plants according to OECD TG 208 and GLP is available. No effect was observed on seed development and growth of *Triticum aestivum*, *Lepidum sativum* and *Brassica alba* up to a concentration of 100 mg DEM/kg soil (Hüls, 1995). In an aerosol exposure study *Pinus echinata*, *Artemisia tridentata* or *Festuca arundinacea* plants were exposed to aerosols containing 1.14 or 0.32 mg DEM/l air for 60 minutes in a sealed air exposure chamber. Visual toxicity symptoms were assessed 0, 2, and 21 days post exposure. At a concentration of 1.14 mg/l chlorosis and burns on the tips of the leaves were observed in *Pinus echinata*, leaf curl and wilting followed by chlorosis in *Artemisia tridentata* and wilting and burn of the leaf tips followed by chlorosis and leaf curl in *Festuca arundinacea* were observed. The effects increased in severity and incidence with time. The NOEC was 0.32 mg/l in all plants (Cataldo et al., 1990).

Soil dwelling organisms

A toxicity test with DEM in artificial soil containing 1000 mg DEM/kg soil according to Dir. 88/302/EEC and GLP using *Eisenia fetida* revealed no mortality after 14 days of exposure (Hüls, 1994).

4.3 Other Environmental Effects

No other relevant data are available.

4.4 Initial Assessment for the Environment

Both, dimethyl- and diethylmalonate are colorless organic liquids with an ester like odor. DMM has a water solubility of about 142 g/l at 20 °C, a vapor pressure of 0.48 - 0.5 hPa at 20 °C and a measured log K_{ow} of -0.05. DEM has a water solubility of 20 g/l at 20 °C, a vapor pressure of 0.36 hPa at 25 °C and a measured log K_{ow} of 0.96. Both substances are readily biodegradable (100 % (DMM) and 98 % (DEM) in a DOC-die away test) and undergo a two-step hydrolytic degradation in a first step to the monoester and in a second step to malonic acid and the corresponding alcohol, methanol or ethanol respectively. The half lives were shortest at pH 9, < 2.4 h (50 °C) for both substances, and increased to 5.7 h (50 °C) and 15.9 h (50 °C) for DMM and DEM respectively at pH 7 and 859 h (50 °C) for DMM at pH 4. At pH 4 and 50 °C DEM showed less than 10 % degradation within 5 days. For photodegradation via oxidation by OH-radicals half-lives of about 31 days for DMM and 4.7 days for DEM in air were estimated. For DEM a 100 % photolytic ozonisation after 40 min under UV-irradiation in water was reported. The generic fugacity model I indicates that both substances are preferably distributed in the water phase (98 % for DMM and 90 % for DEM) with a low amount distributing potentially into air (1.5 and 9.9 % respectively). The fugacity model III however, indicates that a considerable amount may be distributed to the soil if the substances are primarily released into air (36 % for DMM and DEM) or soil (38 % for DMM and 44.5 % for DEM). The measured octanol-water partition coefficients (log K_{ow} -0.05 for DMM and 0.96 for DEM) and soil sorption coefficients (K_{oc} 1,74 for DMM and 10 for DEM) indicate a low potential for bio- or geo-accumulation.

Acute toxicity data for 3 trophic levels of the aquatic environment are available for both substances. The most sensitive species was fish. The 96 h LC₅₀ for fish (*Danio rerio*) was 21 mg/l for DMM and 12 - 17 mg/l for DEM (*Pimephales promelas*). The 48 h EC₅₀ for *Daphnia magna* was

> 728 mg/l for DMM and 179 mg/l for DEM and the 72 h ErC₅₀ for algae (*Desmodesmus subspicatus*) was 240 mg/l for DMM and > 667 mg/l for DEM with nominal NOEC-values of 20 mg/l and 25 mg/l, respectively. Based on the lowest LC₅₀-value for fish of 21 mg/l for DMM and 12 mg/l for DEM, PNEC-values of 21 and 12 µg/l can be derived for DMM and DEM, respectively.

No growth inhibition of DEM to terrestrial plants in soil was observed up to concentrations of > 100 mg/kg soil and no toxicity to *Eisenia fetida* was observed at concentrations of DEM of 1000 mg/kg bw after 14 days of exposure.

5 RECOMMENDATIONS

Human Health:

The chemicals of this category are currently of low priority for further work due to their low hazard profile.

Environment

The chemicals of this category possess properties indicating a hazard for the environment. Although these hazards do not warrant further work (as they are related to acute toxicity which may become evident only at high exposure levels) they should nevertheless be noted by chemical safety professionals and users. The chemicals are currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the sponsor country.

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S I D S

Dossier

Existing Chemical ID: 105-53-3
CAS No. 105-53-3
EINECS Name diethyl malonate
EC No. 203-305-9
TSCA Name Propanedioic acid, diethyl ester
Molecular Formula C7H12O4

Producer Related Part

Company: Degussa AG
Creation date: 04-JUN-2000

Substance Related Part

Company: Degussa AG
Creation date: 04-JUN-2000

Memo: Überarbeitungsversion

Printing date: 26-AUG-2005
Revision date: 19-NOV-2003
Date of last Update: 26-AUG-2005

Number of Pages: 98

Chapter (profile): Chapter: 1.0.1, 1.0.2, 1.0.4, 1.1.0, 1.1.1, 1.2, 1.3, 1.4, 1.5, 1.6.1, 1.6.2, 1.7, 1.7.1, 1.7.2, 1.8, 1.8.1, 1.8.2, 1.8.3, 1.8.4, 1.8.5, 1.8.6, 1.9.1, 1.9.2, 1.10, 1.11, 1.12, 1.13, 2, 3, 4, 5, 6, 10

Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, non confidential, SIDS

1. GENERAL INFORMATION

ID: 105-53-3

DATE: 21.01.2005

1.0.1 Applicant and Company Information

Type: lead organisation
Name: Degussa AG - ZN Wolfgang
Contact Person: Dr. W. Mayr, Dr. S. Jacobi **Date:**
Street: Rodenbacher Chaussee 4
Town: 63457 Hanau
Country: Germany
Phone: +49 6181 59 4139
Telefax: +49 6181 59 2083
Email: wilfried.mayr@degussa.com

29-MAR-2004

Type: other: contact point
Name: Degussa AG, ZN Wolfgang
Contact Person: Dr. Wilfried Mayr **Date:**
Street: Rodenbacher Chaussee 4
Town: 63457 Hanau-Wolfgang
Country: Germany
Phone: +49 6181 59 4139
Telefax: +49 6181 59 2083
Email: wilfried.mayr@degussa.com

1.0.2 Location of Production Site, Importer or Formulator1.0.4 Details on Category/Template

Comment: Dimethylmalonate, CAS No.: 108-59-8, Diethylmalonate, CAS No: 105-53-3

Remark: The category of simple diesters of malonic acid, dimethylmalonate and diethylmalonate has been defined because of the similar properties of the simple esters and their likelihood to be cleaved under physiological conditions yielding malonic acid and the corresponding alcohols. Where data are lacking for one of the members of the category they can reasonably be substituted by data of the other member of the category due to the structural similarity.

The production and use pattern of Diethylmalonate (DEM) and Dimethylmalonate (DMM) are comparable. The two chemicals Diethylmalonate and Dimethylmalonate have very similar physico-chemical properties and both esters are hydrolyzed via a two step reaction to malonic acid and the corresponding alcohol, methanol or ethanol. It is likely that unspecific esterases in the body catalyze the hydrolysis. The alcohols and malonic acid are physiological substances that are metabolized via physiological pathways. Ethanol and Methanol were assessed evaluated in SIAM 19 (OECD, 2004a,b). For ethanol it was concluded that the chemical is currently of low priority for further work, because the hazardous properties of ethanol are manifest only at doses associated with consumption of alcoholic beverages. As it is impossible to reach these exposure levels as a consequence of the manufacture and use of malonates, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. For methanol, SIAM 19 decided that this chemical is a candidate for further

work. Methanol exhibits potential hazardous properties for human health (neurological effects, CNS depression, ocular effects, reproductive and developmental effects, and other organ toxicity). The effects of methanol on the CNS and retina in humans only occur at doses at which formate accumulates due to a rate-limiting conversion to carbon dioxide. In primates, formate accumulation was observed at methanol doses greater than 500 mg/kg bw (which would require a DMM dose of more than 1,000 mg/kg bw). As there were no indications of a methanol associated toxicity from a well performed repeated dose toxicity study with DMM in rodents (which are, however, known to be less sensitive to methanol toxicity than humans), and because methanol toxicity would not be expected up to doses as high as 1,000 mg DMM/kg bw/day, it was concluded that methanol does not make a relevant contribution to the toxicity profile of DMM. A possible mode of action for systemic toxicity of DMM and DEM can only be deduced from the repeated dose study with DMM, indicating a reversible liver hypertrophy at the cellular level at high doses of 1000 mg/kg bw/day. This effect can be an indication of an induction of metabolism in the liver rather than a clear systemic toxicity.

18-AUG-2005

(73) (74)

1.1.0 Substance Identification

IUPAC Name: diethyl malonate
Smiles Code: O=C(OCC)CC(=O)OCC
Mol. Formula: C7H12O4
Mol. Weight: 160.17

21-OCT-2004

1.1.1 General Substance Information

Purity type: typical for marketed substance
Substance type: organic
Physical status: liquid
Purity: ca. 99.8 - % w/w
Colour: ester-like

28-JUL-2005

(29)

1.2 Synonyms and Tradenames

DEM

20-JUL-2005

Dicarbethoxymethane

Diethyl malonate

Diethyl propanedioate

1. GENERAL INFORMATION

ID: 105-53-3

DATE: 21.01.2005

Diethylmalonate

30-NOV-2004

Ethyl malonate

Malonic acid, diethyl ester

Malonsaeurediethylester

Propanedioic acid, diethyl ester

1.3 Impurities

Purity type: typical for marketed substance
CAS-No: 64-17-5
EC-No: 200-578-6
EINECS-Name: ethanol
Contents: ca. .1 - % w/w

28-JUL-2005

(29)

Purity type: typical for marketed substance
CAS-No: 141-78-6
EC-No: 205-500-4
EINECS-Name: ethyl acetate
Contents: ca. .05 - % w/w

28-JUL-2005

(29)

Purity type: typical for marketed substance
EINECS-Name: ethyl methyl malonate
Contents: ca. .05 - % w/w

28-JUL-2005

(29)

1.4 Additives**1.5 Total Quantity****1.6.1 Labelling**

Labelling: no labelling required (no dangerous properties)

Remark: Last update MSDS Chapter 15 "Labelling and Classification" on
2003-07-23.

30-NOV-2004

(5) (28)

1.6.2 Classification

Classified: no classification required (no dangerous properties)

1. GENERAL INFORMATION

ID: 105-53-3

DATE: 21.01.2005

30-NOV-2004

(28)

1.7 Use Pattern

Type: type
Category: Non dispersive use

20-JUL-2005

Type: type
Category: Wide dispersive use

30-NOV-2004

Type: industrial
Category: Chemical industry: used in synthesis

30-NOV-2004

Type: use
Category: Intermediates

30-NOV-2004

Type: use
Category: Odour agents

30-NOV-2004

1.7.1 Detailed Use Pattern**1.7.2 Methods of Manufacture****1.8 Regulatory Measures****1.8.1 Occupational Exposure Limit Values****1.8.2 Acceptable Residues Levels****1.8.3 Water Pollution**

Classified by: other: Huels AG
Labelled by: other: Huels AG
Class of danger: 1 (weakly water polluting)

Country: Germany

30-NOV-2004

(28)

1.8.4 Major Accident Hazards

Legislation: Stoerfallverordnung (DE)
Substance listed: no

1. GENERAL INFORMATION

ID: 105-53-3

DATE: 21.01.2005

Country: Germany
Remark: Stoerfallverordnung 2000, 12. BimSchV, BGBI. I 2000, 603
 30-NOV-2004 (28)

1.8.5 Air Pollution1.8.6 Listings e.g. Chemical Inventories1.9.1 Degradation/Transformation Products1.9.2 Components1.10 Source of Exposure

Source of exposure: Environment: exposure from production
Exposure to the: Substance

Result: Exposure
 From production there are no emissions into water or air. Exhausts are incinerated and waste water is treated in a biological sewage treatment plant. Product containing waste waters (e.g. from maintenance operations) are incinerated. No waste containing DEM is produced.
 From use as flavoring agent no emission data are known.

Flag: Critical study for SIDS endpoint
 20-JUL-2005 (17)

Source of exposure: other: human exposure, product register information

Result: DEM is contained in the SPIN database (2004): For 2002, 79 preparations with an overall amount of 0.3 tonnes are noted for Denmark, 7 preparations with an overall amount of 0.1 tonnes are noted for Norway, and 12 preparations including consumer products with an overall amount of 0.0 tonnes are noted for Sweden. For Finland, confidential data are contained for 2001.

The Swedish product Register (2005) contains data on DEM: 13 products containing 0-2% DEM, 2 of which are consumer products, with a tonnage of 0.0 t/a, and 4 products containing 2-20% DEM, 2 of which are consumer products, with a tonnage of 2.0 t/a. Information on uses of consumer

products

is confidential. Most frequent industrial uses are adhesives, hardeners for adhesive and industrial use, the most common industry category is sales and repair establishments for motor vehicles and motorcycles.

Flag: Critical study for SIDS endpoint
 24-AUG-2005 (88) (91)

Source of exposure: other: human occupational exposure
Exposure to the: Substance

Result: The German producer uses closed systems including gas tight

flunshes for loading and de-loading operations and closed valve-syringe systems for sampling. From the process description very low occupational exposure is anticipated. No data are available for the uses. As the majority of the products are used as intermediates in the chemical industry a controlled exposure situation is anticipated.

Flag: Critical study for SIDS endpoint
20-JUL-2005 (17)

Source of exposure: Human: exposure of the consumer/bystander
Exposure to the: Substance

Result: WHO (2000) evaluated the combined daily intake of 47 flavoring substances including DEM in Europe and the US. The annual production volume of these 47 substances was 200 metric tons in Europe and 1700 metric tons in the US. From this an estimated per capita daily intake of 28 mg in Europe and 300 mg in the US was derived (based on a body weight of 60 kg these intakes would correspond to 0.47 and 5 mg/kg bw/day in Europe and the US, respectively). This intake was considered of no concern.

Flag: Critical study for SIDS endpoint
20-JUL-2005 (97)

1.11 Additional Remarks

1.12 Last Literature Search

Type of Search: Internal and External
Chapters covered: 3, 4, 5
Date of Search: 01-MAY-2000

Remark: DIMDI, CIS
16-AUG-2004

Type of Search: Internal and External
Chapters covered: 3, 4, 5
Date of Search: 24-APR-2003

Remark: DIMDI, CIS, Datastar, Dialog, STN, Beilstein, Update
16-AUG-2004

Type of Search: Internal and External
Chapters covered: 3, 4, 5
Date of Search: 14-MAY-2003

Remark: CIS, STN, DIMDI, Beilstein

1.13 Reviews

2.1 Melting Point

Value: -51.5 degree C

Year: 1934
GLP: no

Test substance: other TS: purified by fractioned distillation until density was constant in two successive fractions

Reliability: (2) valid with restrictions
well documented scientific literature

Flag: Critical study for SIDS endpoint
16-AUG-2004 (94)

Value: = -50 degree C

GLP: no data
Test substance: no data

Reliability: (2) valid with restrictions
Database and handbook data (5) (30) (56) (62) (75) (81) (89)

Value: = -49.2 degree C

Method: other: no data
GLP: no data

Reliability: (2) valid with restrictions
Handbook data
30-NOV-2004 (54)

Value: -48.7 - -49.1 degree C

Year: 1942
GLP: no

Test substance: other TS: purified by fractioned distillation

Reliability: (2) valid with restrictions
well documented scientific literature

Flag: Critical study for SIDS endpoint
16-AUG-2004 (76)

2.2 Boiling Point

Value: 190 degree C

GLP: no data
Test substance: no data

Reliability: (4) not assignable
Database data
20-JUL-2005 (5) (67)

Value: 197 degree C at 1008 hPa

Year: 1894
GLP: no

Test substance: other TS: purified by fractioned distillation

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

Result: Pressure reported as 756.6 mmHg. Boilingn point 97-97.5 °C at 13 mm Hg (17 hPa).
Reliability: (2) valid with restrictions
 Peer reviewed data source.
 16-AUG-2004 (9)

Value: 197 degree C at 1012 hPa
Year: 1934
GLP: no
Test substance: other TS:Synthesized in testing laboratory freshly distilled

Result: Pressure reported as 759 mmHg
Reliability: (2) valid with restrictions
 Peer reviewed data source.
 16-AUG-2004 (95)

Value: 197.8 degree C
Year: 1917
GLP: no
Test substance: no data
Reliability: (2) valid with restrictions
 Peer reviewed data source.
 (56)

Value: 198 - 199 degree C
GLP: no data
Test substance: no data
Reliability: (2) valid with restrictions
 Handbook data
 (75)

Value: 198.5 degree C
Year: 1966
GLP: no
Test substance: no data
Reliability: (2) valid with restrictions
 Scientific literature, no details available
 (35)

Value: = 198.6 degree C at 1013 hPa
Method: other: no data
Year: 1928
GLP: no
Reliability: (2) valid with restrictions
 Scientific literature, no details available
 30-NOV-2004 (60)

Value: = 198.9 degree C
Method: other: no data

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

GLP: no data

Reliability: (2) valid with restrictions
Handbook data

30-NOV-2004 (54)

Value: = 199 degree C at 1013 hPa

Method: other: DIN 51751

GLP: no data

Reliability: (2) valid with restrictions (28)

Value: 199 degree C

GLP: no data

Test substance: no data

Reliability: (2) valid with restrictions
Scientific literature, no details available

Value: 199.3 degree C at 1013 hPa

Year: 1934

GLP: no

Test substance: other TS: purified by fractioned distillation until density was constant in two successive fractions

Reliability: (2) valid with restrictions
well documented scientific literature

Flag: Critical study for SIDS endpoint

16-AUG-2004 (72) (94)

Value: = 199.3 degree C at 1013 hPa

GLP: no

Test substance: other TS: purified, no further data

Reliability: (2) valid with restrictions
well documented scientific literature

30-NOV-2004 (72)

Value: 200 degree C at 1013 hPa

GLP: no data

Test substance: no data

Reliability: (2) valid with restrictions
Database and handbook data (62) (89)

2.3 Density

Type: density

Value: 1.05 g/cm³ at 20 degree C

GLP: no data

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

Test substance: no data

Reliability: (2) valid with restrictions (67)

Type: relative density
Value: 1.0547 at 20 degree C

Year: 1950
GLP: no

Test substance: other TS: purified, no further data

Result: relative density (25 °C) = 1.0494
Reliability: (2) valid with restrictions
 well documented scientific literature

Flag: Critical study for SIDS endpoint
 16-AUG-2004 (72)

Type: density
Value: 1.055 g/cm³ at 20 degree C

GLP: no data
Test substance: no data

Reliability: (2) valid with restrictions
 Database data (5)

Type: relative density
Value: 1.055 at 20 degree C

Method: other: pyrex pycnometer
Year: 1934
GLP: no

Test substance: no data

Result: Densities determined at other temperatures: 1.0104 at 62.2 °C,
 0.9878 at 85.3 °C.

Reliability: (2) valid with restrictions
 well documented scientific literature (95)

Type: relative density
Value: 1.0551 at 20 degree C

Year: 1942
GLP: no

Test substance: other TS: purified by fractioned distillation

Reliability: (2) valid with restrictions
 well documented scientific literature

Flag: Critical study for SIDS endpoint
 16-AUG-2004 (76)

Type: density
Value: 1.0551 g/cm³ at 20 degree C

GLP: no data
Test substance: no data

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

Reliability: (2) valid with restrictions
 Handbook data
 16-AUG-2004 (62) (81)

Type: relative density
Value: 1.0553 at 20 degree C

Year: 1894
GLP: no
Test substance: no data

Reliability: (2) valid with restrictions
 well documented scientific literature (9)

Type: density
Value: ca. 1.06 g/cm³ at 20 degree C

Method: other: DIN 51757
GLP: no data

Reliability: (2) valid with restrictions (28)

Type: relative density
Value: 1.0518 at 25 degree C

Year: 1917
GLP: no
Test substance: no data

Result: Relative density at 50°C: 1.0254.
Reliability: (2) valid with restrictions
 Well documented scientific literature, but details lacking.
 16-AUG-2004 (56)

Type: relative density
Value: 1.0441 at 30 degree C

Year: 1913
GLP: no
Test substance: no data

Result: Relative densities reported at other temperatures:
 1.0655 at 10 °C, 1.0228 at 50 °C.

Reliability: (2) valid with restrictions
 Well documented scientific literature, but details lacking.

Type: relative density
Value: 1.0445 at 30 degree C

Method: other: pycnometer method
Year: 1932
GLP: no
Test substance: no data

Reliability: (2) valid with restrictions (4)

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

Type: relative density
Value: 1.0446 at 30 degree C

Year: 1934
GLP: no
Test substance: other TS: purified by fractioned distillation until density was constant in two successive fractions

Result: At 0 °C: 1.07623
 15 °C: 1.06040
 variation per °C: 0.00105
 dilatation coefficient: 0.00101

Reliability: (2) valid with restrictions
 well documented scientific literature

16-AUG-2004 (94)

Type: relative density
Value: 1.055

GLP: no data
Test substance: no data

Reliability: (2) valid with restrictions
 Handbook data

(30) (54) (75)

2.3.1 Granulometry2.4 Vapour Pressure

Value: = .35 hPa at 20 degree C

GLP: no data
Test substance: no data

Reliability: (4) not assignable
 Database data

20-JUL-2005 (5) (28)

Value: .36 hPa at 25 degree C

Method: other (measured)
Year: 1989
GLP: no data
Test substance: no data

Result: quoted as 0.269 mmHg
Reliability: (2) valid with restrictions
 Peer reviewed data source.

Flag: Critical study for SIDS endpoint

21-OCT-2004 (16)

Value: 1 hPa at 36 degree C

GLP: no data
Test substance: no data

Result: Vapour pressure = 10 hPa [76 °C], 100 hPa [128.5 °C],

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

extrapolated vapour pressures: 0.01 hPa [-23 °C], 0.1 hPa [4 °C].
Reliability: (2) valid with restrictions
 Peer reviewed data source.
 16-AUG-2004 (62)

Value: 1.3 hPa at 40 degree C

GLP: no data

Test substance: no data

Reliability: (4) not assignable
 Database data

20-JUL-2005 (14) (67)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = .703

Method: other (calculated)
Year: 2004

Method: Calculated using Advanced Chemistry Development (ACD/Labs) Software

Result: 0.703 +/- 0.250

30-NOV-2004 (90)

Partition Coeff.: octanol-water
log Pow: .9

GLP: no data

Test substance: no data

Reliability: (4) not assignable
 Database data

(14)

Partition Coeff.: octanol-water
log Pow: .9

Method: other (calculated): KOWWIN (LOGKOW (c)) Program, Version 1.67, Syracuse Research Corporation, Merrill Lane, Syracuse, New York, 13210, U.S.A.

Year: 2004

GLP: no

Reliability: (2) valid with restrictions
 Calculated data, internationally accepted method.

17-AUG-2004 (27)

Partition Coeff.: octanol-water
log Pow: = .96

Method: other (measured)

Year: 1995

Test substance: other TS: Diethylmalonate, no data

Reliability: (2) valid with restrictions

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

Measured, no details, but standard as basis for QSAR calculations.

Flag: Critical study for SIDS endpoint
21-OCT-2004 (41)

Partition Coeff.: octanol-water
log Pow: = .96

Method: other (measured)
Year: 1995
GLP: no data
Test substance: other TS: Diethylmalonate, no data

Reliability: (2) valid with restrictions
Measured, no details
30-NOV-2004 (28) (86) (89)

Partition Coeff.: octanol-water
log Pow: 1.43

Method: other (measured)
GLP: no data
Test substance: no data

Reliability: (4) not assignable
Database data (61)

2.6.1 Solubility in different media

Solubility in: Water
Value: 33.1 g/l at 20 degree C

Reliability: (2) valid with restrictions (28)

Solubility in: Water
Value: 23.2 g/l at 37 degree C

Method: other: measurement
Year: 1992
GLP: no data
Test substance: no data

Reliability: (2) valid with restrictions
Peer reviewed data source.

Flag: Critical study for SIDS endpoint
20-JUL-2005 (98)

Solubility in: Water
Value: 20 g/l at 20 degree C

GLP: no data
Test substance: no data

Reliability: (2) valid with restrictions
Database and handbook data

Flag: Critical study for SIDS endpoint

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

16-AUG-2004

(14) (75)

Solubility in: Water
Value: 20.8 g/l at 20 degree C

Reliability: (2) valid with restrictions
 Database data

16-AUG-2004

(5)

Solubility in: Water
Value: ca. 28 g/l at 20 degree C

Remark: Very soluble in organic solvents.
Result: 2.8 g/100 g water.
Reliability: (4) not assignable
 Handbook data

20-JUL-2005

(54)

2.6.2 Surface Tension

Value: 30.56 mN/m at 30 degree C

Method: other: maximal bubble-pressure method of Sugden
Year: 1932
GLP: no
Test substance: no data

Reliability: (2) valid with restrictions

(4)

Value: 33.03 at 10 degree C

Year: 1913
GLP: no
Test substance: no data

Method: Weight of falling drop
Reliability: (2) valid with restrictions
 Well documented scientific literature, but details lacking.

(71)

Value: 31.9 mN/m at 20 degree C

Year: 1950
GLP: no
Test substance: other TS: purified TS

Result: Surface tension (25 °C) = 31.3 mN/m
Reliability: (2) valid with restrictions
 well documented scientific literature

16-AUG-2004

(72)

Value: 31.71 mN/m at 20 degree C

Year: 1934
GLP: no
Test substance: other TS: purified by fractioned distillation until density

was constant in two successive fractions

Reliability: (2) valid with restrictions
well documented scientific literature

16-AUG-2004 (94)

Value: 31.84 mN/m at 20 degree C

Method: other: method of Richards, Speyers and Carver

Year: 1934

GLP: no

Test substance: other TS: Synthesized in testing laboratory freshly distilled

Reliability: (2) valid with restrictions
well documented scientific literature

16-AUG-2004 (95)

Value: 31 mN/m at 25.2 degree C

Year: 1917

GLP: no

Test substance: no data

Reliability: (2) valid with restrictions

(56)

2.7 Flash Point

Value: 75 degree C
Type: other: no data

Year: 1981

GLP: no data

Reliability: (2) valid with restrictions

30-NOV-2004 (54)

Value: 80 degree C
Type: closed cup

GLP: no data

Test substance: no data

Reliability: (4) not assignable
Database data

(14)

Value: = 90 degree C
Type: closed cup

Method: other: DIN 51758

GLP: no data

Reliability: (2) valid with restrictions

(28) (81)

Value: 93 degree C

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

GLP: no data
Test substance: no data

Reliability: (2) valid with restrictions
 Database data (5) (67)

Value: 93 degree C

GLP: no data
Test substance: other TS: no data

Reliability: (2) valid with restrictions
 Handbook data
Flag: Critical study for SIDS endpoint
 30-NOV-2004 (62)

2.8 Auto Flammability

Value: 424 degree C

GLP: no data
Test substance: no data

Reliability: (4) not assignable
 Database data (14)

Value: = 435 degree C

Method: other: DIN 51794
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 20-JUL-2005 (28)

Value: = 435 degree C

Method: other: no data

Reliability: (2) valid with restrictions
 Database data (5)
 30-NOV-2004

2.9 Flammability2.10 Explosive Properties

Result: other: lower explosion limit = 0.8; upper explosion limit =
 12.8%

Reliability: (2) valid with restrictions (5) (28)

2.11 Oxidizing Properties2.12 Dissociation Constant

Acid-base Const.: pKa = 16.37 +/- 0.06

Test condition: Temperature: 25 °C in dimethylsulfoxide
Spectrophotometric method in dimethylsulfoxid + indicators
30-NOV-2004 (3)

2.13 Viscosity

Value: at 20 degree C

Year: 1950
GLP: no
Test substance: no data

Result: Viscosity (25 °C) = 1.94 mPas
Reliability: (2) valid with restrictions
well documented scientific literature (72)

Value: at 25 degree C

Year: 1934
GLP: no
Test substance: no data

Result: given as 1875 E-05 Poise
at 15 °C: 2.377 mPas (2377E-05 Poise)
at 30 °C: 1.753 mPas (1753E-05 Poise)
Reliability: (2) valid with restrictions
well documented scientific literature (94)

2.14 Additional Remarks

Memo: Dimyristoyl phosphatidylcholine/water partition coefficient

Result: log KDMPC = 0.50
09-AUG-2004 (86)

Memo: Refractive index: 1.4134 (20 °C)
30-NOV-2004 (81)

Memo: Refractive index: 1.4139 (20 °C)
30-NOV-2004 (62) (64) (95)

Memo: Refractive index: 1.4143 (20 °C)
30-NOV-2004 (75)

2. PHYSICO-CHEMICAL DATA

ID: 105-53-3

DATE: 21.01.2005

Memo: Refractive index: 1.4150 (20 °C)

30-NOV-2004

(35)

Memo: Refractive index: 1.4165 (20 °C)

30-NOV-2004

(94)

3.1.1 Photodegradation

Type: air
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: .00000000000341 cm³/(molecule * sec)
Degradation: 50 % after 4.7 day(s)

Method: other (calculated): AOPWIN (AOP(c)) Program, Version 1.90, Syracuse Research Corporation, Merrill Lane, Syracuse, New York, 13210, U.S.A., 2000
Year: 2003
GLP: no
Test substance: no data

Remark: Assumption for the calculation: 24 hours sunlight.
Reliability: (2) valid with restrictions
 Calculated data, internationally accepted method.
Flag: Critical study for SIDS endpoint

(21) (68)

Type: water
Light source: other: UV-lamp
Light spect.: ca. 254 nm
Conc. of subst.: 5 mg/l at 23 degree C
INDIRECT PHOTOLYSIS
Sensitizer: O3
Degradation: ca. 100 % after 35 minute(s)

Method: other (measured): Photolytic Ozonation
Year: 1989
GLP: no data
Test substance: other TS: reagent grade no further purification

Test condition: ozone dose rate: 1.3E-05 mmol/l x min
Flag: Critical study for SIDS endpoint
 01-DEC-2004

(79)

3.1.2 Stability in Water

Type: abiotic
t1/2 pH4: > 120 hour(s) at 50 degree C
t1/2 pH7: = 15.9 hour(s) at 50 degree C
t1/2 pH9: <= 2.4 hour(s) at 50 degree C
t1/2 pH 7 : = 137.5 hour(s) at 25 degree C

Method: Directive 92/69/EEC, C.7
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: OECD TG 111
Result: Results of the main test at pH7:

25 °C: t1/2 = 137.5 h
 50 °C: t1/2 = 15.9 h

Pre-test at pH4:
4.7% Hydrolysis within 5 days at 50 °C.

Pre-test at pH 9: rapid hydrolysis:
81.1 % hydrolysis after 2.4 hours at 50°C.

In the study the intermediate monoester could not be determined due to analytical reasons (decarboxylation in the GC injector block), but by following the formation of the alcohols it was possible to estimate the formation of reaction products, monoester and malonic acid, by performing a mass balance analysis.

At pH 9 the hydrolysis of one ester group occurs first, but the subsequent hydrolysis of the second ester bond also takes place within about 2 half-lives.

At pH 7 the reaction is mainly due to formation of the monoester. However, after longer reaction periods a cleavage of the monoester was also observed.

At pH4 the hydrolysis reaction was much slower, but the initial monoester formation was relatively quickly followed by further hydrolysis to malonic acid.

Reliability:

(1) valid without restriction

Guideline study, GLP

Flag:

20-JUL-2005

Material Safety Dataset, Critical study for SIDS endpoint

(18) (24)

3.1.3 Stability in Soil

Type: laboratory
Radiolabel: no
Content of clay: = 4 %
silt: = 51.4 %
sand: = 45.1 %
Organ. carbon: = .5 %
pH: = 7.4
Cation exch. capac.: = 5.5 meq/100 g soil dry weight
Dissipation time
DT50: = 1.2 - 5.4 hour(s)
Dissipation: > 99 % after 96 hour(s)

Method: other: Persistence Test
Year: 1990
GLP: no data
Test substance: no data

Remark: Diethyl malonate depuration from soil samples was biphasic with an initial rapid loss ($t_{1/2} = 1.2$ h) likely representing volatilization and a second phase exhibiting a slower loss rate ($t_{1/2} = 5.4$ h) due to both volatilization, and abiotic and biotic decomposition.
 The surface diethyl malonate level was reduced to less than 0.1 % of initial dose within 96 h (0.1 $\mu\text{g}/\text{cm}^2$ of 750 $\mu\text{g}/\text{cm}^2$).

Test condition: Exposure of soil samples in Petri dishes to diethyl malonate aerosol concentrations of 0.32 and 1.14 mg/l air, resp. for 60 min in a sealed exposure chamber: average mass loading on soil surfaces was 45.31 +/- 3.26 and 770.45 +/- 386.28 $\mu\text{g}/\text{cm}^2$, resp. (n = 3); no further information available.

Reliability:

(2) valid with restrictions
 well documented scientific literature

01-DEC-2004

(11)

Type: laboratory
Radiolabel: no
Content of clay: = 21.4 %
 silt: = 77.5 %
 sand: = 1.1 %
Organ. carbon: = 1.7 %
pH: = 5.4
Cation exch. capac.: = 23.8 meq/100 g soil dry weight
Dissipation time
 DT50: = 2 - 16 hour(s)
 Dissipation: > 99 % after 96 hour(s)

Method: other: Persistence Test
Year: 1990
GLP: no data
Test substance: no data

Remark: Diethyl malonate depuration from soil samples was biphasic with an initial rapid loss ($t_{1/2} = 2$ h) likely representing volatilization and a second phase exhibiting a slower loss rate ($t_{1/2} = 16$ h) due to both volatilization, and abiotic and biotic decomposition.

Test condition: Exposure of soil samples in Petri dishes to diethyl malonate aerosol concentrations of 0.32 and 1.14 mg/l air, resp. for 60 min in a sealed exposure chamber: average mass loading on soil surfaces was 93.40 +/- 7.68 and 722.77 +/- 226.88 ug/cm², resp. (n = 3); no further information available.

Reliability: (2) valid with restrictions
 well documented scientific literature

01-DEC-2004

(11)

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: water - soil
Method: other: (calculation) PCKOCWIN (PC-KOC (c)) Program, Version 1.66, Syracuse Research Corporation, Merrill Lane, Syracuse, New York, 13210, U.S.A., 2000
Year: 2003

Remark: GLP: no
Result: The soil or sediment adsorption coefficient (Koc) of Diethyl malonate was calculated as Koc = 10.

Reliability: (2) valid with restrictions
 Calculated data, internationally accepted method.

Flag: Critical study for SIDS endpoint

20-JUL-2005

(22)

Type: volatility
Media: water - air
Method: other: (calculation) Henrywin Program, Version 3.10, Syracuse

Research Corporation, Merrill Lane, Syracuse, New York, 13210,
U.S.A., 2000

Year: 2003

Method: Bond estimation method

Remark: GLP: no

Result: Henry's Law Constant [25 °C] = 7.36E-007 atm-m³/mole
= 0.0746 Pa m³/mol
= 3.01E-005 unitless

Reliability: (2) valid with restrictions
Calculated data, internationally accepted method.

Flag: Critical study for SIDS endpoint
20-JUL-2005 (20)

Type: volatility

Media: water - air

Method: other: calculation

Remark: GLP: no

Result: Henry's Law Constant [25 °C] = 2.1E-006 atm-m³/mole = 0.12 Pa
m³/mole

Reliability: (4) not assignable
Database data
20-JUL-2005 (89)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water

Method: Calculation according Mackay, Level III

Year: 2004

Method: Estimation of the Equilibrium Partitioning Characteristics in
the Environment.
Calculation
Mackay Level III, V2.70 Model (2002) Environmental Modelling
Centre, Trent University, Peterborough, Ont. Canada.

Result:

Compartment	Release 100 % in air	Release 100 % in water	Release 100 % in soil
Air	15.2	0.01	0.03
Water	48.8	99.9	55.4
Soil	36.0	0.01	44.5
Sediment	0.02	0.04	0.02

Conclusion:
Under equilibrium steady state flow conditions the substance
distributes to water and soil when released into air and soil,
while the majority of the substance will stay in the water
compartment when released into the water compartment.

Test condition: Input parameters
Molecular mass: 160.17 g/mol
Temperature: 20 °C
log Kow: 0.96
Water solubility: 18 g/l
Vapour pressure: 35 Pa
Melting point: -50 °C
Half-life in air: 113 hours

3. ENVIRONMENTAL FATE AND PATHWAYS

ID: 105-53-3

DATE: 21.01.2005

Reliability: Emission rates default 3000 kg/h to either air, water or soil.
(2) valid with restrictions
Calculated data, internationally accepted method.

Flag: Critical study for SIDS endpoint

01-DEC-2004 (26)

Media: water - air
Method: Calculation according Mackay, Level I
Year: 2004

Result: Air: 9.86 %
Soil: 0.06 %
Water: 90.01 %
Sediment: 0.07 %
Biota: < 0.01 %

Test condition: Data used:
Molar mass: 160.17 g/mol
Data temperature: 20 °C
Log Pow: 0.96
Vapor pressure: 35 Pa
Water solubility: 18.0 g/l
Melting Point: -50 °C

Volumes used:
Air: 6 000 000 000
Soil: 45 000
Water: 7 000 000
Sediment: 21000
Susp. Sediment: 35
Biota: 7
Aerosol: 0.12

Reliability: (2) valid with restrictions
Calculated data, internationally accepted method.

Flag: Critical study for SIDS endpoint

01-DEC-2004 (25)

3.4 Mode of Degradation in Actual Use**3.5 Biodegradation**

Type: aerobic
Inoculum: activated sludge
Concentration: 50 µg/l related to Test substance
Degradation: = 15.1 % after 5 day(s)

Method: other: Biodegradation Test
Year: 1984
GLP: no data
Test substance: no data

Remark: biodegradation related to CO₂ released
Reliability: (4) not assignable
Data insufficient for assessment

01-DEC-2004 (37)

Type: aerobic
Inoculum: activated sludge
Concentration: 10.8 mg/l related to DOC (Dissolved Organic Carbon)

3. ENVIRONMENTAL FATE AND PATHWAYS

ID: 105-53-3

DATE: 21.01.2005

Degradation: = 98 % after 28 day(s)
Result: readily biodegradable
Kinetic: 7 day(s) = 92 %

Method: other: Directive 79/831/EEC, Appendix V, Part C: DOC-DIE AWAY Test, Method C.4-A
Year: 1993
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Kinetics of biodegradation: % decrease of DOC
 day FE1(%) FE2(%) FC1(%) FC2(%)

day	FE1(%)	FE2(%)	FC1(%)	FC2(%)
0	0	0	0	0
7	90	94	100	99
14	95	98	96	96
21	99	100	99	100
27	101	99	101	100
28	99	98	98	98

FE1 and FE2: Flasks with test substance and inoculum
 FC1 and FC2: Falsks control substance and inoculum

- Breakdown product: no
- Remarks: The difference of biodegradation between the two flasks containing the test substance at the end of the test is less than 20%
- More than 70% of biodegradation was reached within 14 days in the control flasks containing sodium benzoate (99% degradation after 7 days): the inoculum activity is sufficient.

The test substance was degraded to 90-94% after 7 days and 95 to 100% after 14 to 28 days.

Conclusions:

The test substance is readily biodegradable under the test conditions.

Test condition:

INOCULUM/TEST ORGANISM

- Type of sludge: activated sludge, predominantly domestic
- Source: Sewage plant Marl-Ost
- Sampling site: activated sludge basin
- Preparation of inoculum: Centrifugation 10 min at 1100 x g, the supernatant is discarded and the sludge resuspended with mineral medium, further centrifugation for 10 min at 1100 x g Resuspension of the activated sludge (2.79 g/l dry mass of activated sludge)
- Initial cell concentration: 27.9 mg/l

TEST SYSTEM

- Culturing apparatus: 2000 ml Erlenmeyer flask with slight aluminium foil closure
- Aeration device: shaking machine
- Measuring equipment: Carbon analyzer (Schimadzu)

INITIAL TEST SUBSTANCE CONCENTRATION: 9.56 mgDOC/l in the test flasks, 10.64 mg DOC/l in the control flasks

METHOD OF PREPARATION OF TEST SOLUTION: Stock solution: 1000 mg/l (512 mg DOC/l)

DURATION OF THE TEST: 28 days

ANALYTICAL PARAMETER: Dissolved organic carbon (DOC)

SAMPLING: After 0, 7, 14, 21, 27, 28 days.

TEST CONDITIONS

- Composition of stock nutrient solutions:

- a) 8.5 g/l KH₂PO₄
21.75 g/l K₂HPO₄
33.3 g/l Na₂HPO₄ * 2 H₂O
20.0 g/l (NH₄)Cl
- b) 22.5 g/l MgSO₄ * 7 H₂O
- c) 27.5 g/l CaCl₂
- d) 0.25 g/l FeCl₃ * 6 H₂O

- Additional substrate: No

- Test temperature: 21.8 - 22.1 °C

- Aeration of dilution water: no

- Concentration of suspended solids: 27.9 mg/l

- Addition of Stock nutrient solutions: a): 20 ml, b) - d): 2ml each.

CONTROLS: 1 Flask without test substance, but with inoculum,
REFERENCE SUBSTANCE: 2 Flasks with Benzoic acid, sodium salt,
10.64 mg DOC/l and inoculum.

No abiotic control (with test substance, without inoculum) and no inhibitory control was included in the test

Test substance:

FACTORS AFFECTING TEST:

- Stability: see hydrolysis as function of pH, section 3.1.2 stability in water

- Vapor pressure: 0.35 hPa (20 °C)

- Water solubility: 18 g/l (20 °C)

- Adsorption potential (log Pow): 0.96

- Toxicity to microorganisms: EC₅₀ = 3097 mg/l

Reliability:

(1) valid without restriction

Guideline study, GLP

Flag:

WGK (DE), Material Safety Dataset, Critical study for SIDS endpoint

20-JUL-2005

(48)

Type:

aerobic

Inoculum:

other bacteria: Streptomyces nitrificans

Concentration:

770 mg/l related to Test substance

Degradation:

= 34 % after 110 minute(s)

Method:

other: Warburg Respirometric Experiment

Year:

1957

GLP:

no data

Test substance:

no data

Remark:

inoculum: homogenized mycelium of urethan-grown Streptomyces nitrificans

Test condition:

pH 7.0; 30 degree C

Reliability:

(2) valid with restrictions

No standard test system

(85)

3.6 BOD₅, COD or BOD₅/COD Ratio**3.7 Bioaccumulation****3.8 Additional Remarks**

Remark:

Reaction of ozone with diethyl malonate in water:

rate constant: $0.06 \text{ M}^{-1} \times \text{s}^{-1}$;

test substance concentration: 8 or 70 mM diethyl malonate;

diethyl malonate/O₃ ratio: $\geq 10 \text{ mol/mol}$; pH 2; 20 degree C

01-DEC-2004

(43)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 12
LC100: = 25

Method: other: Acute Toxicity Test
Year: 1985
GLP: no data

Remark: age of fish: 28 d

Result: RESULTS: EXPOSED
 - Nominal/measured concentrations:

	0	5.58	8.58	13.2	20.3	31.2
Nominal	0	5.58	8.58	13.2	20.3	31.2
Day 0	<0.22	5.88	8.59	13.7	19.7	33.4
Day 1	<0.22	6.73	10.9	17.2	26.3	35.1
Day 2	<0.22	4.75	7.29	12.4	22.4	-
Day 3	<0.22	7.31	9.61	15.3	23.5	-
Day 4	<0.22	10.0	12.6	24.5	34.0	-
corr.						
average	<0.22	6.78	9.59	16.3	24.6	33.5

Percent recovery: 102.2 (+-4.38)%

- Effect data (Mortality):

96 h LC50 = 11.8 mg/l
 confidence limit: 10.3-13.4 mg/l
 96 h LC100 = 24.6 mg/l

- Concentration / response curve:
 (20 fish per concentration)

Conc. (mg/l)	No. surviving	No dead	% mortality
control	20	0	0
6.78	18	2	10
9.59	15	5	25
16.3	3	17	85
24.6	0	20	100
33.5	0	20	100

Other observations: affected fish lost schooling behavior, swam near the surface. They were hyperactive. The fish lost equilibrium prior to death.

During the last day dissolved oxygen was below 60% in the low concentration group.

Test condition: TEST ORGANISMS

- Strain: Pimephales promelas (fathead minnow)
 - Age/size/weight/loading: 28 d, mean length: 22.0 (+- 1.432) mm, mean weight: 0.165 (+-0.0338)g, loading: 2.75 g/l (20 fish per chamber)
 - Chamber volume: 1.2 l

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Dispersion: Stock solution: 500 mg/l in water

STABILITY OF THE TEST CHEMICAL SOLUTIONS: confirmed by analysis (gas-liquid chromatography) (102.2 % recovery)

DILUTION WATER

- Alkalinity: 41.1 (+-3.01) mg/l as CaCO₃
 - Hardness: 53.0 (+- 2.55) mg/l as CaCO₃
 - Oxygen content: 7.0 (+-0.48) mg/l
 - pH: 7.27 (+- 0.06)
 - Temperature: 23.9 (+- 1.13) °C
 Test Type: flow through, 12 volume additions per day.

Test substance: Diethyl malonate, from Aldrich, 99% purity
Reliability: (2) valid with restrictions
 Study well documented, meets generally accepted scientific principles, acceptable for assessment.
Flag: Critical study for SIDS endpoint
 26-AUG-2005 (39)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 15
LC100: = 33

Method: other: Acute Toxicity Test
Year: 1984
GLP: no data

Result: RESULTS: EXPOSED
 - Nominal/measured concentrations:
 Experiment 1

Nominal	0	10.6	17.8	29.6	49.3	82.2
Day 0	0	5.8	10.3	15.8	35.1	63.6
Day 1	0	6.65	12.9	23.0	39.0	69.7
Day 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 3	n.d.	4.3	9.4	16.0	n.d.	n.d.
Day 4	0	4.4	8.6	14.0	n.d.	n.d.
corr.						
average	0	5.4	10.4	17.4	37.5	67.5

n.d. = not determined

Experiment 2

Nominal	0	10.6	17.8	29.6	49.3	82.2
Day 0	0	5.5	9.5	20.4	34.2	62.8
Day 1	0	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	n.d.	5.0	9.2	18.3	31.5	n.d.
Day 3	0	n.d.	n.d.	n.d.	n.d.	n.d.
Day 4	0	4.5	8.0	15.5	n.d.	n.d.
corr.						
average	0	5.1	9.0	18.3	33.2	63.6

n.d. = not determined
 Recovery: 98.8 (+-3.8)%
 - Effect data (Mortality):
 96 h LC50 = 15.4 mg/l
 confidence limit: 14.1-16.9 mg/l
 96 h LC100 = 33 mg/l
 - Concentration / response curve:
 Experiment 1: (25 fish per concentration)

Conc. (mg/l)	No. surviving	No dead	% mortality
control	25	0	0
5.4	25	0	0
10.4	24	1	4
17.4	6	19	76
37.5	0	25	100
67.5	0	25	100

Experiment 2: (25 fish per concentration)

Conc. (mg/l)	No. surviving	No dead	% mortality
control	25	0	0
5.1	25	0	0
9.0	24	1	4
18.3	8	17	68
33.2	0	25	100
63.6	0	25	100

- Other effects: Affected fish lost equilibrium prior to death.

Test condition:

TEST ORGANISMS
 - Strain: Pimephales promelas (fathead minnow)
 - Supplier: EPA Duluth or UW-Superior culture units, EPA Duluth brood stock.
 - Age/size/weight/loading: 33 d, mean length: 22 (+- 2.708) mm, mean weight: 0.152 (+-0.0703)g, loading: 0.603 g/l (25 fish per chamber)
 - Chamber volume: 6.3 l
 - Feeding: Tetramin commercial fish food and brine shrimp (Artemia salina)
 - Pretreatment: none
 - Feeding during test: no

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Dispersion: Stock solution: 15.8 g/l in water
 STABILITY OF THE TEST CHEMICAL SOLUTIONS: confirmed by analysis (gas-liquid chromatography) (98.8 % recovery)

DILUTION WATER

- Source: Lake Superior water
 - Aeration: no
 - Alkalinity: 44 mg/l as CaCO₃
 - Hardness: 45 mg/l as CaCO₃
 - Oxygen content: 7.5 (+-1.08) mg/l
 - pH: 7.42 (+- 0.05)
 - Temperature: 25.4 (+- 1.53) °C
 - Illumination: 16 fluorescent light/ 8 hour dark
 Test Type: flow through, 5.7 volume additions per day.

Test substance:

Diethyl malonate, from Aldrich, 99% purity

Reliability:

(2) valid with restrictions
 Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag:

Critical study for SIDS endpoint

24-AUG-2005

(8) (10)

Type:

flow through

Species:

Pimephales promelas (Fish, fresh water)

Exposure period:

96 hour(s)

Unit:

mg/l

Analytical monitoring: yes

LC50:

= 17

LC100:

= 33

Method:

other: Acute Toxicity Test

Year:

1986

GLP:

no data

Remark:

For MSDS 11.8 - 17.4 mg/l
 This value was used by Admans et al., 2001 and Martin and Young, 2001, to develop a QSAR for molecules that had a

toxicity exceeding baseline QSAR predictions for "polar or unpolar" narcotics. The authors used an artificial neuronal network model and a multiple regression model. The fit of the neuronal network model was better than that of the multiple regression model.

Result:

RESULTS: EXPOSED

- Nominal/measured concentrations:

Nominal	0	9.1	14.0	21.6	33.2	51.0
Day 0	<5.0	-	11.0	16.0	25.0	46.0
Day 1	<5.0	-	12.0	17.0	28.0	51.0
Day 2	<1.0	6.5	9.5	22.8	37.0	49.2
Day 3	<1.0	11.7	15.2	21.3	32.9	59.4
Day 4	<1.0	11.3	14.6	20.3	34.9	53.0
corr.						
average	<2.8	10.4	13.2	20.6	33.3	54.6

Percent recovery: 94.7 (+-1.27)%

- Effect data (Mortality):

96 h LC50 = 17.4 mg/l

confidence limit: 15.4-19.5 mg/l

96 h LC100 = 33 mg/l

- Concentration / response curve:

(20 fish per concentration)

Conc. (mg/l)	No. surviving	No dead	% mortality
control	0	0	0
10.4	20	0	0
13.2	15	5	25
20.6	6	14	30
33.3	0	20	100
54.6	0	20	100

Other observations: affected fish lost schooling behavior, swam near the bottom, were hypoactive and unreactive to external stimuli. The fish lost equilibrium prior to death.

Test condition:

TEST ORGANISMS

- Strain: Pimephales promelas (fathead minnow)

- Age/size/weight/loading: 28 d, mean length: 19.8 (+- 2.149) mm, mean weight: 0.120 (+-0.0324)g, loading: 1.2 g/l (20 fish

per chamber)

- Chamber volume: 2.0 l

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Dispersion: Stock solution: 260 mg/l in water

STABILITY OF THE TEST CHEMICAL SOLUTIONS: confirmed by analysis (gas-liquid chromatography) (94.7 % recovery)

DILUTION WATER

- Alkalinity: 38.6 (+-1.6) mg/l as CaCO3

- Hardness: 37.4 (+- 0.25) mg/l as CaCO3

- Oxygen content: 7.1 (+-0.09) mg/l

- pH: 7.42 (+- 0.05)

- Temperature: 25.1 (+- 0.13) °C

Test Type: flow through, 18 volume additions per day.

Test substance:

Diethyl malonate, from Aldrich, 99% purity

Reliability:

(2) valid with restrictions

Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag:

WGK (DE), Material Safety Dataset, Critical study for SIDS endpoint

26-AUG-2005

(1) (38) (66)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 16 calculated

Method: other: acute toxicity test
Year: 1995
GLP: no data
Test substance: other TS: Diethyl malonate purity >= 95%

Result: "Non-polar narcosis" was identified as the primary mechanism of action by the authors.

Test condition: TEST ORGANISMS
- Strain: Pimephales promelas (fathead minnow)
- Age: 26-33 d
- Pretreatment: none
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- no data
DILUTION WATER
- Source: Lake Superior water
- Aeration: no
- Alkalinity: 42 mg/l as CaCO₃
- Hardness: 45 mg/l as CaCO₃
- pH: 7.8
- Temperature: 25 °C
Statistical Method: Spearman-Kärber

24-AUG-2005

(7)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 15
Limit Test: no

Method: other: scientific method
Year: 1984
GLP: no data

Remark: This reference possibly refers to the same results as Brooke et al. 1984, but the details are not all the same.

Result: Measured concentrations:
5.06, 9.51, 17.2, 34.3, 64.1 mg/l.
LC50 (96 h): 14.9 mg/l
95% confidence limits: 13.7-16.3 mg/l.
Affected fish lost equilibrium.

Test condition: TEST ORGANISMS
- Strain: Pimephales promelas (fathead minnow)
- Supplier: EPA Duluth or UW-Superior culture units, EPA Duluth brood stock.
- Age/size/weight/loading: 33 d, mean length: 22 (+- 3) mm, mean weight: 0.152 (+-0.070)g, loading: 25 fish per chamber
- Chamber volume: 6.3 l
- Feeding: Tetramin commercial fish food and brine shrimp (Salinus artemia)
- Pretreatment: none
- Feeding during test: no
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- no data

STABILITY OF THE TEST CHEMICAL SOLUTIONS: confirmed by analysis (gas-liquid chromatography) (98.8 % recovery)
DILUTION WATER
- Source: Lake Superior water
- Aeration: no
- Alkalinity: 45 mg/l as CaCO₃
- Hardness: 44 mg/l as CaCO₃
- Oxygen content: 87.6 +- 10.7%
- pH: 7.42 (+- 0.04)
- Temperature: 25.4 (+- 1.4) °C
- Illumination: 16 fluorescent light/ 8 hour dark
Test Type: flow through, 1.2 to 5.8 volume additions per day.

Test substance: Diethyl malonate, from Aldrich, 99% purity
Reliability: (2) valid with restrictions
Details lacking, but scientifically relevant reference.

Flag: Critical study for SIDS endpoint
24-AUG-2005 (10)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 73

Method: other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: RESULTS: EXPOSED
- Nominal/measured concentrations: Nominal only, no details available.
- Effect data (Mortality): LC50 = 73 mg/l
- Concentration / response curve: no details available.

Test condition: TEST ORGANISMS
- Strain: Leuciscus idus melanotus
No further data available, as specified in DIN 38412 part 2
STOCK AND TEST SOLUTION AND THEIR PREPARATION
No details available
STABILITY OF THE TEST CHEMICAL SOLUTIONS:

DILUTION WATER
No details available, as specified in DIN 38412 part 2
TEST SYSTEM
- Test type: static
- Concentrations: no details available
- Number of replicates, fish per replicate:
No details available, as specified in DIN 38412 part 2
- Test temperature:
No details available, as specified in DIN 38412 part 2
- Dissolved oxygen: no details available
- pH: no details available
- Intensity of irradiation: no details available
- Photoperiod: no details available
DURATION OF THE TEST: 48 hours
TEST PARAMETER: mortality
MONITORING OF TEST SUBSTANCE CONCENTRATION: no

Reliability: (2) valid with restrictions
Standard national method 48 h exposure only, no details

reported.
Flag: Material Safety Dataset, Critical study for SIDS endpoint
 24-AUG-2005 (45)

Type: static
Species: other: Oncorhynchus kisutch, Ptychocheilus oregonesis,
 Oncorhynchus tshawytscha
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0 : > 10

Method: other: Acute Toxicity Test
Year: 1969
GLP: no data
Test substance: no data

Result: None of the fish of the different species died or lost their equilibrium.
Test condition: TEST ORGANISMS
 - Strain: Oncorhynchus kisutch, Ptychocheilus oregonesis, Oncorhynchus tshawytscha.
 - Wild caught: For Salmonids: Eagle Creek National Fish Hatchery, Portland Oregon.
 For Ptychocheilus oregonesis: St. Maries river Sanata Creek.
 - Size: 5 to 10 cm
 - loading: 5 g/l, one fish of each species was placed together in one vessel with 4 l of water.
 - Feeding: non during acclimatization and treatment.
 STOCK AND TEST SOLUTION AND THEIR PREPARATION
 - Dispersion: The chemical was dissolved in minimal amounts of water and added directly to the test vessels.
 - Vehicle, solvent: water
 STABILITY OF THE TEST CHEMICAL SOLUTIONS: not indicated
 DILUTION WATER
 - Source: Water from Rochat Creek
 - Aeration: yes
 - Alkalinity: 7 ppm (presumably as CaCO3)
 - Hardness: 0-17 ppm (presumably as CaCO3)
 - pH: 7.2
 - Oxygen content:
 - Temperature: 17.8 °C

Reliability: (3) invalid
 Limited number of animals, only one concentration tested.
 24-AUG-2005 (65)

Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: g/l **Analytical monitoring:**
LC50: >= 14

GLP: no data
Test substance: no data

Reliability: (4) not assignable
 Original publication not available
 24-AUG-2005 (31)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 100
EC50: = 202
EC100: = 400
Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: 1984
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: RESULTS: EXPOSED
 - Nominal concentrations:
 100, 140, 200, 280, 400, 560, 800 mg
 - Effect data (immobilization) (48 h): EC50: 202.3 mg/l 95%
 confidence limit (mg/l): 175.2 - 233.6
 - Concentration / response curve: 48 h

concentration total (mg/l)	No.	No. mobile	No. immobile	%immobile
control	20	20	0	0
100	20	20	0	0
140	20	17	3	15
200	20	11	9	45
280	20	3	17	85
400	20	0	20	100
560	20	0	20	100
800	21	0	21	100

- Effect data (immobilization) (24 h): EC50 = 285.8 mg/l, 95%
 confidence limit (mg/l): 244.6 - 333.9
 - Concentration / response curve: 24 h

concentration total (mg/l)	No.	No. mobile	No. immobile	%immobile
control	20	20	0	0
100	20	20	0	0
140	20	20	0	0
200	20	15	5	25
280	20	12	8	40
400	20	4	16	80
560	20	0	20	100
800	21	0	21	100

- Cumulative immobilization: 400 mg/l (48 h) (lowest
 concentration with 100% immobilization)

RESULTS CONTROL:
 RESULTS: TEST WITH REFERENCE SUBSTANCE
 - Concentrations:
 - Results (immobilization) (24 h):

concentration % immobilized daphnids
(mg/l)

0.9	20
1.9	100

Based on effective concentrations (corrected for hydrolysis)
the EC50 48 h is 179 mg/l.

Test condition:

TEST ORGANISMS

- Strain: Daphnia magna Straus Clone 5
- Source/supplier: Hüls AG
- Breeding method: Breeding method according to Elenedt (1990) in M4-medium in 1l beakers, water exchange every 2 to 3 days.
- Age: < 1 day
- Feeding: Desmodesmus subspicatus
- Feeding during test: none
- Control group: negative control (water only), positive control: potassium dichromate

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Vehicle, solvent: water
- Concentration of vehicle/ solvent: 1000 mg/l

STABILITY OF THE TEST CHEMICAL SOLUTIONS: see stability information (hydrolysis as function of pH).

DILUTION WATER

- Source: Synthetic fresh water
- Aeration: no
- Hardness: CaCl₂ x 2 H₂O: 294 mg/l, MgSO₄ x 7 H₂O: 123 mg/l
- Salinity: KCl: 5.5 mg/l
- Ca/Mg ratio: 4 : 1
- Na/K ratio: 10 : 1
- pH: 7.0 to 7.5
- Oxygen content: 6.6 to 7.8 mg/l

TEST SYSTEM

- Test type: static
- Concentrations: (nominal): 100, 140, 200, 280, 400, 560, 800 mg/l
- Exposure vessel type: round bottom flasks
- Number of replicates, individuals per replicate: 4 replicates, 5 individuals
- Test temperature: 20 +/- 2°C
- Dissolved oxygen: 6.6 - 7.8 mg/l
- pH: 7.0 - 7.5
- Adjustment of pH: no
- Intensity of irradiation: dark

DURATION OF THE TEST: 48 hours

TEST PARAMETER: Immobilization

MONITORING OF TEST SUBSTANCE CONCENTRATION: not performed, nominal concentrations used.

Statistical Analysis: Probit analysis according to Cavalli-Sforza (1972)

Reliability:

(2) valid with restrictions
Guideline study, GLP, but no analytical substance determination.

Flag:

WGK (DE), Material Safety Dataset, Critical study for SIDS endpoint

26-AUG-2005

(49)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 25
EC10: = 115
EC50: > 800

Method: other: Guideline 88/302/EEC
Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: cell growth: EC10 = 30.1; EC50 = 508.2
 growth rate: EC10 = 115.1; EC50 > 800 mg/l (highest concentration tested)

Result: RESULTS:
 Nominal concentrations only
 - Effect data/Element values:
 Experiment 1
 - Cell density data:
 Cell density in cells x 10exp4/ml (standard deviation)
 (at 24, 48, and 72 h mean values of 8 parallel experiments for controls and 5 experiments for test substance concentrations)
 0 h:

Control	36	60	100	170	300	500
0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l

2	2	2	2	2	2	2
---	---	---	---	---	---	---

Time	Control	36	60	100	170	300	500
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l

24 h	7	6	7	6	6	6	5
(s.d.)	(0.5)	(0.4)	(0.5)	(0.6)	(0.7)	(0.4)	(0.5)

48 h	24	23	21	20	21	20	16
(s.d.)	(2.1)	(1.9)	(1.6)	(2.1)	(1.9)	(1)	(1.9)

72 h	82	70	67	57	51	43	31
(s.d.)	(3)	(6.3)	(2.7)	(0.7)	(3.8)	(2.4)	(2.4)

- Growth curves:

Area under the growth curve and % inhibition

	Control	36	60	100	170	300	500
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l

Area	67	59	56.5	49.5	47.5	42.5	31.5
% inhib.		11.9	15.7	26.1	29.1	36.6	53

Growth rate (u)
 0-72 h

Control	36	60	100	170	300	500
0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l

u	1.24	1.19	1.17	1.12	1.06	1.02	0.91
% inhib.		4.3	5.4	9.8	12.8	17.4	26.2

pH-development during the test:

	Control	36	60	100	170	300	500
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0 h	7.4	7.6	7.4	7.6	7.5	7.5	7.5
72 h	9.2	6.9	7.1	6.8	6.9	7.7	8.5

Experiment 2:

- Cell density data:

Cell density in cells x 10exp4/ml (standard deviation)
(at 24, 48, and 72 h mean values of 8 parallel experiments for controls and 5 experiments for test substance concentrations)

0 h:

Control	12.5	25	50	100	200	400	800
0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l

2	2	2	2	2	2	2	2
---	---	---	---	---	---	---	---

Time	Control	12.5	25	50	100	200	400	800
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l

24 h	5	5	4	5	5	5	5	4
(s.d.)	(0.7)	(0)	(0.4)	(0)	(0.4)	(0.4)	(0.4)	(0.4)

48 h	17	18	17	16	17	18	12	8
(s.d.)	(1.5)	(0.5)	(1.4)	(1)	(0.4)	(1.9)	(2.3)	(1.9)

72 h	54	59	55	45	44	47	29	18
(s.d.)	(6.6)	(2.9)	(4)	(5.5)	(4.5)	(5.9)	(4.7)	(3.4)

- Growth curves:

Area under the growth curve and % inhibition

	Control	12.5	25	50	100	200	400	800
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Area	44	47.5	44.5	37.5	39	41.5	26.5	16
% inhib.		-8	-1.1	14.8	11.4	5.7	0.89	0.73

Growth rate (u)
0-72 h

	Control	12.5	25	50	100	200	400	800
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
u	1.099	1.13	1.11	1.10	1.03	1.05	0.89	0.73
% inhib.		-2.6	-0.5	5.6	6.3	4.3	18.9	33.4

pH-development during the test:

	Control	12.5	25	50	100	200	400	800
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0 h	7.3	7.5	7.5	7.5	7.5	7.5	7.5	7.2
72 h	8.8	8.9	8.7	8	7.6	7.7	8.5	7.6

In some of the test vials including controls the pH values were increased during the test. As the growth was not influenced by the increase in pH this does not compromise the quality of the data according to the authors.

STATISTICAL RESULTS:

Cell growth (biomass):

72 h EbC50: 508.2 mg/l

72 h EbC10: 30.1 mg/l

72 h EbC90: > highest tested concentration of 800 mg/l

Growth rates:

72 h ErC50: > highest tested concentration of 800 mg/l

72 h ErC10: 115.1 mg/l

72 h ErC90: > highest tested concentration of 800 mg/l

Based on effective concentrations corrected for hydrolysis the 72 h EC50 would be > 667 mg/l (growth rate) and 424 mg/l (biomass).

Test condition:

TEST ORGANISMS

- Strain: *Desmodesmus subspicatus* (*Scenedesmus subspicatus*), 86.81 SAG

- Source/supplier: Institut für Wasser-, Boden- und Lufthygiene, Berlin and Hüls AG, own breeding

- Laboratory culture: From a stem culture, prepared 3 days prior to the start of the experiment.

- Method of cultivation: Cell density: 20000 cells/ml, culture in sterile Erlenmeyer flasks on light-tables, light intensity: 8000 Lux, white, medium according to EC-guideline 88/302/EEC, temperature: 24 +/- 2 °C

- Controls: without test substance

- Initial cell concentration: 2x 10^{exp4} cells/ml

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- 1 g/l in water

STABILITY OF THE TEST CHEMICAL SOLUTIONS: see stability information (hydrolysis as function of pH).

DILUTION WATER

- Source: Deionized water

- Aeration: no

TEST SYSTEM

- Test type: static

- Number of replicates: 5 to 8, 2 independent experiments

- Concentrations:

Experiment 1: 36, 60, 100, 170, 300, 500 mg/l (nominal)

Experiment 2: 12.5, 25, 50, 100, 200, 400, 800 mg/l

(nominal)

- Test temperature: 24 +/- 2 °C

- pH: pH at the beginning of the test: 7.4 to 7.6, at the end of the test: 6.8 to 9.2.

- Intensity of irradiation: 8000 Lux

MONITORING OF TEST SUBSTANCE CONCENTRATION: not performed, nominal concentrations used.

Statistical Method:

Probit analysis according to Cavalli and Sforza, 1972.

Reliability:

(2) valid with restrictions

Guideline study, GLP, but no analytical substance determination.

Flag:

WGK (DE), Material Safety Dataset, Critical study for SIDS endpoint

26-AUG-2005

(51)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 1092
EC50: = 3097

Method: other: DIN 38412 Teil 8 (DE)
Year: 1993
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Temperature: 21 +/- 1 degree C
Reliability: (1) valid without restriction
Flag: Material Safety Dataset, Critical study for SIDS endpoint

09-AUG-2004

(47)

Type: aquatic
Species: Tetrahymena pyriformis (Protozoa)
Unit: mmol/l **Analytical monitoring:** no data
EC50: = 10

Method: other
Year: 1997
GLP: no data
Test substance: other TS: no data

Method: 2-dimensional static 50% inhibition growth concentration (IGC50) for axenic cultures of the ciliate Tetrahymena pyriformis according to Schultz, 1996.

Remark: The data were used to develop a QSAR model.
Test condition: Stock solutions: In DMSO at concentrations of 5 to 50 mg/l.
Flag: Critical study for SIDS endpoint

24-AUG-2005

(57)

Type: aquatic
Species: Tetrahymena pyriformis (Protozoa)
Exposure period: 40 hour(s)
Unit: mmol/l **Analytical monitoring:** no data
EC50: = 10.7

Method: other: Tetratox, 1997
Year: 2000
GLP: no data
Test substance: other TS: no data

Remark: The value was used to derive QSAR relationships for aquatic toxicity data that tested the use of dimyristoyl phosphatidylcholine/water partition coefficients in place of octanol/water partition coefficients to get a better fit of the data.

Test condition: pH: 7.35, not adjusted.
Organic amended medium.

24-AUG-2005

(86)

Type: aquatic

Species: other bacteria: nitrifying bacteria
Unit: mg/l **Analytical monitoring:** no data
EC0: <= 50

Method: other: no data
Year: 1973
GLP: no data
Test substance: no data

Remark: no further information available (12)

Type: aquatic
Species: other protozoa: Infusoria
Unit: **Analytical monitoring:** no data

Method: other: no data
Year: 1973
GLP: no data
Test substance: no data

Remark: 1000 mg diethyl malonate/l was lethal to infusoria (no further information available). (12)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

Species: other terrestrial plant: Brassica alba, Lepidium sativum, Triticum aestivum
Endpoint: other: emergence and growth
Method: OECD Guide-line 208 "Terrestrial Plants, Growth Test"
Year: 1995
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Emergence of seeds:

plant	controls, No.			100 mg/kg, No.		
	planted	emerged	%	planted	emerged	%
Triticum aestivum	20	19	95	20	17	85
Lepidium sativum	20	19	95	20	17	85
Brassica alba	20	19	95	20	18	90

Plant growth:

Given as weight of the aboveground parts directly after the end of the experiment. (17 days)

100 mg/kg weight (mg/kg) (s.d.)	plant		controls	
	weight (mg/kg)	(s.d.)	weight (mg/kg)	(s.d.)
Triticum aestivum	343	(109)	352	(138)
Lepidium sativum	86	(20)	99	(18)
Brassica alba	202	(36)	216	(64)

No effects were observed on seed development and growth of all 3 plant species up to a concentration of 100 mg/kg.

Test condition: Test endpoints: emergence and growth of terrestrial higher plants.

Test plants: Triticum aestivum, Lepidium sativum, Brassica alba.

Test vials: Flowerpots, diameter: bottom: 6 cm, top: 8 cm height: 6 cm.

Number of replicates: 4 per species with 5 seeds each.

Maximum water capacity: 60 %

Watering: Once during the test.

Substrate: origin: LUFA Speyer, according to OECD guideline 208: sieved, 0.5 mesh, organic carbon content: < 1.5%, particles < 20 micro-m: 10 to 20%

pH: 5 to 7.5

Stock mixture: 0.160 g test substance on 20 g silica sand were mixed with 1580 g substrate.

Growth conditions: in a partly climatized room with an average humidity of 98%.

Light intensity: 16 h: 1300-1800, 8 h 50-100

Temperature: 23-24 °C

Statistical methods: Standard t-test.

Reliability: Test type: limit test with 100 mg test substance/kg substrate.
(1) valid without restriction
Flag: Material Safety Dataset, Critical study for SIDS endpoint
24-AUG-2005 (53)

Species: other terrestrial plant: Spinacea oleracea
Endpoint: other: photosynthetic electron transport
Unit: mg/kg soil dw
NOEC: >= 100

Method: other: Measurement of photosynthetic electron transport in isolated chloroplasts
Year: 1988
GLP: no data
Test substance: no data

Result: Neither the electron transport in the whole photosynthetic chain, nor the photosystems I or II were affected by an in vitro incubation of isolated spinach chloroplasts with 100 mg diethyl malonate/l for 5 min compared to control.

Test condition: Temperature: 23 - 24 °C
light: day (16 h) 1300 - 1800 Lux
night (8 h) 50 - 100 Lux

16-AUG-2004 (34)

Species: other terrestrial plant: Pinus echinata
Endpoint: other: visual toxicity symptoms
Unit: mg/l
NOEC: .32

Method: other: Aerosol Exposure Test
Year: 1990
GLP: no data
Test substance: no data

Result: 1.14 mg/l aerosol concentration:
0 d: old and new growth developed healthy
2 d: chlorosis and tip or leaf edge burn:
5 - 25 % of foliage affected
21 d: chlorosis and tip or leaf edge burn:
25 - 50 % of foliage affected
0.32 mg/l aerosol concentration:
old and new growth developed healthy within the post-exposure period.

Test condition: average foliar mass loading (n = 3): 0.20 +/- 0.12 ug/cm2 (diethyl malonate exposure: 0.32 mg/l air) and 17.08 +/- 2.94 ug/cm2 (diethyl malonate exposure: 1.14 mg/l air); relative humidity: 35 %; temperature: 24 degree C (low concentration) and 25 degree C (high concentration)
Short-needle pine plants were aerosol-exposed for 60 min in a sealed exposure chamber and visual toxicity symptoms were assessed at 0, 2, and 21 d post-exposure.

Reliability: (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag: Critical study for SIDS endpoint
24-AUG-2005 (11)

Species: other terrestrial plant: Artemisia tridentata
Endpoint: other: visual toxicity symptoms

Unit: mg/l
NOEC: .32

Method: other: Aerosol Exposure Test
Year: 1990
GLP: no data
Test substance: no data

Result: 1.14 mg/l aerosol concentration:
0 d: old and new growth developed healthy
2 d: leaf curl and wilting:
25 - 50 % of foliage affected
21 d: chlorosis, leaf curl, growing tip dieback:
75 - 95 % of foliage affected
0.32 mg/l aerosol concentration:
old and new growth developed healthy within the
post-exposure period

Test condition: average foliar mass loading (n = 3): 11.69 +/- 0.54 ug/cm2
(diethyl malonate exposure: 0.32 mg/l air) and 114.79 +/-
29.44 ug/cm2 (diethyl malonate exposure: 1.14 mg/l air);
relative humidity: 35 %; 24 degree C (low concentration)
and 25 degree C (high concentration)
Sagebrush plants were aerosol-exposed for 60 min in a sealed
exposure chamber and visual toxicity symptoms were assessed at
0, 2, and 21 d post-exposure.

Reliability: (2) valid with restrictions
Study well documented, meets generally accepted scientific
principles, acceptable for assessment.

Flag: Critical study for SIDS endpoint

24-AUG-2005 (11)

Species: other terrestrial plant: Festuca arundinacea
Endpoint: other: visual toxicity symptoms and growth
Unit: mg/l
NOEC: .32

Method: other: Aerosol Exposure Test
Year: 1990
GLP: no data
Test substance: no data

Result: 1.14 mg/l aerosol concentration:
0 d: wilting: 25 - 50 % of foliage affected
2 d: wilting and tip or leaf edge burn:
95 - 100 % of foliage affected
21 d: wilting, chlorosis, leaf curl and tip of leaf edge
burn: 95 - 100 % of foliage affected
Post exposure:
first harvest: 46 % of the control value;
second harvest: 62 % of the control value.
0.32 mg/l aerosol concentration:
old and new growth developed healthy within the
post-exposure period, dry matter production at 30 and 60
days post-exposure was not affected.

Test condition: average foliar mass loading (n = 3): 8.32 +/- 4.45 ug/cm2
(diethyl malonate exposure: 0.32 mg/l air) and 193.56 +/-
63.41 ug/cm2 (diethyl malonate exposure: 1.14 mg/l air);
relative humidity: 35 %; temperature: 24 degree C (low
concentration) and 25 degree C (high concentration)

Tall fescue plants were aerosol-exposed for 60 min in a sealed exposure chamber and visual toxicity symptoms were assessed at 0, 2 and 21 d post-exposure. Residual treatment effects were assessed at 30 and 60 days post-exposure. Plant canopies were harvested and dry matter production was determined at 30 days post exposure; plants were allowed to regrow for a second harvest at 60 days post-exposure.

Reliability: (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag: Critical study for SIDS endpoint
21-OCT-2004 (11)

4.6.3 Toxicity to Soil Dwelling Organisms

Type: artificial soil
Species: Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint: mortality
Exposure period: 14 day(s)
Unit: mg/kg soil dw
LC0: > 1000
LC50: > 1000

Method: other: Directive 88/302/EEC, 1988
Year: 1994
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Test condition: temperature: 20 +/- 2 °C
pH: 5.9
light: 500 - 700 lux permanent light

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
09-AUG-2004 (52)

Type: artificial soil
Species: Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint: mortality

Method: other: Earthworm Bioassay
Year: 1990
GLP: no data
Test substance: no data

Result: Survival:

	(a)	(b)
days post-exposure 1:	30/30	30/30
7:	27/30	23/30
12:	26/30	20/30

Test condition: pH 6.5; 35 % soil moisture (related to dry weight); no further information available
Artificial soil containing 6 earthworms/25 cm² was contaminated with diethyl malonate by aerosol treatment, resulting in soil mass loading of (a) 107.5 +/- 65.0 ug/cm² and (b) 204.1 +/- 39.6 ug/cm², resp.; survival of earthworms was recorded over 12 days.

16-AUG-2004 (11)

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

Memo: Olfactory function in snails

Method: Aspects of olfactory sensitivity of the pulmonate *Helix pomatia* L. were studied by neurophysiological and behavioral methods. Different chemicals (odors) were added to a continuous air stream and single fiber recordings performed in the olfactory nerve of the posterior tentacles.

Result: Behavioral test: Diethyl malonate had no negative but a slightly positive effect, according to the authors due to the fruity odor similar to their diet. It did not influence movement in 8 of 20 snails. 7 of 20 moved towards the inlet and 5 of 20 withdrew.

Physiological experiment:
84% of the fibres were inresponsive to diethylmalonate. 12% weakly sensitive and 2 % showed medium or high sensitivity.

Test condition: Species: adult *Helix pomatia* L. collected in the field.
Acclimatization: 3 weeks, at constant temperature, 12 h light 12 h dark.
Feed: ad libitum: lettuce, carrots, cucumber, eggshells.

Neurolophysiological study:

Preparation: The snails were decapitated and the preparation placed in Ringer's solution. CNS and nerves were prepared. All nerves except the 3 pairs of lip nerves and tentacle nerves were sectioned. The preparation was fixed in a 2 chambered plexiglass device and the 2 posterior tentacles were put through holes in the separating plexiglass wall and fixed without damage of the nerve tissue. One of the tentacle nerves was placed on a microelectrode. The front chamber contained 2 tubes, one control and one for the inlet of the odors. Air could be directed through either of the tubes.

Stimuli: Ethanol, pentanol, hexanol, octanol, ethylacetate, diethyl malonate (all undiluted) and vanillin (1%aqueous solution).

Stimulation: 1. 30 seconds air without stimulus, 2. 60 s air led through one of the substances, 3 minutes air without stimulus. The order of the stimuli was changed at random. Neuronal responses (impuls frequencies of 3 independent nerve fibres) were recorded.

Behavioral studies:
Chamber: plastic, 6.5 cm wide, 4.5 cm high, open on the top, inlets for control air stream and olfactory stimulus, outlet on the opposite side. The air stream was let to the bottom of the chamber. Constant light conditions by artificial illumination.

Experiment: individual snails (total number 20) starved for 5 days were placed 24 cm from the inlet. When the tentacles were evaginated the air stream was switched from control to stimulus. The behavior was observed for 10 min. Air stream between stimuli for 3 min. Stimuli were applied at random. If the snail moved at least 5 cm towards the inlet the stimulus was judged positive, movement of at least 5 cm in the opposite direction or withdrawal into the shell was judged as an avoidance behavior.

26-AUG-2005

(96)

Remark: A concentration of 2 % diethyl malonate completely inhibited the cellulase activity in gut extracts of the termites *Termes obesus* and *Heterotermes indicola*.

(69)

Remark: Growth and proteolytic activity of the fungus *Ctenomyces* were inhibited by the addition of 1.5 - 2 % diethyl malonate. The proteolytic activity was determined by measuring the degradation of woollen fabric by the loss in weight of the fabric.

(2)

Remark: The addition of 13 mmol diethyl malonate/l to a 3 h old culture of *Bacillus cereus* decreased the sporulation yield to 0.1 % of the control value.

(40)

Remark: Ecological dose causing 50 % inhibition of soil dehydrogenase activity in silt loam and sandy loam:
EcD50 ca. 2.5 mg/g dry soil after 3 days of incubation
and > 2.5 mg/g dry soil after 28 days of incubation of soil samples with diethyl malonate at 22 degree C in the dark.

Test substance: 99 % purity

(11)

Remark: Ecological dose causing 50 % inhibition of soil acid phosphatase activity in silt loam and sandy loam:
EcD50 > 2.5 mg/g dry soil after 3 days and 28 days of incubation of soil samples with diethyl malonate at 22 degree C in the dark.

Test substance: 99 % purity

5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo: In vivo
Type: Metabolism
Species: rat
Doses, males: 5 µl/0.5 mCi
Doses, females: 5 µl/0.5 mCi
Route of administration: other: intracerebral injection

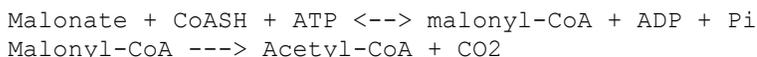
Deg. product: yes

Method: other
Year: 1978
GLP: no data

Test substance: other TS: C1 or C2 14-C-radiolabeled malonic acid, specific activity 12 mCi and 42 mCi respectively

Result: The authors verified that the decarboxylation of malonic acid to acetyl-CoA by various mammalian tissues also occurs in vivo after intracerebral injection. A rapid reflux of unreacted malonic acid in venous blood was reported. Labeled 14CO₂ was recovered from venous blood and the expired air after administration of C-1 labeled product, but not after C-2 labeled product. High radioactivity was present in glutamate, aspartate and GABA. Sequential degradation of glutamate and aspartate proved that labeling of these amino acids occurred from [1-14C]acetyl-CoA and [2-14C]acetyl-CoA respectively via the Krebs-cycle. Malonate activation and decarboxylation were similar to in vitro experiments with isolated mitochondria from different tissues. In vitro the radiolabel was however not incorporated into amino acids. In the in vivo experiment a minor amount of radioactivity was also incorporated in brain lipids.

The authors conclude that malonic acid is metabolised via the following route:



In vitro:
 Acetyl-CoA → acetate + CoASH

In vivo: Acetyl-CoA enters the Krebs cycle and is used for the formation of aspartate, glutamate and GABA. A minor amount may also be incorporated into lipids.

Test condition: Intracerebral injection of either C1- or C2- 14C- radiolabeled malonic acid to anesthetized adult male and female rats. The rats were killed after 2, 5, 10, 15 or 30 min and the brains removed, weighed, homogenized and analysed for radiolabeled reaction products. Venous blood and expired air was also analysed for radioactivity.

Reliability: (2) valid with restrictions
 Well documented scientific reference

Flag: Critical study for SIDS endpoint
 16-AUG-2005

(58)

In Vitro/in vivo: In vitro

Type: Absorption
Species: human
Doses, males: 4 µl/0.8 cm² of skin
Vehicle: other: undiluted
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other: in vitro flow through skin penetration test
Year: 1999
GLP: no data
Test substance: other TS

Result: After 24 h 16% of the applied dose (642.06 +-23.43 µg) had penetrated through the skin. The maximum flux rate was obtained after 5 hr: 280 µg/h (350µg/cm²/h). The majority evaporated from the skin (45-50%), while the residual part (34-39%) rested on the skin. The mean penetration rate was 99.21 +- 16.46 µg/h (120 µg/cm²/h).

When skin was washed longer than 1 h after treatment, remnant diethylmalonate on the skin penetrated at an accelerated rate due to skin hydration.

Test condition: Skin samples:
Human cadaver skin, male Chinese, 60 to 80 years of age. Split thickness skin, 600 µm thickness (epidermis and uppermost layer of dermis). After removal skin samples were transported on ice/salt, circular pieces of 20 mm diameter were prepared and stored at -30 °C for up to 1 month. Before use the samples were thawed rapidly in a 37°C water bath.

Diffusion experiment:
Flow through cell, 10 mm diameter, 4 mm height. Total diffusion volume 0.32 cm³.
Surface area of exposed skin: 0.8 cm².
Perfusion: continuously, steady flow of 8 ml/h with 0.9% saline.
The small receptacle volume and relatively high perfusion rate created a sink condition beneath the skin layer to avoid a diffusion lag.

A small constant air flow was maintained above the surface to maintain non-occlusive condition.

Temperature: 32 °C
The outer chamber with the skin surface was sealed and covered with a tenax tube to collect evaporating test substance.
Sampling of receptor fluid: every 2 h.
Duration: 24 h
Volume of application: 4 µl of neat test substance.
Extraction of amounts in skin: with 10 ml of ethanol for 2 h.
Analysis: GC-FID.

Additional experiment:
influence of decontamination with different solutions at 0.25, 0.5, 1 h post exposure.
Test substance: Diethylmalonate, 99%, Merck, Singapore
Reliability: (2) valid with restrictions

Flag: well documented scientific literature
Critical study for SIDS endpoint
20-JUL-2005 (63)

In Vitro/in vivo: In vivo
Type: Absorption
Species: other: pig, hairless dogs, mice, human and pig
skin grafted on mice
Route of administration: dermal

Result: The percutaneous penetration was estimated from the recovery of radioactivity in the urine and feces and corrected for incompleteness of excretion by the recovery of radioactivity in urine and feces recovered from parenteral administration (percentage of topically applied radioactive dose):
human skin grafted athymic nude mouse: 4 % +- 2%
pig skin grafted athymic nude mouse: 6 % +- 1%
athymic nude mouse: 15 % +- 2%
weanling pig: 2.5 % +- 0.2%
hairless dog: 4 % +- 2%

Test condition: The percutaneous penetration of radiolabelled diethyl malonate was assayed in different animal models by nonocclusive dermal application of 0.1 mg diethyl [2-14C]malonate/cm² for 24 (mouse) or 48 h (dog, pig) with subsequent decontamination of the skin surface with ethanol.

Skin area: mice (clipped), human and pig skin grafted on mice: 1.27 cm², pigs (clipped) and hairless dogs: 25 cm².
Patch: mice: non occlusive protective patch.
Patch pigs, dogs: non-occlusive, replaced after 24 h.
Animals were housed individually in metabolic cages.
Urine, skin, subcutaneous fat, liver, kidney and spleen were analysed for radioactivity.

Test substance: Diethylmalonate, Sigma chemical Co., St. Louis Mo., no further data. Diethyl [2-14C]malonate (specific activity 3 mCi/mmol), Amersham corporation, Arlington Heights, III.

Reliability: (2) valid with restrictions
well documented scientific literature

Flag: Critical study for SIDS endpoint
20-JUL-2005 (83)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig

Result: Percentage of applied radioactive dose appearing in the acceptor cell over 50 h:
dose A: application of 100 ug diethyl [2-14C]malonate/cm²:
3 % +- 1%
dose B: application of 100 ug diethyl [2-14C]malonate diluted in ethanol/cm² (12.5 mg/ml): 6 % +- 3%
dose C: application of 4 ug diethyl [2-14C]malonate diluted in ethanol/cm² (0.5 mg/ml): 10 % +- 3%
Percentage of radioactivity remaining in the skin after 50 h:
dose A: 8.8 % +- 0.5%
dose B: 13 % +- 2%
dose C: 30 % +- 10%

25 - 50 % of the applied radioactivity was lost due to evaporation.

Test condition: The percutaneous penetration of radiolabelled diethyl malonate was assayed in a diffusion cell with freshly prepared skin of weanling Yorkshire pigs.

Skin sample: 1.8 cm diameter, thickness: 1.9 mm (full thickness skin, subcutaneous fat removed).
Receptor fluid: tyrodes solution.

Temperature acceptor cell: 37 degree C;
Acceptor cell fluid flow rate: 5 ml/h; donor cell: 24 degree C

Test substance: Diethylmalonate, Sigma chemical Co., St. Louis Mo., purity > 97%. Diethyl [2-14C]malonate (specific activity 3 mCi/mmol), Amersham corporation, Arlington Heights, III. Radiochemical purity >= 95%.

Reliability: (2) valid with restrictions
well documented scientific literature

Flag: Critical study for SIDS endpoint
20-JUL-2005 (42)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Result: Total recovery of radiolabel ranged from 50 to 80%. Some radiolabel was lost due to volatilisation.

Percentage of the applied radioactive dose recovered in the acceptor cell, in the skin and on the skin surface over 24 h:
diethyl malonate in the acceptor cell: 0.2 - 1.6 %;
diethyl malonate in the skin: 0.2 - 0.9 %;
diethyl malonate in the skin surface: 0.6 - 0.7 %.
The skin mediated hydrolysis of radiolabelled diethyl malonate to monoethyl malonate and malonic acid amounted to 15 - 35 % of the applied radioactivity dose, corrected for hydrolysis products in the starting solution.
Percentage of hydrolysis products recovered in the acceptor cell: 20 - 21 % of the applied dose; percentage of hydrolysis products recovered in the skin: 3-5% and on the skin surface: 2 - 4 % of the applied dose.
The maximum penetration rate of hydrolysis products was reached after 5 h and amounted to ca. 2 % of the applied dose/h.

Heat treated skin: DEM in receptor fluid:
24-42 % of applied dose
in skin: 0.1-2.3%
on skin surface: 0.2 - 1%
Hydrolysis products:
In receptor fluid: 2-6%
in skin: 2-4%
on skin surface: 2-3%

No indication of the ratio of the hydrolysis products in the receptor fluid is given. A varying amount of hydrolysis products could also be detected in the receptor fluid controls (1.2 to 16%).

Preincubation of skin samples for 5 min in an 80 degree C water bath increased the penetration rate of diethyl malonate and decreased the amount and penetration rate of hydrolysis products.
The total recovery of radioactivity amounted to 50 - 80 % of the applied dose.

Test condition: The percutaneous penetration of radiolabelled diethyl malonate was assayed in a diffusion cell with freshly prepared skin of weanling Yorkshire pigs. Skin samples of 1 mm thickness were incubated with 1 mg diethyl [2-14C]-malonate in acetone.

Skin: clipped split thickness skin (containing epidermis and part of the dermis).
Receptor medium: oxygenated solution of Rose Park Memorial Insitute (RPMI) media 1640 formula 78-5117, Gibco, NY.
Application: 1 mg/cm² in 10 micro-l of acetone.
Skin area: 0.8 cm²
Temperature: 37 degree C; acceptor cell fluid flow rate: 5 ml/h; acceptor cell fluid collection: hourly between hours 1 and 12, 23 and 24, bihourly from hours 12 to 22
Analysis of hydrolysis products: TLC and scintillation counting of the areas with Rf-values corresponding to the respective control substances of the hydrolysis products. To determine possible hydrolysis in the acceptor fluid controls were prepared by adding aliquots of 10 micro-l of the test compund to the receptor fluid. These solutions were also analysed for the hydrolysis products.

Test substance: Diethylmalonate, Aldrich chemical Co., Milwaukee, Wi.,purity > 98%. Diethyl [2-14C]malonate (specific acitivity 14 mCi/mmol), Amersham corporation, Arlington Heights, LI. Radiochemical purity >= 98%.

Reliability: (2) valid with restrictions
well documented scientific literature

Flag: Critical study for SIDS endpoint

20-JUL-2005 (13)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig

Result: Percentage of applied radioactive dose appearing in the acceptor cell over 15 min: 0.09 % +- 0.03%;
radioactivity remaining in the skin: 2.4 %+-0.7%;
radioactivity recovered from the skin surface by scrubbing and rinsing with 1 % aqueous surfactant solution: 13.8 % +- 2.0%.
evaporation loss accounted for 63.1 % +- 3.0% of the applied dose.
Decontamination assay: The skin was rinsed twice for 1 s with water after 15 min of incubation with radiolabelled diethyl malonate. The decontamination water contained 13.6 % +- 3.2%
of the applied dose and the amount of radioactivity on the skin surface decreased to 2.3 % +- 0.5%. Penetration was essentially unaltered (0.1 +- 0.05%), scrub: 2.1 +- 0.8%. Application of thickened diethyl [2-14C]malonate (200 centistokes) did not alter the radioactivity distribution.

The overall recovery of the radioactive dose in these experiments was ca. 70 %.

Test condition: The percutaneous penetration of radiolabelled diethyl malonate was assayed in a diffusion cell with skin of weanling Yorkshire pigs. The skin was stored frozen for approximately 1 month and was subsequently incubated with 0.1 mg diethyl [2-14C]malonate/cm² for 15 min.
Temperatures:
acceptor cell: 37 degree C; skin surface temperature: 25 - 27 degree C
Skin area: 2.85 cm².

Reliability: (3) invalid
Study not suitable for evaluation of dermal absorption. Contact time too short. The purpose of the study was to investigate decontamination agents with malonates as non-toxic model substance.

21-OCT-2004 (84)

In Vitro/in vivo: In vivo
Type: Metabolism

Remark: DEM is likely to be metabolized by unspecific (serine-) esterases of different tissues, in particular in the liver to the mono esters and finally to malonic acid and the corresponding alcohols, methanol and ethanol respectively. This is corroborated by the findings of the abiotic hydrolysis, in particular at alkaline pH that can be regarded as qualitatively similar to the hydrolysis catalyzed by unspecific esterases (Jacobi and Hoffmann, 1989). The hydrolysis products are likely to be metabolized via physiological pathways as the tricarboxylic acid cycle because they are part of the normal intermediate metabolism (WHO, 2000).

20-JUL-2005 (55) (97)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Value: = 15794 mg/kg bw

Method: other: as described by Smyth et al., Amer. Ind. Hyg. Assoc. J. 23, 95-107
Year: 1962
GLP: no
Test substance: no data

Reliability: (4) not assignable
Details lacking.

Flag: Material Safety Dataset, Critical study for SIDS endpoint

14-JAN-2005 (87)

5.1.2 Acute Inhalation Toxicity

Species: rat

Method: other: as described by Smyth et al., Amer. Ind. Hyg. Assoc. J. 23, 95-107

Year: 1962

GLP: no

Test substance: no data

Remark: concentrated vapour inhalation; maximal inhalation period for no death: 8 h

Reliability: (4) not assignable
Details lacking.

Flag: Material Safety Dataset, Critical study for SIDS endpoint
14-JAN-2005 (87)

5.1.3 Acute Dermal Toxicity

Type: LD50

Species: rabbit

Value: > 16960 mg/kg bw

Method: other: According to 24-hour cuff method of Draize et al.

Year: 1944

GLP: no

Test substance: no data

Remark: contact period: 24 h

Reliability: (4) not assignable
Details lacking.

Flag: Material Safety Dataset, Critical study for SIDS endpoint
14-JAN-2005 (87)

5.1.4 Acute Toxicity, other Routes

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit

Result: not irritating

Method: other: as described by Smyth et al., Amer. Ind. Hyg. Assoc. J. 23, 95-107

Year: 1962

GLP: no

Test substance: no data

Remark: uncovered application of 0.01 ml diethyl malonate on the rabbit belly; injury grade 2 of 10 (grade 1 indicates no irritation and grade 2 the least visible capillary injection from the undiluted chemical, grade 6 indicates necrosis when undiluted and grade 10 indicates necrosis from a 0.01% solution);
not classifiable according to current EEC directives

Result: Irritation grade 2 of 10. No further information.

Reliability: (4) not assignable
Details lacking.

14-JAN-2005

(87)

Species: rabbit
Concentration: undiluted
Exposure: Occlusive
Exposure Time: 24 hour(s)
Result: slightly irritating
EC classificat.: not irritating

Method: other: no data
Year: 1976
GLP: no
Test substance: other TS: no data

Reliability: (2) valid with restrictions
Flag: Material Safety Dataset, Critical study for SIDS endpoint
Species: guinea pig

Method: other: no data
Year: 1976
GLP: no
Test substance: other TS: no data

Result: At doses of 10 ml/kg no skin irritation.

(33)

5.2.2 Eye Irritation

Species: rabbit
Result: highly irritating

Method: other: as described by Smyth et al., Amer. Ind. Hyg. Assoc. J. 23, 95-107
Year: 1962
GLP: no
Test substance: no data

Remark: Scoring system: grade 1 indicates a very small area of necrosis resulting from 0.5 ml of undiluted chemical in the eye, grade 5 indicates a so-called severe burn from 0.005 ml and grade 10 indicates a severe burn from 0.5 ml of a 1% solution in water or propylene glycol (Smyth et al. Range finding toxicity data: List VI, Amer. Ind. Hyg. J. 23: 95 (1962). The scoring system is not comparable to the Draize score.

The result is in contradiction to other studies. As no details of the test substance and the procedure are available the relevance of the finding is doubtful.

Result: application of 0.005 - 0.5 ml diethyl malonate to the centre of the cornea; examination after 18 - 24 h; injury grade 5 of 10.

Reliability: (2) valid with restrictions
Well documented scientific literature, but details lacking.

01-DEC-2004

(87)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 504 hour(s)

Comment: not rinsed
No. of Animals: 6
Result: slightly irritating
EC classificat.: not irritating

Method: other: according to U.S.A. Environmental Protection Agency Guidelines
Year: 1989
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: AVERAGE SCORE (24, 48, 72 hours)
- Cornea: 0.6
- Iris: 0.8
- Conjunctivae (Redness): 1.7
- Conjunctivae (Chemosis): 1.3
- Overall irritation score: (Draize score) 25.8 of 110

DESCRIPTION OF LESIONS:

1 h p.a.: dulling of the corneal surface in 4 animals, sloughing of corneal epithelium in 1 animal. Slight iriditis was observed in 2 animals. Minimal to moderate conjunctival redness and chemosis in all animals. 5 animals showed slight conjunctival discharge.

24 h p.a.: Dulling of the cornea was seen in 5 animals. Diffuse corneal opacity was observed in one animal. Slight iriditis was observed in all animals. Minimal to moderate conjunctival redness and chemosis was observed in all treated eyes. Minimal to moderate discharge was seen in five animals.

48 h p.a.: Diffuse or translucent corneal opacity was observed in 5 animals. Slight iriditis was observed in all animals. Minimal to moderate conjunctival redness and chemosis was observed in all treated eyes. Minimal to moderate discharge was seen in 2 animals, severe discharge in 1 animal.

72 h p.a.: Diffuse or translucent corneal opacity was observed in 3 animals. Slight iriditis was observed in all animals. Minimal to moderate conjunctival redness and chemosis was observed in all treated eyes. Minimal to moderate discharge was seen in 2 animals.

day 7: Translucent corneal opacity was observed in one treated eye, Iriditis was observed in one animal, minimal to moderate conjunctival redness in 2 animals.

14 d p.a.: Minimal conjunctival redness was observed in one treated eye.

REVERSIBILITY: All effects were reversible within 21 days.

Test condition: OTHER EFFECTS: No other effects were reported.
TEST ANIMALS: Rabbits
- Strain: New Zealand White
- Sex: male and female
- Source: David Percival Ltd. U.K.
- Age: 12 to 16 weeks
- Weight at study initiation: 2.54 to 3.12 kg
- Number of animals: 6
- Controls: second eye

ADMINISTRATION/EXPOSURE

- Preparation of test substance: used undiluted
- Amount of substance instilled: 0.1 ml
- Vehicle: none

EXAMINATIONS

- Ophthalmoscopic examination: standard ophthalmoscope
- Scoring system: Draize
- Observation period: 21 days

Reliability: (1) valid without restriction
Flag: Material Safety Dataset, Critical study for SIDS endpoint
01-DEC-2004 (46)

5.3 Sensitization

Type: other: human maximisation test
Species: human

Method: other: no data
Year: 1972

Result: A maximisation test according to Kligman, 1966 and Kligman and Eppstein, 1975 was reported in 23 volunteers. 4% of the test substance in petrolatum produced no sensitization reactions.

Reliability: (4) not assignable
Original publication not available
Flag: Critical study for SIDS endpoint
20-JUL-2005 (32)

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: Charles River CD
Route of administration: oral feed
Exposure period: 90 d
Frequency of treatment: daily
Doses: males: 35.93 mg/kg b.w./d; females: 41.14 mg/kg b.w./d
Control Group: yes, concurrent no treatment

Method: other: as described by author
Year: 1967
GLP: no
Test substance: other TS: purity > 90%

Remark: No substance-related differences were found between control and test rats with respect to growth, food intake, haematological and clinical chemistry parameters, blood-urea level, organ weights or organ pathology;

Test condition: TEST ORGANISMS
- Number of animals: 10 to 16 males and females
ADMINISTRATION / EXPOSURE
- Duration of test/exposure: 90 days
- Type of exposure: dietary, the test material was incorporated into the diet either as 16.7 % emulsion in gummi arabicum, as 16.7 % adsorbate on microcrystalline cellulose or as 10% solution in ethanol or peanut oil. (Not specified). The dietary levels were adjusted during the study so that animals received on a mg/kg per day basis a level in excess of 100-fold the maximum estimated dietary intake of humans from flavoring agents. This level was exceeded by 10 to 40% during

the study.
- Doses: 35.93 mg/kg bw for males, 41.14 mg/kg bw for females (average doses)

CLINICAL OBSERVATIONS AND FREQUENCY:

Body weight and food intake were recorded weekly.
Haematological evaluations and blood urea determination were performed on 50% of the animals in week 7 and on 50% at termination (week 13).

At autopsy liver and kidneys were weighed and histopathological examination was performed on a wide range of organs (not further specified).

Reliability:

(2) valid with restrictions
Scientific study, but methodological deficiency, one dose only tested. Used as corroborative evidence.

Flag:

Critical study for SIDS endpoint

01-DEC-2004

(82)

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: gavage
Exposure period: 39 to 51 days (from 14 days before mating to day 3 of lactation)
Frequency of treatment: daily, 7 days per week
Post exposure period: 14 days
Doses: 0, 100, 300, 1000 mg/kg bw per day
Control Group: yes, concurrent vehicle
NOAEL: = 300 mg/kg bw
LOAEL: = 1000 mg/kg bw

Method: OECD combined study TG422
Year: 2004
GLP: yes
Test substance: other TS: Dimethyl malonate

Method: OECD Combined Repeated Dose and Reproduction/Developmental Screening Test.

Result: Mortality: No mortality was observed in any of the dose groups.

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

- Clinical signs:
No test item related clinical signs were observed throughout the test and recovery period in any of the dose groups.
- FOB:
No treatment related changes were observed.
- Body weight gain:
No treatment related effects on body weight and body weight gain were observed.
- Food consumption:
No treatment related effects were observed.
- Clinical chemistry:
No treatment related effects were observed.
- Haematology:
No treatment related changes were observed.
- Organ weights:
No treatment related effects were observed.
- Gross pathology:
No treatment related effects were observed.

Test condition:

- Histopathology:

1000 mg/kg bw: Livers of males and females showed a significantly increased incidence of hepatocellular hypertrophy. The change was considered reversible as the incidence was not significantly increased in the high dose recovery animals.

300 and 100 mg/kg bw: No treatment related changes of the liver were observed.

All other histopathological findings were not considered treatment related.

STATISTICAL RESULTS: Significantly increased hepatocellular hypertrophy in the high dose group only.

TEST ORGANISMS

- HSDCpb-WU rats

- Age at start of treatment: 11-12 weeks

- Weight at study initiation: males: 377-379 g, females: 210-219 g

- Number of animals: Main group: 10 m, 10 f, recovery groups: 5 m, 5 f

ADMINISTRATION / EXPOSURE

- Duration of test/exposure:

Males: Test groups: 39 days, male recovery group: 39 exposure days, 45 test days.

Females: Treatment groups: 51 +- 7 days, recovery group: 39 exposure days, 45 test days.

- Type of exposure: Oral gavage

- Post exposure period: 14 days

- Vehicle: Double distilled water

- Concentration in vehicle: 10 mg/ml, 30 mg/ml, 100 mg/ml

- Total volume applied: 10 ml/kg bw

- Doses: 100, 300, 1000 mg/kg bw

Treatment:

Male rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period and approximately 2 weeks post mating.

Female rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period, pregnancy and up to lactation day 4.

For the high dose recovery group animals (males and females) the test item was administered once daily, 7 days per week by gavage throughout the treatment period.

CLINICAL OBSERVATIONS AND FREQUENCY:

- Daily observations for appearance, behaviour, clinical signs and preterminal deaths. Females were observed for signs of difficult and prolonged parturition.

- Twice daily: morbidity and mortality.

- Detailed clinical observations: once before exposure and at least once per week thereafter. Signs included were changes in skin, fur, eyes, mucous membranes, occurrence of secretions or excretions and autonomic activity, changes in gait, posture, response to handling, behavioural changes, difficult or prolonged parturition.

Functional observation battery (FOB): Examinations were performed in randomly selected 5 animals of each group at the end of the dosing period for males and during the lactation period for females and included homecage observations, handling observations, open field tests, sensory observations, neuromuscular observations and physiological observations (body temperature).

Body weights were recorded at the beginning of the study, at least weekly thereafter and at termination. All dams were weighed on gestation days 0, 7, 14 and 20 and lactation days 0 and 4.

Food consumption was recorded weekly.

The fertility index for males and females was determined.

LITTER DATA: All pups from each litter were examined for any external deformities, litter size and sex distribution was determined. Pup weights were recorded on day 0 and 4. All pups were examined for malformations and subject to gross pathological examination. Pup survival index up to lactation day 4 was determined.

HAEMATOLOGY/CLINICAL CHEMISTRY: Standard haematological and clinical chemistry parameters were determined at the end of the pre-mating period and the recovery period in 5 randomly selected males and females of each group.

ORGAN WEIGHTS: Organ weights of liver, adrenals, kidneys, thymus spleen, brain and heart were determined of 5 males and females of each group. Testes and epididymis weights of all adult males of each group were also determined.

GROSS PATHOLOGY.

All adult animals and pups were examined for any structural abnormalities and pathological changes.

HISTOPATHOLOGY:

The following tissues of 5 males and females of the control and high dose group as well as all animals of the recovery and recovery control groups were examined microscopically: all gross lesions, brain, spinal cord, gastrointestinal tract, liver, kidney, adrenals, spleen, heart, thymus, thyroid, trachea, lungs, testes (fixed in Bouins fluid), epididymes (fixed in Bouins fluid), ovaries, uterus, seminal vesicles, coagulating glands, prostate, urinary bladder, axillary lymph nodes, mesenteric lymph nodes, sciatic nerve, femur with marrow, bone marrow smear. Stages of spermatogenesis and interstitial testicular structure in male gonads were determined additionally. Livers of 5 males and females in the mid and low dose groups and testes of 5 males of the mid and low dose groups were also examined.

STATISTICAL ANALYSIS:

Dunnett's t-Test: body weight, body weight change, food intake, haematology, clinical chemistry, organ weight, FOB, gestation length, litter size, No. corpora lutea, No.

implantation.

Z-Test/Student's t-Test: Mating performance, conception rate, fertility index, gestation index, live birth index, viability index, sex ratio, pup survival data, No. littered, No. dead pups, No. live pups, Pup survival data, Pre- and Post-implantation loss. Histopathological data.

t-Test/ANOVA: dose correlation

Test substance:

Dimethyl malonate, purity: 99.8%.

Reliability:

(1) valid without restriction

Guideline study, GLP

Flag:

Material Safety Dataset, Critical study for SIDS endpoint

11-AUG-2004

(19)

5.5 Genetic Toxicity 'in Vitro'

Type: Cytogenetic assay
System of testing: Human peripheral lymphocytes
Concentration: 312.5, 625, 1250, 2500, 5000 µg/ml medium
Cytotoxic Concentration: 5000 µg/ml
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 473
Year: 2003
GLP: yes
Test substance: other TS: Dimethyl malonate

Result: GENOTOXIC EFFECTS:
- With metabolic activation:
The mean incidence of chromosomal aberrations excluding gaps at concentrations from 625 to 5000 µg/ml ranged from 1.5% to 3.5% and was comparable to control rates and within the historical control range of 0 to 5%. There was no dose related increase in chromosomal aberrations. No polyploidy was noted.
- Without metabolic activation: The mean incidence of chromosomal aberrations excluding gaps at concentrations from 625 to 5000 µg/ml ranged from 1.0% to 3% and was comparable to control rates and within the historical control range of 0 to 5%. There was no dose related increase in chromosomal aberrations. No polyploidy was noted.
All positive and negative controls gave the expected results that were within the ranges of the laboratory and consistent with those reported in the literature.

MITOTIC INDEX:
Pretest:
Without S9 mix, 24 h exposure:
At concentrations up to 250 µg/ml \geq 1.
1000 µg/ml: 0.44
2500 µg/ml: 0.72
5000 µg/ml: 0
With S9, 4 h exposure:
At concentrations up to 1000 µg/ml: 0.72 to 1.0 (not concentration dependent)
2500 µg/ml: 0.44
5000 µg/ml: 0
Main experiment:
Without S9, 4 h exposure: not significantly reduced (0.88 to 1.0)
Without S9, 24 h exposure: \geq 1 up to 625 µg/ml.
1250 µg/ml: 0.79
2500 µg/ml: 0.54
With S9:
Not significantly reduced up to 1250 µg/ml: (0.92 to 1.22)
2500 µg/ml 0.72 and 0.87
5000 µg/ml: 0 in both experiments
CYTOTOXIC CONCENTRATION:
- With metabolic activation: Pretest and main test: 5000 µg/ml
- Without metabolic activation: Pretest and main test: 5000 µg/ml after 24 h.

STATISTICAL RESULTS: The incidence of chromosomal aberrations (excluding gaps) was not significantly different from the controls with and without metabolic activations at all tested dose levels using Fisher exact test ($P \leq 0.05$). The positive controls induced a statistically significant increase in chromosomal aberrations excluding gaps.

Test condition:

Cell culture:

Human peripheral blood was obtained by venipuncture from healthy donors without medication and collected in heparinised vessels. 0.5 ml samples of whole blood were added to tubes containing 5 ml of complete culture medium and incubated at 37 °C with occasional shaking.

Solvent: DMSO

Negative control: Solvent: DMSO

Positive control: Mitomycin C in the absence of metabolic activation (0.1 and 0.2 µg/ml medium), cyclophosphamide in the presence of metabolic activation (10 to 20 µg/ml medium).

Metabolic activation system: Postmitochondrial (S9) fraction of rats treated with Arochlor 1254

Preliminary cytotoxicity test:

With concentrations from 10 to 5000 µg/ml medium with and without metabolic activation, 48 h after culture establishment, 24 h incubation. Examination of 1 slide per culture, 1000 lymphocytes per culture. Calculation of mitotic index.

Main study:

Experiment 1:

The test item or test item plus S9 mix was added to the cultures after 48 h of culture and incubated for 4 h at 37 °C.

After centrifugation and washing the resuspended cell pellet was incubated for further 20 h in the dark. Colcemid was added to arrest cell division and the cells incubated for a further 2 h. The cells were harvested, fixed in freshly prepared methanol: glacial acetic acid (4: 1) and slides prepared.

Experiment 2: With S9: same procedure as experiment 1.

Without S9:

In the absence of S9 a continuous treatment for 24 h was performed. After centrifugation and washing the resuspended cell pellet was incubated for further 20 h in the dark. Colcemid was added to arrest cell division and the cells incubated for a further 2 h. The cells were harvested, fixed in freshly prepared methanol: glacial acetic acid (4: 1) and slides prepared.

All cultures were run in duplicate using blood from a different donor.

Stain: Giemsa

For each treatment and culture 100 metaphases per plate were examined.

For the determination of cytotoxicity 1000 cells were scored and the mitotic index determined as percentage of cells in metaphase.

Statistical evaluation:

Fisher Exact Test.

Test substance:

Dimethyl malonate, purity: 99.8%.

Reliability:

(1) valid without restriction

Flag:

Material Safety Dataset, Critical study for SIDS endpoint

17-AUG-2004

(23)

Type: Ames test
System of testing: Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentration: 481 µg/plate (maximal concentration)
Metabolic activation: with and without
Result: negative

Method: other: According to Ames et al., Mut. Res. 31(6), 347-364
Year: 1975
GLP: no data
Test substance: no data

Remark: solvent: ethanol
Type: Ames test
System of testing: Salmonella typhimurium TA 97, TA 98, TA 100
Concentration: 5000 µg/plate (maximal concentration)
Cytotoxic Concentration: > 5000 µg/plate
Metabolic activation: with and without
Result: negative

Method: other: no data
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: GENOTOXIC EFFECTS:
- With metabolic activation: negative
- Without metabolic activation: negative
Up to 5000 µg/plate increase of revertants per plate that exceeded the control rate of revertants by a factor of 2 or more was observed in any of the strains tested with and without metabolic activation.
All positive and negative controls gave the expected results that were within the ranges of the laboratory and consistent with those reported in the literature.

CYTOTOXIC CONCENTRATION:
- With metabolic activation: > 5000 µg/plate
- Without metabolic activation: > 5000 µg/plate
No cytotoxicity was observed up to the highest concentration level.

Test condition: SYSTEM OF TESTING
- Species/cell type: Salmonella typhimurium TA 97, TA 98, TA 100
- Metabolic activation system: Aroclor-1254 induced male Wistar rat liver post-mitochondrial fraction (S-9)
Preexperiment for toxicity in S. Typhimurium TA 100.
Solvent: Dimethylsulfoxide (DMSO)
- Number of replicates: One experiment 3 replicates per concentration and strain.
- Application:
- Positive and negative control groups:
Positive controls:
Without S9:
TA98: 2-Nitrofluorene (50 µg/plate)
TA100: Sodium azide (2 µg/plate)
TA97: 9-Aminoacridine (50 µg/plate)
With S9:
All strains: 2-Aminoanthracene (5 µg/plate)
Negative control: solvent: DMSO
CRITERIA FOR EVALUATING RESULTS:
A two-fold increase in revertants compared to concurrent negative controls indicates a positive response.

STATISTICAL METHODS: If two-fold increase is reached or exceeded: Analysis of variance (F-Test) and regression analysis.

Reliability: (2) valid with restrictions
3 strains only tested
Flag: Critical study for SIDS endpoint

23-JUL-2004

(44)

Type: Escherichia coli reverse mutation assay
System of testing: Escherichia coli Sd-4-73
Metabolic activation: without
Result: negative
Method: other: Paper disk method with streptomycin-dependent E. coli
Year: 1958
GLP: no
Test substance: no data

(92)

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA 100, TA 1535, TA 1537, TA 1538
Concentration: 5000 µg/plate (maximal concentration)
Cytotoxic Concentration: > 5000 µg/plate
Metabolic activation: with and without
Result: negative

Method: Directive 84/449/EEC, B.14
Year: 1984
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: GENOTOXIC EFFECTS:
- With and without metabolic activation: No increase in the number of revertants per plate compared to controls was observed in any of the tested strains and concentrations. All positive and negative controls gave the expected results that were within the ranges of the laboratory and consistent with those reported in the literature.

CYTOTOXIC CONCENTRATION:

- With metabolic activation: > 5000 µg/plate
- Without metabolic activation: > 5000 µg/plate
No cytotoxicity was observed up to the maximum test concentration of 5000 µg/plate.

Test condition: SYSTEM OF TESTING
- Species/cell type: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
- Metabolic activation system: phenobarbiturate induced rat liver S9 fraction
- Solvent: DMSO
- Number of replicates: 2 main studies, one plate incorporation, one pre-incubation test. Three replicates per experiment.
- Concentrations tested: 8, 40, 200, 1000, 5000 µg/plate
- Positive and negative controls:
- Negative control: solvent DMSO
- Positive control:
without S9 mix:

- TA 98, 1538: Nitrofluorene (111-304 µg/plate)
 - TA 100, 1535 Sodium azide (295-372 µg/plate)
 - TA 1537: Aminocridine (78-194 µg/plate)
 With S9 mix:
 Cyclophosphamide (93-114 µg/plate)
 - Pre-incubation time: 30 min at 37 °C
 STATISTICAL METHODS: Mean values and standard deviations by Biosys software.

Reliability: (1) valid without restriction
Flag: Material Safety Dataset, Critical study for SIDS endpoint
 23-JUL-2004 (50)

5.6 Genetic Toxicity 'in Vivo'

5.7 Carcinogenicity

5.8.1 Toxicity to Fertility

Type: other: OECD Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Screening Test
Species: rat
Sex: male/female
Strain: Wistar
Route of administration: gavage
Exposure Period: 39 to 51 days (from 14 days before mating to day 3 of lactation)
Frequency of treatment: daily, 7 days per week
Premating Exposure Period
 male: 2 weeks
 female: 2 weeks
Duration of test: Males: 39 days, females 51 +- 7 days, recovery groups: 39 days
Doses: 100, 300, 1000 mg/kg bw
Control Group: yes, concurrent vehicle
NOAEL Parental: = 300 mg/kg bw
NOAEL F1 Offspring: > 1000 mg/kg bw
Result: No treatment related effects on fertility

Method: OECD combined repeated dose and reproductive/developmental toxicity screening test
Year: 2004
GLP: yes
Test substance: other TS: Dimethyl malonate

Method: OECD Combined Repeated Dose and Reproduction/Developmental Screening Test.
Result: For effects on parent animals: See section 5.4, repeated dose toxicity.
 Reproductive results
 - Fertility index:
 Males, all dose groups: 100%
 Females, all dose groups: 100%
 - Duration of gestation:

Dose (mg/kg bw)	Duration (days) +- SD
0	22 +- 0.3
100	23 +- 0.5
300	23 +- 0.5

1000 22 +- 0.4

- Gestation index:
100 % in all dose groups.

- Parturition: 100 % in all dose groups

- Effects on sperm: No treatment related effects

- Number of implantations:

Dose (mg/kg bw)	No.	Percent
0	12.3	88.1
100	11.8	84.7
300	12.0	88.9
1000	11.6	88.6

- Number of corpora lutea:

Dose (mg/kg bw)	No.
0	14.0
100	13.9
300	13.5
1000	13.1

Percentage pre-implantation loss

Dose (mg/kg bw)	Percent
0	11.9
100	15.3
300	11.1
1000	11.4

Percentage post-implantation loss

Dose (mg/kg bw)	Percent
0	8.1
100	19.1
300	10.4
1000	15.1

Litter results:

- Number of pups born

Dose (mg/kg bw)	No.
0	103
100	76
300	86
1000	84

No of live litters

Dose (mg/kg bw)	No.
0	9
100	8
300	8
1000	8

Mean litter size index

Dose (mg/kg bw):	
0	11.4
100	9.5
300	10.8
1000	10.5

Mean viable litter size:

Dose (mg/kg bw):	
0	11.3
100	9.5
300	10.8
1000	9.9

No. of pups alive on day 0

Dose (mg/kg bw)	No.
0	102
100	76
300	86

1000 79
Live birth index:
Dose (mg/kg bw)
0 99
100 100
300 100
1000 94
Sex ratio at birth (no of males/total number born x 100)
Dose (mg/kg bw)
0 46.6
100 60.5
300 54.7
1000 46.4
24 hour survival: 100% all dose groups.
No of pups alive on day 4 of lactation
Dose (mg/kg bw) No.
0 101
100 75
300 83
1000 78
Day 4 survival index:
Dose (mg/kg bw):
0 99.0
100 98.7
300 96.5
1000 98.7
Sex ratio day 4
Dose (mg/kg bw):
0 44.7
100 60.5
300 53.5
1000 41.7
No of pups dead or cannibalised up to day 4
Dose (mg/kg bw):
0 2
100 1
300 3
1000 6

Observations and necropsy findings on pups:
No treatment related effects were observed.

STATISTICAL RESULTS:

Fertility indices for males and females were not statistically different from controls in all dose groups.

In the low dose group post implantation loss and consequently the percentage of live pups born was significantly reduced compared to controls ($P \leq 0.05$). These changes were considered incidental and not treatment related as the effects were not observed at the higher dose groups.

No statistical significant differences from controls were observed for the number of pregnancies, number littered, number of live litters, mean litter size, mean viable litter size, sex ratio at birth, number of pups dead at first observation or day 2 to 4, number of live pups on day 0,3 and 4 and the associated survival indices, external abnormalities of life and dead pups at all dose levels.

A significantly higher percentage of male rats in the low dose group on day 4 was considered incidental and not treatment related as a similar change was not found in the higher dose groups.

The mean number and the mean weight of male and female (and

both sexes combined) pups during different intervals of the lactation period were not statistically significantly different from controls except from a significantly lower ($P \leq 0.05$) mean number of female pups on lactation day 4 in the low dose group which was considered incidental and not related to treatment.

Test condition:

TEST ORGANISMS

- HSDCpb-WU rats
- Age at start of treatment: 11-12 weeks
- Weight at study initiation: males: 377-379 g, females: 210-219 g
- Number of animals: Main group: 10 m, 10 f, recovery groups: 5 m, 5 f

ADMINISTRATION / EXPOSURE

- Duration of test/exposure:

Males: Test groups: 39 days, male recovery group: 39 exposure days, 45 test days.

Females: Treatment groups: 51 +- 7 days, recovery group: 39 exposure days, 45 test days.

- Type of exposure: Oral gavage
- Post exposure period: 14 days
- Vehicle: Double distilled water
- Concentration in vehicle: 10 mg/ml, 30 mg/ml, 100 mg/ml
- Total volume applied: 10 ml/kg bw
- Doses: 100, 300, 1000 mg/kg bw

Treatment:

Male rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period and approximately 2 weeks post mating.

Female rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period, pregnancy and up to lactation day 4.

For the high dose recovery group animals (males and females) the test item was administered once daily, 7 days per week by gavage throughout the treatment period.

MATING PROCEDURES:

Male/female ratio: 1:1 per cage. Cohabitation period until evidence of pregnancy (sperm in vaginal smear) was observed.

CLINICAL OBSERVATIONS AND FREQUENCY:

- Daily observations for appearance, behaviour, clinical signs and preterminal deaths. Females were observed for signs of difficult and prolonged parturition.
- Twice daily: morbidity and mortality.
- Detailed clinical observations: once before exposure and at least once per week thereafter. Signs included were changes in skin, fur, eyes, mucous membranes, occurrence of secretions or excretions and autonomic activity, changes in gait, posture, response to handling, behavioural changes, difficult or prolonged parturition.

Functional observation battery (FOB): Examinations were performed in randomly selected 5 animals of each group at the end of the dosing period for males and during the lactation period for females and included homecage observations, handling observations, open field tests, sensory observations, neuromuscular observations and physiological observations (body temperature).

Body weights were recorded at the beginning of the study, at

least weekly thereafter and at termination. All dams were weighed on gestation days 0, 7, 14 and 20 and lactation days 0 and 4.

Food consumption was recorded weekly.

The fertility index for males and females was determined.

LITTER DATA: All pups from each litter were examined for any external deformities, litter size and sex distribution was determined. Pup weights were recorded on day 0 and 4. All pups were examined for malformations and subject to gross pathological examination. Pup survival index up to lactation day 4 was determined.

HAEMATOLOGY/CLINICAL CHEMISTRY: Standard haematological and clinical chemistry parameters were determined at the end of the pre-mating period and the recovery period in 5 randomly selected males and females of each group.

ORGAN WEIGHTS: Organ weights of liver, adrenals, kidneys, thymus spleen, brain and heart were determined of 5 males and females of each group. Testes and epididymis weights of all adult males of each group were also determined.

GROSS PATHOLOGY.

All adult animals and pups were examined for any structural abnormalities and pathological changes.

HISTOPATHOLOGY:

The following tissues of 5 males and females of the control and high dose group as well as all animals of the recovery and recovery control groups were examined microscopically: all gross lesions, brain, spinal cord, gastrointestinal tract, liver, kidney, adrenals, spleen, heart, thymus, thyroid, trachea, lungs, testes (fixed in Bouins fluid), epididymes (fixed in Bouins fluid), ovaries, uterus, seminal vesicles, coagulating glands, prostate, urinary bladder, axillary lymph nodes, mesenteric lymph nodes, sciatic nerve, femur with marrow, bone marrow smear. Stages of spermatogenesis and interstitial testicular structure in male gonads were determined additionally. Livers of 5 males and females in the mid and low dose groups and testes of 5 males of the mid and low dose groups were also examined.

STATISTICAL ANALYSIS:

Dunnett's t-Test: body weight, body weight change, food intake, haematology, clinical chemistry, organ weight, FOB, gestation length, litter size, No. corpora lutea, No. implantation.

Z-Test/Student's t-Test: Mating performance, conception rate, fertility index, gestation index, live birth index, viability index, sex ratio, pup survival data, No. littered, No. dead pups, No. live pups, Pup survival data, Pre- and Post-implantation loss. Histopathological data.

t-Test/ANOVA: dose correlation

Dimethyl malonate, purity: 99.8%.

(1) valid without restriction

Material Safety Dataset, Critical study for SIDS endpoint

Test substance:

Reliability:

Flag:

11-AUG-2004

(19)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: gavage
Exposure period: 39 to 51 days (from 14 days before mating to day 3 of lactation)
Frequency of treatment: daily, 7 days per week
Duration of test: Males: 39 days, females 51 +/- 7 days, recovery groups: 39 days
Doses: 100, 300, 1000 mg/kg bw
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: = 300 mg/kg bw
NOAEL Teratogenicity: >= 1000 mg/kg bw

Method: OECD Combined Repeated Dose and Reproduction/Developmental Screening Test.

Result: For effects on parent animals: See section 5.4, repeated dose toxicity.

Reproductive results

- Fertility index:

Males, all dose groups: 100%

Females, all dose groups: 100%

- Duration of gestation:

Dose (mg/kg bw)	Duration (days) +/- SD
0	22 +/- 0.3
100	23 +/- 0.5
300	23 +/- 0.5
1000	22 +/- 0.4

- Gestation index:

100 % in all dose groups.

- Parturition: 100 % in all dose groups

- Effects on sperm: No treatment related effects

- Number of implantations:

Dose (mg/kg bw)	No.	Percent
0	12.3	88.1
100	11.8	84.7
300	12.0	88.9
1000	11.6	88.6

- Number of corpora lutea:

Dose (mg/kg bw)	No.
0	14.0
100	13.9
300	13.5
1000	13.1

Percentage pre-implantation loss

Dose (mg/kg bw)	Percent
0	11.9
100	15.3
300	11.1
1000	11.4

Percentage post-implantation loss

Dose (mg/kg bw)	Percent
0	8.1
100	19.1
300	10.4
1000	15.1

Litter results:

- Number of pups born

Dose (mg/kg bw)	No.
0	103
100	76
300	86
1000	84

No of live litters

Dose (mg/kg bw)	No.
0	9
100	8
300	8
1000	8

Mean litter size index

Dose (mg/kg bw):	
0	11.4
100	9.5
300	10.8
1000	10.5

Mean viable litter size:

Dose (mg/kg bw):	
0	11.3
100	9.5
300	10.8
1000	9.9

No. of pups alive on day 0

Dose (mg/kg bw)	No.
0	102
100	76
300	86
1000	79

Live birth index:

Dose (mg/kg bw)	
0	99
100	100
300	100
1000	94

Sex ratio at birth (no of males/total number born x 100)

Dose (mg/kg bw)	
0	46.6
100	60.5
300	54.7
1000	46.4

24 hour survival: 100% all dose groups.

No of pups alive on day 4 of lactation

Dose (mg/kg bw)	No.
0	101
100	75
300	83
1000	78

Day 4 survival index:

Dose (mg/kg bw):	
0	99.0
100	98.7
300	96.5
1000	98.7

Sex ratio day 4

Dose (mg/kg bw):	
0	44.7
100	60.5

300	53.5
1000	41.7

No of pups dead or cannibalised up to day 4

Dose (mg/kg bw):

0	2
100	1
300	3
1000	6

Observations and necropsy findings on pups:
No treatment related effects were observed.

STATISTICAL RESULTS:

Fertility indices for males and females were not statistically different from controls in all dose groups.

In the low dose group post implantation loss and consequently the percentage of live pups born was significantly reduced compared to controls ($P \leq 0.05$). These changes were considered incidental and not treatment related as the effects were not observed at the higher dose groups.

No statistical significant differences from controls were observed for the number of pregnancies, number littered, number of live litters, mean litter size, mean viable litter size, sex ratio at birth, number of pups dead at first observation or day 2 to 4, number of live pups on day 0,3 and 4 and the associated survival indices, external abnormalities of life and dead pups at all dose levels.

A significantly higher percentage of male rats in the low dose group on day 4 was considered incidental and not treatment related as a similar change was not found in the higher dose groups.

The mean number and the mean weight of male and female (and both sexes combined) pups during different intervals of the lactation period were not statistically significantly different from controls except from a significantly lower ($P \leq 0.05$) mean number of female pups on lactation day 4 in the low dose group which was considered incidental and not related to treatment.

Test condition:

TEST ORGANISMS

- HSDCpb-WU rats
- Age at start of treatment: 11-12 weeks
- Weight at study initiation: males: 377-379 g, females: 210-219 g
- Number of animals: Main group: 10 m, 10 f, recovery groups: 5 m, 5 f

ADMINISTRATION / EXPOSURE

- Duration of test/exposure:
Males: Test groups: 39 days, male recovery group: 39 exposure days, 45 test days.
Females: Treatment groups: 51 +- 7 days, recovery group: 39 exposure days, 45 test days.
- Type of exposure: Oral gavage
- Post exposure period: 14 days
- Vehicle: Double distilled water
- Concentration in vehicle: 10 mg/ml, 30 mg/ml, 100 mg/ml
- Total volume applied: 10 ml/kg bw
- Doses: 100, 300, 1000 mg/kg bw

Treatment:

Male rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period and approximately 2 weeks post mating.

Female rats: The test item was administered once daily, 7 days

per week by gavage for 2 weeks prior to mating, during the mating period, pregnancy and up to lactation day 4. For the high dose recovery group animals (males and females) the test item was administered once daily, 7 days per week by gavage throughout the treatment period.

MATING PROCEDURES:

Male/female ratio: 1:1 per cage. Cohabitation period until evidence of pregnancy (sperm in vaginal smear) was observed.

CLINICAL OBSERVATIONS AND FREQUENCY:

- Daily observations for appearance, behaviour, clinical signs and preterminal deaths. Females were observed for signs of difficult and prolonged parturition.
- Twice daily: morbidity and mortality.
- Detailed clinical observations: once before exposure and at least once per week thereafter. Signs included were changes in skin, fur, eyes, mucous membranes, occurrence of secretions or excretions and autonomic activity, changes in gait, posture, response to handling, behavioural changes, difficult or prolonged parturition.

Functional observation battery (FOB): Examinations were performed in randomly selected 5 animals of each group at the end of the dosing period for males and during the lactation period for females and included homecage observations, handling observations, open field tests, sensory observations, neuromuscular observations and physiological observations (body temperature).

Body weights were recorded at the beginning of the study, at least weekly thereafter and at termination. All dams were weighed on gestation days 0, 7, 14 and 20 and lactation days 0 and 4.

Food consumption was recorded weekly.

The fertility index for males and females was determined.

LITTER DATA: All pups from each litter were examined for any external deformities, litter size and sex distribution was determined. Pup weights were recorded on day 0 and 4. All pups were examined for malformations and subject to gross pathological examination. Pup survival index up to lactation day 4 was determined.

HAEMATOLOGY/CLINICAL CHEMISTRY: Standard haematological and clinical chemistry parameters were determined at the end of the pre-mating period and the recovery period in 5 randomly selected males and females of each group.

ORGAN WEIGHTS: Organ weights of liver, adrenals, kidneys, thymus spleen, brain and heart were determined of 5 males and females of each group. Testes and epididymis weights of all adult males of each group were also determined.

GROSS PATHOLOGY.

All adult animals and pups were examined for any structural abnormalities and pathological changes.

HISTOPATHOLOGY:

The following tissues of 5 males and females of the control and high dose group as well as all animals of the recovery and recovery control groups were examined microscopically: all

gross lesions, brain, spinal cord, gastrointestinal tract, liver, kidney, adrenals, spleen, heart, thymus, thyroid, trachea, lungs, testes (fixed in Bouins fluid), epididymes (fixed in Bouins fluid), ovaries, uterus, seminal vesicles, coagulating glands, prostate, urinary bladder, axillary lymph nodes, mesenteric lymph nodes, sciatic nerve, femur with marrow, bone marrow smear. Stages of spermatogenesis and interstitial testicular structure in male gonads were determined additionally. Livers of 5 males and females in the mid and low dose groups and testes of 5 males of the mid and low dose groups were also examined.

STATISTICAL ANALYSIS:

Dunnett's t-Test: body weight, body weight change, food intake, haematology, clinical chemistry, organ weight, FOB, gestation length, litter size, No. corpora lutea, No. implantation.

Z-Test/Student's t-Test: Mating performance, conception rate, fertility index, gestation index, live birth index, viability index, sex ratio, pup survival data, No. littered, No. dead pups, No. live pups, Pup survival data, Pre- and Post-implantation loss. Histopathological data.

t-Test/ANOVA: dose correlation

Dimethyl malonate, purity: 99.8%.

(1) valid without restriction

Test substance:

Reliability:

Flag:

11-AUG-2004

Material Safety Dataset, Critical study for SIDS endpoint

(19)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

5.11 Additional Remarks

Type: Biochemical or cellular interactions

Remark: Administration of 1000 mg diethyl malonate/kg b.w. s.c. to rats decreased the hepatic glutathione concentration. 1 h after application the value approximated 60 % of the control value, and this new steady state was maintained for about 2.5 h. In fed rats the depletion of glutathione increased the rate constant of glutathione turnover by 319 %. In contrast, administration of diethyl malonate to fasted rats resulted in only a 76 % increase in the fractional rate of glutathione turnover.

(59)

Type: Cytotoxicity

Remark: In vitro incubation of ascites sarcoma BP8 cells with 1 mM diethyl malonate for 48 h led to 5 % inhibition of growth rate.

(80)

Type: Cytotoxicity

Remark: In vitro incubation of human diploid embryonic lung fibroblasts (MRC-5) with 25 mM diethyl malonate for 30 min did not lead to any membrane damage.

(93)

Type: Cytotoxicity

Remark: In vitro incubation of isolated brown fat cells from adult hamsters with 1 mM diethyl malonate for 5 min led to 59 % inhibition of the noradrenaline induced respiration.

(77)

Type: Cytotoxicity

Remark: In vitro incubation of chicken tracheal segments with 5 mM diethyl malonate for 60 min did not inhibit the ciliary activity.

Type: Metabolism

Remark: In vitro incubation of 10 umoles diethyl malonate with 2 ug purified lipase from pork adipose tissue for 20 min yielded 1.9 umoles acid (no further specification; 37 degree C; pH 7.0).

10-AUG-2004

(64)

Type: Metabolism

Remark: In vitro incubation of 29.5 mg diethyl malonate/ml with 0.5 mg alpha-Chymotrypsin/ml for 20 h yielded 73 % monoethyl malonate; 25 degree C; pH 7.2 (enzyme source not specified).

10-AUG-2004

(15)

Type: other: dermal adsorption in vitro

Remark: Percentage of the applied radioactive dose recovered in the acceptor cell, in the skin and on the skin surface over 24 h:
diethyl malonate in the acceptor cell: 0.2 - 1.6 %;
diethyl malonate in the skin: 0.2 - 0.9 %;
diethyl malonate in the skin surface: 0.6 - 0.7 %.
The skin mediated hydrolysis of radiolabelled diethyl malonate to monoethyl malonate and malonic acid amounted to 15 - 35 % of the applied radioactivity dose, corrected for hydrolysis products in the starting solution.
Percentage of hydrolysis products recovered in the acceptor cell: 20 - 21 % of the applied dose; percentage of hydrolysis products recovered in the skin and on the skin surface: 3 - 4 % of the applied dose each.
The maximum penetration rate of hydrolysis products was reached after 5 h and amounted to ca. 2 % of the applied dose/h.

Preincubation of skin samples for 5 min in an 80 degree C water bath increased the penetration rate of diethyl malonate and decreased the amount and penetration rate of hydrolysis products.

The total recovery of radioactivity amounted to 50 - 80 % of the applied dose.

Test conditions:

37 degree C; acceptor cell fluid flow rate: 5 ml/h; acceptor cell fluid collection: hourly between hours

1 and 12, 23 and 24, bihourly from hours 12 to 22

18-OCT-2004

Type: other: inhibition of tumour growth

Result: A 27% inhibition of tumour growth compared to controls was seen for grafted tumours and a 25% inhibition for spontaneous carcinoma.

Test condition: Groups of 5 Dilute Brown mice either grafted with sarcoma or with a primary tumor induced by injection of 1 mg methylcholanthrene recieved daily doses of 1/4 of the LD50 (40 mg/kg bw) by gavage 6d/week for 14 days.

10-AUG-2004

(6)

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S I D S

Dossier

Existing Chemical ID: 108-59-8
CAS No. 108-59-8
EINECS Name dimethyl malonate
EC No. 203-597-8
Molecular Weight 132.12
Structural Formula COC(=O)CC(=O)OC
Molecular Formula C5H8O4

Producer Related Part

Company: Degussa AG
Creation date: 19-JUN-2001

Substance Related Part

Company: Degussa AG
Creation date: 19-JUN-2001

Memo: Überarbeitungsversion

Printing date: 26-AUG-2005
Revision date: 28-JUN-2004
Date of last Update: 26-AUG-2005

Number of Pages: 73

Chapter (profile): Chapter: 1.0.1, 1.0.2, 1.0.4, 1.1.0, 1.1.1, 1.2, 1.3, 1.4, 1.5, 1.6.1, 1.6.2, 1.7, 1.7.1, 1.7.2, 1.8, 1.8.1, 1.8.2, 1.8.3, 1.8.4, 1.8.5, 1.8.6, 1.9.1, 1.9.2, 1.10, 1.11, 1.12, 1.13, 2, 3, 4, 5, 6, 10

Reliability (profile): Reliability: without reliability, 1, 2, 3, 4

Flags (profile): Flags: without flag, non confidential, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
Name: Degussa AG - ZN Wolfgang
Contact Person: Dr. W. Mayr, Dr. S. Jacobi **Date:**
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Type: other: contact point
Name: Degussa AG - ZN Wolfgang
Contact Person: Dr. W. Mayr **Date:**
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Country: Germany
Phone: +49 6181 59 4139
Telefax: +49 6181 59 2083
Email: wilfried.mayr@degussa.com

1.0.2 Location of Production Site, Importer or Formulator**1.0.4 Details on Category/Template**

Comment: Dimethylmalonate, CAS No.: 108-59-8, Diethylmalonate, CAS No.: 105-53-3

Remark: The category of simple diesters of malonic acid, dimethylmalonate and diethylmalonate has been defined because of the similar properties of the simple esters and their likelihood to be cleaved under physiological conditions yielding malonic acid and the corresponding alcohols. Where data are lacking for one of the members of the category they can reasonably be substituted by data of the other member of the category due to the structural similarity. The production and use pattern of Diethylmalonate (DEM) and Dimethylmalonate (DMM) are comparable. The two chemicals Diethylmalonate and Dimethylmalonate have very similar physico-chemical properties and both esters are hydrolyzed via a two step reaction to malonic acid and the corresponding alcohol, methanol or ethanol. It is likely that unspecific esterases in the body catalyze the hydrolysis. The alcohols and malonic acid are physiological substances that are metabolized via physiological pathways. Ethanol and Methanol were assessed evaluated in SIAM 19 (OECD, 2004a,b). For ethanol it was concluded that the chemical is currently of low priority for further work, because the hazardous properties of ethanol are manifest only at doses associated with consumption of alcoholic beverages. As it is impossible to reach these exposure levels as a consequence of the manufacture and use of malonates, it can be expected that malonic acid will be the metabolite that determines the toxicity of DEM. For methanol, SIAM 19 decided that this chemical is a candidate for further work. Methanol exhibits potential hazardous properties for human health (neurological effects, CNS depression, ocular

1. GENERAL INFORMATION

ID: 108-59-8

DATE: 21.01.2005

effects, reproductive and developmental effects, and other organ toxicity). The effects of methanol on the CNS and retina in humans only occur at doses at which formate accumulates due to a rate-limiting conversion to carbon dioxide. In primates, formate accumulation was observed at methanol doses greater than 500 mg/kg bw (which would require a DMM dose of more than 1,000 mg/kg bw). As there were no indications of a methanol associated toxicity from a well performed repeated dose toxicity study with DMM in rodents (which are, however, known to be less sensitive to methanol toxicity than humans), and because methanol toxicity would not be expected up to doses as high as 1,000 mg DMM/kg bw/day, it was concluded that methanol does not make a relevant contribution to the toxicity profile of DMM. A possible mode of action for systemic toxicity of DMM and DEM can only be deduced from the repeated dose study with DMM, indicating a reversible liver hypertrophy at the cellular level at high doses of 1000 mg/kg bw/day. This effect can be an indication of an induction of metabolism in the liver rather than a clear systemic toxicity.

18-AUG-2005

(58) (59)

1.1.0 Substance Identification

IUPAC Name: dimethyl malonate
Smiles Code: O=C(OC)CC(=O)OC
Mol. Formula: C5H8O4
Mol. Weight: 132.12

1.1.1 General Substance Information

Purity type: typical for marketed substance
Substance type: organic
Physical status: liquid
Purity: ca. 99.5 - % w/w
Colour: colourless
Odour: slightly ester-like

28-JUL-2005

(22)

1.2 Synonyms and Tradenames

Dimethyl malonate

29-NOV-2004

Dimethyl propanedioate

21-JUL-2005

(64)

Dimethylmalonate

29-NOV-2004

DMM

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DMM

1. GENERAL INFORMATION

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Malonic acid, dimethylester

21-JUL-2005

Malonsaeuredimethylester

Methandicarbonsaueuredimethylester

Methyl malonate

21-JUL-2005

(64)

Propandisaeuredimethylester

Propanedioic acid, dimethyl ester

21-JUL-2005

(64)

1.3 Impurities**Purity type:** typical for marketed substance**CAS-No:** 67-56-1**EC-No:** 200-659-6**EINECS-Name:** methanol**Contents:** ca. .3 - % w/w

28-JUL-2005

(22)

Purity type: typical for marketed substance**CAS-No:** 609-02-9**EC-No:** 210-173-6**EINECS-Name:** dimethyl methylmalonate**Contents:** ca. .2 - % w/w

28-JUL-2005

(22)

1.4 Additives**1.5 Total Quantity****1.6.1 Labelling****Labelling:** no labelling required (no dangerous properties)**Remark:** Last update MSDS Chapter 15 "Labelling and Classification" on 2003-07-23.

29-NOV-2004

(21) (51)

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DATE: 21.01.2005

1.6.2 Classification

Classified: no classification required (no dangerous properties)

29-NOV-2004

(21) (51)

1.7 Use Pattern

Type: type
Category: Non dispersive use

Type: type
Category: Wide dispersive use

29-NOV-2004

(60)

Type: industrial
Category: Chemical industry: used in synthesis

Type: use
Category: Intermediates

(26) (60)

1.7.1 Detailed Use Pattern1.7.2 Methods of Manufacture1.8 Regulatory Measures1.8.1 Occupational Exposure Limit Values1.8.2 Acceptable Residues Levels1.8.3 Water Pollution

Classified by: KBwS (DE)
Labelled by: KBwS (DE)
Class of danger: 1 (weakly water polluting)

Country: Germany
Remark: No. 3353 in catalogue

(21)

1.8.4 Major Accident Hazards

Legislation: Stoerfallverordnung (DE)
Substance listed: no

Country: Germany

1. GENERAL INFORMATION

ID: 108-59-8

DATE: 21.01.2005

Remark: Stoerfallverordnung 2000, (12. BischV, BGBI. I, 2000, 631)
29-NOV-2004 (21)

1.8.5 Air Pollution1.8.6 Listings e.g. Chemical Inventories1.9.1 Degradation/Transformation Products1.9.2 Components1.10 Source of Exposure

Source of exposure: Environment: exposure from production
Exposure to the: Substance

Result: From production there are no emissions into water or air. Exhausts are incinerated and waste water is treated in a biological sewage treatment plant. Product containing waste waters (e.g. from maintenance operations) are incinerated. No waste containing DMM or DEM is produced. From use as flavoring agent no emission data are known.
21-JUL-2005 (12)

Source of exposure: other: human exposure, product register information
Exposure to the: Substance

Result: The Swedish Product Register (2004) contains confidential data on DMM on the whole and the note that there are no consumer products containing DMM. One entry on DMM is contained in the Swiss Product Register (2004): 1 commercial product with a DMM-content of 100 %, i.e. the pure chemical. The SPIN database (2004) does not contain any entries on DMM.
21-JUL-2005 (65) (67) (68)

Source of exposure: other: human occupational exposure
Exposure to the: Substance

Result: The German producer uses closed systems including gas tight flunshes for loading and de-loading operations and closed valve-syringe systems for sampling. From the process description very low occupational exposure is anticipated. No data are available for the uses. As the majority of the products are used as intermediates in the chemical industry a controlled exposure situation is anticipated.
21-JUL-2005 (12)

Source of exposure: Human: exposure of the consumer/bystander
Exposure to the: Substance

Result: WHO (2000) evaluated the combined daily intake of 47 flavoring substances including DEM in Europe and the US. The annual production volume of these 47 substances was 200 metric tons in Europe and 1700 metric tons in the US. From

this an estimated per capita daily intake of 28 mg in Europe and 300 mg in the US was derived (based on a body weight of 60 kg these intakes would correspond to 0.47 and 5 mg/kg bw/day in Europe and the US, respectively). This intake was considered of no concern.

21-JUL-2005

(72)

1.11 Additional Remarks

Memo: Dimethyl malonate could also be detected in traces < 5 µg/100 g extract in banana (*Musa sapientum*) aroma.

Flag: Critical study for SIDS endpoint
12-AUG-2004 (6)

Memo: Dimethyl malonate could be detected in traces as aroma compound in fresh blackberries

Flag: Critical study for SIDS endpoint
12-AUG-2004 (24)

Memo: Dimethyl malonate could be identified as a volatile constituent of green and ripened pineapple (*Ananas comosus* [L.] Merr.)

Result: Umamo et al.(1993) report concentrations of total volatiles and dimethyl malonate in green and ripe pineapples:

Fruit	total volatiles	dimethyl malonate
Green pineapples	0.0006% = 6mg/kg	0.3% of total volatiles = 18 micro-g/kg
Ripe pineapples	0.0009% = 9mg/kg	0.18% of total volatiles = 17 micro-g/kg

Flag: Critical study for SIDS endpoint
12-AUG-2004 (9) (70)

Memo: In blended pineapple pulp dimethyl malonate occurs in concentrations of 19 ppb and is a volatile flavor component

Flag: Critical study for SIDS endpoint
12-AUG-2004 (69)

Memo: In traces dimethyl malonate could be detected as aroma compound of fresh blackberries (*Rubus laciniata* L.).

Flag: Critical study for SIDS endpoint
12-AUG-2004 (24)

Remark: Dimethyl malonate is a volatile flavor component of *Astragali radix* (= the root of *Astragalus membranaceus*, Bunge) and occurs in the oily extract in quantities of 3.4 % of all 35 flavor components. Dimethyl malonate has a mild fruity and winey odor.

Flag: Critical study for SIDS endpoint
12-AUG-2004 (55)

1.12 Last Literature Search

Type of Search: Internal and External

Chapters covered: 3, 4, 5

Date of Search: 13-JUL-2000

Remark: CIS, DIMDI

18-AUG-2004

Type of Search: Internal and External

Chapters covered: 3, 4, 5

Date of Search: 17-AUG-2004

Remark: CIS, DIMDI, STN, Dialog

18-AUG-2004

1.13 Reviews

2. PHYSICO-CHEMICAL DATA

ID: 108-59-8

DATE: 21.01.2005

2.1 Melting Point**Value:** = -62 degree C**Decomposition:** no at degree C**Sublimation:** no**GLP:** no**Reliability:** (2) valid with restrictions
Data of different handbooks

(7) (26) (51) (61) (63)

Value: = -62 degree C**Year:** 1916**GLP:** no**Test substance:** no data**Reliability:** (2) valid with restrictions
Data from handbook (Beilstein)**Flag:** Critical study for SIDS endpoint

(43)

Value: -61.9 degree C**Reliability:** (2) valid with restrictions
Data from handbook

(38) (50)

Value: -61.9 degree C**Year:** 1942**GLP:** no**Test substance:** no data**Reliability:** (2) valid with restrictions
Well documented scientific reference**Flag:** Critical study for SIDS endpoint

(62)

2.2 Boiling Point**Value:** 180 - 181 degree C**Reliability:** (2) valid with restrictions

(26) (61)

Value: 180 degree C at 1026 hPa**Year:** 1934**GLP:** no**Test substance:** no data**Reliability:** (2) valid with restrictions

(71)

Value: 180.7 degree C

2. PHYSICO-CHEMICAL DATA

ID: 108-59-8

DATE: 21.01.2005

Year: 1889
GLP: no
Test substance: no data

Reliability: (2) valid with restrictions (73)

Value: = 181 degree C at 1013 hPa
Decomposition: no

Method: other: DIN 51751
GLP: no

Reliability: (2) valid with restrictions (21)

Value: 181 degree C at 988 hPa

Year: 1894
GLP: no
Test substance: no data

Remark: Boiling point 84 °C at 13 mm Hg (17 hPa).
Reliability: (2) valid with restrictions (8)

Value: 181 degree C

Reliability: (2) valid with restrictions (7)
 29-NOV-2004

Value: 181.4 degree C

Reliability: (2) valid with restrictions (63)

Value: 181.4 degree C at 1013 hPa

Reliability: (2) valid with restrictions
 Data from handbook (38) (50)

Value: = 181.4 degree C at 1013 hPa

Test substance: no data

Reliability: (2) valid with restrictions
 Data from handbook (Beilstein)

Flag: Critical study for SIDS endpoint (48)
 21-JUL-2005

2.3 Density

Type: density
Value: ca. 1.15 g/cm³ at 20 degree C

2. PHYSICO-CHEMICAL DATA

ID: 108-59-8

DATE: 21.01.2005

Method:	other: DIN 51757	
GLP:	no	
Reliability:	(2) valid with restrictions	(21) (51)
29-NOV-2004		
Type:	density	
Value:	1.15 g/cm ³ at 20 degree C	
Reliability:	(2) valid with restrictions	(51)
29-NOV-2004		
Type:	relative density	
Value:	1.1527 at 20 degree C	
Method:	other: pycnometer-method	
Year:	1934	
GLP:	no	
Test substance:	no data	
Remark:	Relative densities determined at other temperatures: 1.1066 at 63.4 °C, 1.0774 at 86.4 °C.	
Reliability:	(2) valid with restrictions	
Flag:	Critical study for SIDS endpoint	(71)
Type:	relative density	
Value:	1.1528 at 20 degree C	
Reliability:	(2) valid with restrictions	(63)
Type:	relative density	
Value:	1.1528 at 20 degree C	
Year:	1942	
GLP:	no	
Test substance:	no data	
Reliability:	(2) valid with restrictions	
Flag:	Critical study for SIDS endpoint	(62)
Type:	density	
Value:	= 1.1539 g/cm ³ at 20 degree C	
Reliability:	(2) valid with restrictions Data from handbook (Beilstein)	(41)
Type:	density	
Value:	1.154 g/cm ³ at 20 degree C	
Reliability:	(2) valid with restrictions	(7) (61)
29-NOV-2004		

2. PHYSICO-CHEMICAL DATA

ID: 108-59-8

DATE: 21.01.2005

Type: relative density
Value: 1.1544 at 20 degree C

Year: 1894
GLP: no
Test substance: no data

Reliability: (2) valid with restrictions (8)

Type: density
Value: 1.153 g/cm³ at 25 degree C

Reliability: (2) valid with restrictions (38)

Type: relative density
Value: 1.1447 at 30 degree C

Year: 1932
GLP: no
Test substance: no data

Reliability: (2) valid with restrictions (5)

Type: relative density
Value: 1.1465 at 30 degree C

Year: 1913
GLP: no
Test substance: no data

Remark: Relative densities at other temperatures: 1.1649 at 10 °C,
 1.128 at 50 °C.
Reliability: (2) valid with restrictions (56)

2.3.1 Granulometry2.4 Vapour Pressure

Value: .15 hPa at 20 degree C

Reliability: (4) not assignable (7) (51)
 21-JUL-2005

Value: ca. .48 hPa at 20 degree C

Method: other (calculated)
Year: 1967
GLP: no data
Test substance: no data

Remark: The value at 20 deg C is an estimate obtained by
 interpolation of the data given in the reference using the

2. PHYSICO-CHEMICAL DATA

ID: 108-59-8

DATE: 21.01.2005

Clausius-Clapeyron equation ($\log VP = -\Delta h(\text{vap})/2.3 R \times 1/T + \text{const}$ (see standard textbooks of physics), i.e. linear regression of $\log VP$ versus $1/T$ (K)).

180.7 degree C: 760 Torr = 1013 hPa
 121.9 degree C: 100 Torr = 133 hPa
 72.0 degree C: 10 Torr = 13.3 hPa
 35.0 degree C: 1 Torr = 1.33 hPa

Reliability: $\log VP = -2762 * (1/T) + 9.1061$ (T in K, VP in hPa)
 (2) valid with restrictions
 Data from handbook

Flag: Critical study for SIDS endpoint
 21-JUL-2005 (11)

Value: = .5 hPa at 20 degree C

GLP: no

Reliability: (4) not assignable
 21-JUL-2005 (21)

Value: ca. .5 at 20 degree C

Method: other (measured)
Year: 2003
GLP: no data
Test substance: no data

Result: The value at 20 degree C is an estimate obtained by interpolation of the data given in the reference using the Clausius-Clapeyron equation ($\log VP = -\Delta h(\text{vap})/2.3 R \times 1/T + \text{const}$ (see standard textbooks of physics), i.e. linear regression of $\log VP$ versus $1/T$ (K)).

Vapour pressure (hPa)	Temperature (°C)
1.0	30
10.0	66.7
100	114.7
1000	180.2

The resulting regression equation is:
 $\log VP = -2745 \times 1/T + 9.065$

The authors also quote extrapolated values for the following temperatures:

Vapour pressure (hPa)	Temperature (°C)
0.01	- 22
0.1	1

Reliability: (2) valid with restrictions
 Data from handbook

Flag: Critical study for SIDS endpoint
 29-NOV-2004 (50)

Value: 19.6 hPa at 25 degree C

Method: other (calculated)
Year: 1985
GLP: no data
Test substance: no data

Remark: Vapour pressure reported as 14.7 mm Hg

2. PHYSICO-CHEMICAL DATA

ID: 108-59-8

DATE: 21.01.2005

Reliability: (4) not assignable
Primary reference containing information on the estimation method was not obtained.
29-NOV-2004 (57)

Value: = 60.65 hPa at 101 degree C

Remark: Other vapour pressures reported: 429.6 hPa at 152.1 °C, 2042 hPa at 202 °C.

Reliability: (2) valid with restrictions
Data from handbook (Beilstein) (3)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = -.36

Method: other (calculated): Calculated using advanced chemistry development (ACD/Labs) Software
Year: 2004

Reliability: (2) valid with restrictions
Calculated data, internationally accepted method.
30-NOV-2004 (66)

Partition Coeff.: octanol-water
log Pow: = -.09

Method: other (calculated): KOWWIN (LOGKOW (c)) Program Version 1.66
Syracuse Research corporation, Merrill Lane, Syracuse, New York, 13210 USA
Year: 2004
GLP: no

Reliability: (2) valid with restrictions
Calculated data, internationally accepted method.
30-NOV-2004

Partition Coeff.: octanol-water
log Pow: -.05

Method: other (measured)
Year: 1995
GLP: no data
Test substance: no data

Reliability: (2) valid with restrictions
Measured, no details, but standard as basis for QSAR calculations.

Flag: Critical study for SIDS endpoint
20-AUG-2004 (25) (51)

Partition Coeff.: octanol-water
log Pow: = 1.43

Remark: no further information available. Probably calculated with the

2. PHYSICO-CHEMICAL DATA

ID: 108-59-8

DATE: 21.01.2005

Reliability: substituent method.
(4) not assignable
No details reported.
21-JUL-2005 (4)

2.6.1 Solubility in different media

Solubility in: Water

Remark: slightly soluble in water, miscible with alcohol, ether, oils; no further specification of alcohol or ether given
Reliability: (2) valid with restrictions (61) (63)

Solubility in: Water

Remark: very slightly soluble in water; soluble in alcohol and ether; no further specification of alcohol or ether given
Reliability: (2) valid with restrictions (26)

Solubility in: Water

Remark: not miscible with water
Reliability: (2) valid with restrictions (51)

Solubility in: Water
Value: = 142 g/l at 20 degree C
Descr.: of very high solubility

GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
21-JUL-2005 (21)

Solubility in: Water
Value: 99.5 g/l at 25 degree C

Method: other: estimated data

Year: 1996

GLP: no data

Test substance: no data

Reliability: (4) not assignable
Peer reviewed data base.
Flag: Critical study for SIDS endpoint
30-NOV-2004 (53)

Solubility in: Water
Value: 160 g/l at 20 degree C

Reliability: (4) not assignable
database data

21-JUL-2005

(7)

2.6.2 Surface Tension**Value:** 35.9 mN/m at 30 degree C**Method:** other: maximal bubble-pressure method by Sugden**Year:** 1932**GLP:** no**Test substance:** no data**Reliability:** (2) valid with restrictions

30-NOV-2004

(5)

Value: 38.24 mN/m**Year:** 1913**GLP:** no**Test substance:** no data**Reliability:** (2) valid with restrictions

30-NOV-2004

(56)

2.7 Flash Point**Value:** = 90 degree C**Type:** other: no data**Method:** other: no data**Year:** 1981**GLP:** no data**Test substance:** no data**Reliability:** (2) valid with restrictions**Flag:** Critical study for SIDS endpoint

21-JUL-2005

(7) (26) (51)

Value: = 90 degree C**Type:** closed cup**Method:** other: DIN 51758**Year:** 1978**GLP:** no data**Reliability:** (2) valid with restrictions

(21) (63)

Value: = 90 degree C**Type:** other: no data**Method:** other: no data**Year:** 1981**GLP:** no data**Reliability:** (2) valid with restrictions

(38)

2.8 Auto Flammability

Value: = 440 degree C

Method: other: DIN 51794
GLP: no

Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
 21-JUL-2005 (21)

Value: 440 degree C

Reliability: (4) not assignable
 No details.
 20-AUG-2004 (7) (51)

2.9 Flammability

Result: flammable

Reliability: (4) not assignable
 20-AUG-2004 (51)

2.10 Explosive Properties

Result: other: 1.3 - 17.4%

Reliability: (2) valid with restrictions (7) (21) (51)

Result: other: no data

Remark: combustibile
Reliability: (2) valid with restrictions (26)

2.11 Oxidizing Properties

Result: other: irreversible oxidation potential (Eox) of potassium enolate of dimethyl malonate = - 0.293 +- 0.006 V relative to ferrocene (reversible Eox of ferrocene) at 25 degree C in dimethyl sulfoxide

Method: other: Enolization, alkylation and redox potentials for some beta-di-and tri-carbonyl compounds
Year: 1987
GLP: no data

Remark: The data give the degree of enolization relative to ferrocene.
Test condition: purified commercially available substance

30-NOV-2004

(1)

2.12 Dissociation Constant**Acid-base Const.:** pKa = 15.88 +- 0.06**Result:** Dimethyl malonate has a pKa of 15.88 +- 0.06 in dimethyl sulfoxide at a temperature of 25 °C.

12-AUG-2004

(1) (2)

2.13 Viscosity**2.14 Additional Remarks****Remark:** refractive index: 1.4140 (no temperature mentioned) (26)**Remark:** refractive index: 1.4149 (17 degree C) (61)**Remark:** refractive index: 1.4135 (20 degree C) (63)**Remark:** refractive index: 1.4138 (20 degree C) (38)

3.1.1 Photodegradation

Type: air
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: .000000000000525 cm³/(molecule * sec)
Degradation: 50 % after 30.6 day(s)

Method: other (calculated): AOPWIN (AOP(c)) Program, Version 1.90, Syracuse Research Corporation, Merrill Lane, Syracuse, New York, 13210, U.S.A., 2000
Year: 2003
GLP: no
Test substance: no data

Remark: Assumption for the calculation: 24 hours sunlight.
Reliability: (2) valid with restrictions
 Calculated data, internationally accepted method.
Flag: Critical study for SIDS endpoint

(15) (52)

3.1.2 Stability in Water

Type: abiotic
t1/2 pH7: = 5.7 hour(s) at 50 degree C
t1/2 pH9: <= 2.4 hour(s) at 50 degree C

Method: Directive 92/69/EEC, C.7
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: OECD TG 111
Result: Results of the main test at pH4:

50 °C: t1/2 = 858.7 h
 65 °C: t1/2 = 294.9 h
 76 °C: t1/2 = 114.7 h

The extrapolated value for 25 °C: t1/2 = 8422 h.

At pH 7:
 50 °C: t1/2 = 5.7 h
 25 °C: t1/2 = 52.5 h

At pH 9 only the pretest was performed indicating rapid hydrolysis:

50 °C: 95.9% hydrolysis within 2.4 h.

Reliability: (1) valid without restriction
 Guideline study, GLP

Flag: Material Safety Dataset, Critical study for SIDS endpoint
 21-JUL-2005

(17)

3.1.3 Stability in Soil3.2.1 Monitoring Data (Environment)

Type of measurement: other: natural occurrence

Medium: biota

Remark: see data reported in 1.11

12-AUG-2004

3.2.2 Field Studies3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: water - soil
Method: other: (calculation) PCKOCWIN (PC-KOC (c)) Program, Version 1.66, Syracuse Research Corporation, Merrill Lane, Syracuse, New York, 13210, U.S.A., 2000
Year: 2003

Remark: no GLP

Result: The soil or sediment adsorption coefficient (Koc) of Dimethyl malonate was calculated as Koc = 1.74.

Reliability: (2) valid with restrictions
 Calculated data, internationally accepted method.

Flag: Critical study for SIDS endpoint

21-JUL-2005

(18)

Type: volatility
Media: water - air
Method: other: (calculation) Henrywin Program, Version 3.10, Syracuse Research Corporation, Merrill Lane, Syracuse, New York, 13210, U.S.A., 2000
Year: 2003

Method: Bond Estimation Method

Remark: no GLP

Result: Henry's Law Constant [25 °C] = 4.17E-007 atm·m³/mole
 = 0.0422 Pa m³/mole
 = 1.71E-005 unitless

Reliability: (2) valid with restrictions
 Calculated data, internationally accepted method.

Flag: Critical study for SIDS endpoint

21-JUL-2005

(14)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water

Method: Calculation according Mackay, Level I

Year: 2004

Result: Air: 1.55 %
 Soil: < 0.01 %
 Water: 98.44 %
 Sediment: < 0.01 %

3. ENVIRONMENTAL FATE AND PATHWAYS

ID: 108-59-8

DATE: 21.01.2005

	Biota:	< 0.01 %		
Test condition:	Data used:			
	Molar mass:	132.12 g/mol		
	Vapour pressure:	48 Pa		
	Water solubility:	142 g/l		
	Melting Point:	-62 °C		
	logKow:	-0.05		
	Volumes used:			
	Air:	6 000 000 000		
	Soil:	45 000		
	Water:	7 000 000		
	Sediment:	21 000		
	Susp. Sediment:	35		
	Biota:	7		
	Aerosol:	0.12		
Reliability:	(2) valid with restrictions			
	Calculated data, internationally accepted method.			
Flag:	Critical study for SIDS endpoint			
24-MAR-2005				(19)
Media:	air - biota - sediment(s) - soil - water			
Method:	Calculation according Mackay, Level III			
Year:	2004			
Method:	Estimation of the Equilibrium Partitioning Characteristics in the Environment.			
	Calculation Mackay Level III, V2.70 Model (2002) Environmental Modelling Centre, Trent University, Peterborough, Ont. Canada			
Result:	Compartment	Release	Release	Release
		100 % in air	100 % in water	100 % in soil
	Air	2.63	0	0.02
	Water	61.3	99.9	61.7
	Soil	36.0	0.01	38.3
	Sediment	0.02	0.04	0.02
	Conclusion:			
	Under equilibrium steady state flow conditions the substance distributes to water and soil when released into the air or soil compartment, while the majority of the substance will stay in the water compartment when released into water.			
Test condition:	Input parameters			
	Molecular mass:	132.12 g/mol		
	Temperature:	20 °C		
	logKow:	-0.05		
	Water solubility:	142 g/l		
	Vapour pressure:	48 Pa		
	Melting Point:	-62 °C		
	Half-life in air:	734 hours		
	Emission rates default 3000 kg/h to either air, water or soil.			
Reliability:	(2) valid with restrictions			
	Calculated data, internationally accepted method.			
Flag:	Critical study for SIDS endpoint			
01-DEC-2004				(20)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic, non-adapted
Concentration: 10.6 mg/l related to DOC (Dissolved Organic Carbon)
Degradation: = 100 % after 28 day(s)
Result: readily biodegradable

Method: other: DOC DIE AWAY-Test, Directive 92/69/EEC, part II, C.4-A
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Kinetics of biodegradation: % decrease of DOC
 day FE1(%) FE2(%) FC1(%) FC2(%)

day	FE1(%)	FE2(%)	FC1(%)	FC2(%)
0	0	0	0	0
7	86	87	99	99
14	100	100	99	99
21	95	99	98	98
27	95	99	100	100
28	99	100	99	98

FE1 and FE2: Flasks with test substance and inoculum
 FC1 and FC2: Flasks control substance (sodium benzoate) and inoculum

- Breakdown product: no
- Remarks: The difference of biodegradation between the two flasks containing the test substance at the end of the test is less than 20%
- More than 70% of biodegradation was reached within 14 days in the control flasks containing sodium benzoate (99% degradation after 7 days): the inoculum activity is sufficient.

The test substance was degraded to 86-87% after 7 days and 95 to 100% after 14 to 28 days.

Conclusions:

The test substance is readily biodegradable under the test conditions.

Test condition: INOCULUM/TEST ORGANISM

- Type of sludge: activated sludge, predominantly domestic
- Source: Sewage plant Marl-Ost
- Sampling site: activated sludge basin
- Preparation of inoculum: Centrifugation 10 min at 1100 x g, the supernatant is discarded and the sludge resuspended with mineral medium, further centrifugation for 10 min at 1100 x g
- Resuspension of the activated sludge (4.9 g/l dry mass of activated sludge)
- Initial cell concentration: 24.5 mg/l

TEST SYSTEM

- Culturing apparatus: 2000 ml Erlenmeyer flask with slight aluminium foil closure
- Number of culture flasks per concentration: 2
- Aeration device: shaking machine
- Measuring equipment: Carbon analyzer (Schimadzu)

INITIAL TEST SUBSTANCE CONCENTRATION: 9.2 mgDOC/l in the test flasks, 10.42 mg DOC/l in the control flasks

METHOD OF PREPARATION OF TEST SOLUTION: Stock solution: 1000 mg/l (459 mg DOC/l)

DURATION OF THE TEST: 28 days

ANALYTICAL PARAMETER: Dissolved organic carbon (DOC)

SAMPLING: After 0, 7, 14, 21, 27, 28 days.

TEST CONDITIONS

- Composition of stock nutrient solution:

- a) 8.5 g/l KH₂PO₄
21.75 g/l K₂HPO₄
33.3 g/l Na₂HPO₄ * 2 H₂O
20.0 g/l (NH₄)Cl
- b) 22.5 g/l MgSO₄ * 7 H₂O
- c) 27.5 g/l CaCl₂
- d) 0.25 g/l FeCl₃ * 6 H₂O

- Additional substrate: No

- Test temperature: 22 +- 0.2 °C

- Addition of stock solutions: a) 20 ml, b) - d): 2 ml each

- Aeration of dilution water: no

- Concentration of suspended solids: 24.5 mg/l

CONTROLS: 2 Flasks without test substance, but with inoculum,

REFERENCE SUBSTANCE: 2 Flasks with Benzoic acid, sodium salt, 10.42 mg DOC/l and inoculum.

No abiotic control (with test substance, without inoculum) and no inhibitory control was included in the test.

Test substance:

FACTORS AFFECTING TEST:

- Stability: see hydrolysis as function of pH, section 3.1.2 stability in water
- Vapor pressure: 0.5 hPa (20 °C)
- Water solubility: 142 g/l (20 °C)
- Adsorption potential (log Pow): -0.05
- Toxicity to microorganisms: >= 177 g/l

Reliability:

(1) valid without restriction

Guideline study, GLP

Flag:

WGK (DE), Material Safety Dataset, Critical study for SIDS endpoint

21-JUL-2005

(32)

3.6 BOD₅, COD or BOD₅/COD Ratio

3.7 Bioaccumulation

3.8 Additional Remarks

Memo:

Uptake into clouds

Result:

The droplet train technique was used to investigate the uptake of gaseous dicarboxylic acids including DMM into water droplets. The uptake coefficients were calculated to be in the range of 0.04 to 0.09 at temperatures between 265 and 285 K indicating an efficient capture in the droplets according to the authors. This could be a principal elimination way from the atmosphere. Hydrolysis of the esters could additionally lead to the presence of dicarboxylic acids in the cloud that could be a source of cloud condensation nuclei.

01-DEC-2004

(42)

Remark:

In the MITI-List dimethyl malonate is not mentioned in the sections biodegradation and bioaccumulation. (54)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC0: = 7
LC50: = 21
LC100: = 50
Method: Directive 84/449/EEC, C.1 "Acute toxicity for fish"
Year: 1984
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: RESULTS: EXPOSED
 - Nominal/measured concentrations:

Nominal (mg/l) measured (mg/l) (mean of 5 days)

25	7.0
36	13.8
50	23.0
70	50.0
100	74.5

- Effect data (Mortality):

LC50 (96 h): 21.0 mg/l
 LC0 (96 h): 7.0 mg/l
 LC100 (96 h): 50.0 mg/l

(graphical evaluation)

- Concentration / response curve:

24 h

Conc. (mg/l) No. surviving No dead % mortality

control	10	0	0
7.0	10	0	0
13.8	10	0	0
23.0	6	4	40
50.0	0	10	100
74.5	0	10	100

48 h

Conc. (mg/l) No. surviving No dead % mortality

control	10	0	0
7.0	10	0	0
13.8	9	1	10
23.0	4	6	60
50.0	0	10	100
74.5	0	10	100

72 h

Conc. (mg/l) No. surviving No dead % mortality

control	10	0	0
7.0	10	0	0
13.8	9	1	10

23.0	4	6	60
50.0	0	10	100
74.5	0	10	100

96 h

Conc. (mg/l) No. surviving No dead % mortality

control	10	0	0
7.0	10	0	0
13.8	9	1	10
23.0	4	6	60
50.0	0	10	100
74.5	0	10	100

Test condition:

TEST ORGANISMS

- Strain: Danio rerio (Brachydanio rerio)
- Supplier: West Aquarium, Bad Lauterberg
- Age/size/weight/loading: 3 cm +- 0.5 cm/0.41 g
- Feeding: TetraMin 1 % of the body weight
- Pretreatment: treatment 3 times a week with Malachitgreen
14 days of quarantine
- Feeding during test: no

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Solution: 301.2 g/2.5 l
 - Vehicle, solvent: deionized water
- STABILITY OF THE TEST CHEMICAL SOLUTIONS: The test substance was not completely stable and the deviations from the nominal concentrations were on average more than 20 %. Therefore the mean values of the measured concentrations were used.

DILUTION WATER

- Source: Drinking water of Gelsenwasser AG
- Aeration: Continuously
- Hardness: 13.6° dH
- pH: 7.3 - 7,7
- Oxygen content: 8.6 - 9.4 mg/l
- Holding water: Dechlorinated drinking water (Gelsenwasser AG)

TEST SYSTEM

- Test type: flow through test
- Concentrations: 25, 36, 50, 70, 100 mg/l
- Dosing rate: 6.9 +- 0.1 ml/10 min
- Flow rate: 10 l/h
- Exposure vessel type: 45 l aquaria
- Number of replicates, fish per replicate: 1, 10
- Test temperature: 20 +- 1 °C
- Dissolved oxygen: 8.6 - 9.4 mg/l
- pH: 7.3 - 7.6
- Adjustment of pH: no
- Photoperiod: light/dark: 16 h/8 h

DURATION OF THE TEST: 96 hours

TEST PARAMETER: Mortality

SAMPLING: at 0, 24, 48, 72, and 96 h.

MONITORING OF TEST SUBSTANCE CONCENTRATION:

By HPLC analysis

Reliability:

(1) valid without restriction

Flag:

Guideline study, GLP, substance specific analysis
WGK (DE), Material Safety Dataset, Critical study for SIDS
endpoint

24-AUG-2005

(35)

Type: other: no data
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 17

Method: other: Quantitative structure-activity relationship (QSAR)
Year: 1991
GLP: no data

Remark: Probably also quoted by Eldred et al., 1999 for a QSAR development.
 Results are recalculated from the log (1/LC50) value (QSAR = 0.1264 mmol/l).

24-AUG-2005

(10) (23)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: >= 1000
EC50: > 1000

Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: 1984
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: RESULTS: EXPOSED
 - Nominal/measured concentrations: 250, 350, 500, 700, 1000 mg/l (nominal only)
 - Effect data (immobilization) (48 h): EC50: > 1000 mg/l (highest concentration tested)
 - Concentration / response curve: 48 h

concentration total No. No. mobile No. immobile %immobile (mg/l)

control	20	20	0	0
250	20	20	0	0
350	21	20	1	5
500	20	20	0	0
700	20	20	0	0
1000	21	21	0	0

- Effect data (immobilization) (24 h): EC50 > 1000 mg/l (highest concentration tested)
 - Concentration / response curve: 24 h

concentration total No. No. mobile No. immobile %immobile (mg/l)

control	20	20	0	0
250	20	20	0	0
350	21	20	1	5
500	20	20	0	0
700	20	20	0	0

1000 21 21 0 0

- Cumulative immobilization: \geq 1000 mg/l
- Effect concentration vs. test substance solubility:
water solubility: 142 g/l (20°C)

Based on the effective concentration corrected for potential hydrolysis an EC50 of $>$ 728 mg/l can be calculated. This may however be a conservative approach, as due to the lower pH during the test hydrolysis might actually have been slower than the hydrolysis rate at pH7 used for the calculation.

RESULTS CONTROL:

RESULTS: TEST WITH REFERENCE SUBSTANCE

- Concentrations:
- Results (immobilization) (24 h):
concentration % immobilized daphnids
(mg/l)

0.9	30
1.9	100

Test condition:

TEST ORGANISMS

- Strain: Daphnia magna Straus IRCHA
- Source/supplier: Hüls AG
- Breeding method: Breeding method according to Elendt (1990) in M4-medium in 1l beakers water exchange every 2 to 3 days.
- Age: $<$ 1 day
- Feeding: Desmodesmus subspicatus
- Feeding during test: none
- Control group: negative control (water only), positive control: potassium dichromate

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Vehicle, solvent: water
- Concentration of vehicle/ solvent: 2000 mg/l

STABILITY OF THE TEST CHEMICAL SOLUTIONS: see stability information (hydrolysis as function of pH).

DILUTION WATER

- Source: Synthetic fresh water
- Aeration: no
- Hardness: CaCl₂ x 2 H₂O: 294 mg/l, MgSO₄ x 7 H₂O: 123 mg/l
- Salinity: KCl: 5.5 mg/l
- Ca/Mg ratio: 4 : 1
- Na/K ratio: 10 : 1
- pH: 6.4 to 6.8
- Oxygen content: 7.7 to 8.4 mg/l

TEST SYSTEM

- Test type: static
- Concentrations: (nominal): 250, 350, 500, 700, 1000 mg/l
- Exposure vessel type: round bottom flasks
- Number of replicates, individuals per replicate: 4 replicates, 5 individuals
- Test temperature: 20 \pm 2°C
- Dissolved oxygen: 7.7 - 8.4 mg/l
- pH: 6.4 - 6.8
- Adjustment of pH: no
- Intensity of irradiation: dark

DURATION OF THE TEST: 48 hours

TEST PARAMETER: Immobilization

MONITORING OF TEST SUBSTANCE CONCENTRATION: not performed, nominal concentrations used.

Statistical Analysis: Probit analysis according to Cavalli-Sforza (1972).

Reliability: (2) valid with restrictions
Guideline study, GLP, but no analytical substance determination.

Flag: WGK (DE), Material Safety Dataset, Critical study for SIDS endpoint

24-AUG-2005 (33)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 20
EC10: = 68
EC50: = 386

Method: Directive 87/302/EEC, part C, p. 89 "Algal inhibition test"
Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Method: 88/302/EEC or OECD 201
Result: RESULTS:
 Nominal concentrations only
 EXPOSED
 - Effect data/Element values:
 - Cell density data:
 Cell density in cells x 10exp4/ml (standard deviation)
 (at 24, 48, and 72 h mean values of 8 parallel experiments for controls and 5 experiments for test substance concentrations)

0 h:	Control	10	20	40	80	160	320
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
	2	2	2	2	2	2	2
Time	Control	10	20	40	80	160	320
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
24 h	7	6	7	7	6	6	5
(s.d.)	(1.1)	(1)	(1.4)	(1.4)	(1.2)	(1.3)	(0.5)
48 h	27	29	32	31	26	20	14
(s.d.)	(4.1)	(5.5)	(5.5)	(5.8)	(3.2)	(2.5)	(1.2)
72 h	134	141	129	113	71	43	23
(s.d.)	(6.1)	(8.1)	(7.1)	(4.5)	(2.8)	(2.5)	(3.6)

- Growth curves:
 Area under the growth curve and % inhibition

	Control	10	20	40	80	160	320
	0 mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Area	96	100.5	98.5	89.5	62.5	42.5	25.5
% inhib.		-4.7	-2.6	6.8	34.9	55.7	73.4

Growth rate (u)
0-72 h

	Control 0 mg/l	10 mg/l	20 mg/l	40 mg/l	80 mg/l	160 mg/l	320 mg/l
u	1.40	1.42	1.39	1.35	1.19	1.02	0.81
% inhib.		-1.2	0.9	4.1	15.1	27	41.9

pH-development during the test:

	Control 0 mg/l	10 mg/l	20 mg/l	40 mg/l	80 mg/l	160 mg/l	320 mg/l
0 h	7.3	7.3	7.3	7.3	7.3	7.3	7.1
72 h	9	9	8.5	5.6	5	4.7	4.7

Due to the lower pH in the test substance vials compared to controls a hydrolysis of the test substance during the study cannot be completely excluded.

STATISTICAL RESULTS:

Cell growth (biomass):

72 h EbC50: 147.9 mg/l

72 h EbC10: 42.2 mg/l

72 h EbC90: > highest tested concentration of 320 mg/l

Growth rates:

72 h ErC50: 386.4 mg/l

72 h ErC10: 68.1 mg/l

72 h ErC90: > highest test concentration of 320 mg/l

Recalculation of the results taking into account potential hydrolysis at pH7 leads to 72 h EC50 values of 240 mg/l for growth rate and 92 mg/l for biomass. This may however be a conservative approach, as due to the lowered pH during the test hydrolysis might actually have been slower than the hydrolysis rate at pH7 used for the calculation.

Test condition:

TEST ORGANISMS

- Strain: *Desmodesmus subspicatus* (*Scenedesmus subspicatus*), 86.81 SAG

- Source/supplier: Institut für Wasser-, Boden- und Lufthygiene, Berlin and Hüls AG, own breeding

- Laboratory culture: From a stem culture, prepared 3 days prior to the start of the experiment.

- Method of cultivation: Cell density: 20000 cells/ml, culture in sterile Erlenmeyer flasks on light-tables, light intensity: 8000 Lux, white, medium according to EC-guideline 88/302/EEC, temperature: 24 +/- 2 °C

- Controls: without test substance

- Initial cell concentration: 2x 10exp4 cells/ml

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- 1 g/l in water

STABILITY OF THE TEST CHEMICAL SOLUTIONS: see stability information (hydrolysis as function of pH).

DILUTION WATER

- Source: Deionized water

- Aeration: no

TEST SYSTEM

- Test type: static
 - Number of replicates: 5 to 8
 - Concentrations: 10, 20, 40, 80, 160, 320 mg/l
 (nominal)
 - Test temperature: 24 +- 2 °C
 - pH: pH at the beginning of the test: 7.1
 to 7.3, at the end of the test: 4.7 to 9.0.
 - Intensity of irradiation: 8000 Lux
 MONITORING OF TEST SUBSTANCE CONCENTRATION: not performed,
 nominal concentrations used.
 Statistical Method:
 Probit analysis according to Cavalli and Sforza, 1972.
Reliability: (2) valid with restrictions
 Guideline study, GLP, but no analytical substance
 determination.
Flag: WGK (DE), Material Safety Dataset, Critical study for SIDS
 endpoint
 24-AUG-2005 (37)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 5 minute(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 38
Method: other: Microtox
Year: 1982
GLP: no data
Test substance: other TS: Dimethyl malonate, unknown purity
Remark: Results are recalculated from $\log 1/EC50 = 0.288$ mmol/l.
Test substance: stock solution contained 2 % NaCl
 26-AUG-2005 (10)

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 18 hour(s)
Unit: g/l **Analytical monitoring:** no
EC10: = 6
EC50: >= 177
Method: other: DIN 38412, part 8
GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Remark: EC50 values extrapolated beyond the range of concentrations
 used in experiment (≤ 6.2 g/l)
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
 24-AUG-2005 (36)

Type: aquatic
Species: Tetrahymena pyriformis (Protozoa)
Unit: mmol/l **Analytical monitoring:** no data
EC50: = 20
Method: other

Year: 1997
GLP: no data

Method: 2-dimensional static 50% inhibition growth concentration (IGC50) for axenic cultures of the ciliate *Tetrahymena pyriformis* according to Schultz, 1996.

Remark: The data were used to develop a QSAR model.

Test condition: Stock solutions: In DMSO at concentrations of 5 to 50 mg/l. Population density measured photometrically at 540 nm.

Test substance: Dimethyl malonate, purity $\geq 95\%$

Flag: Critical study for SIDS endpoint

26-AUG-2005 (40)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS4.6.1 Toxicity to Sediment Dwelling Organisms4.6.2 Toxicity to Terrestrial Plants4.6.3 Toxicity to Soil Dwelling Organisms4.6.4 Toxicity to other Non-Mamm. Terrestrial Species4.7 Biological Effects Monitoring4.8 Biotransformation and Kinetics4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo: In vivo
Type: Metabolism
Species: rat
Doses, males: 5 µl/0.5 mCi
Doses, females: 5 µl/0.5 mCi
Route of administration: other: intracerebral injection

Deg. product: yes

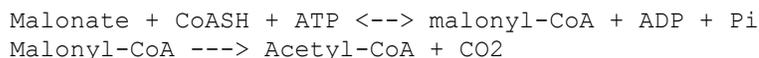
Method: other
Year: 1978
GLP: no data

Test substance: other TS: C1 or C2 14-C-radiolabeled malonic acid, specific activity 12 mCi and 42 mCi respectively

Result: The authors verified that the decarboxylation of malonic acid to acetyl-CoA by various mammalian tissues also occurs in vivo after intracerebral injection.

A rapid reflux of unreacted malonic acid in venous blood was reported. Labeled ¹⁴C¹⁴O₂ was recovered from venous blood and the expired air after administration of C-1 labeled product, but not after C-2 labeled product. High radioactivity was present in glutamate, aspartate and GABA. Sequential degradation of glutamate and aspartate proved that labeling of these amino acids occurred from [1-¹⁴C]acetyl-CoA and [2-¹⁴C]acetyl-CoA respectively via the Krebs-cycle. Malonate activation and decarboxylation were similar to in vitro experiments with isolated mitochondria from different tissues. In vitro the radiolabel was however not incorporated into amino acids. In the in vivo experiment a minor amount of radioactivity was also incorporated in brain lipids.

The authors conclude that malonic acid is metabolised via the following route:



In vitro:
 Acetyl-CoA → acetate + CoASH

In vivo: Acetyl-CoA enters the Krebs cycle and is used for the formation of aspartate, glutamate and GABA. A minor amount may also be incorporated into lipids.

Test condition: Intracerebral injection of either C1- or C2- ¹⁴C- radiolabeled malonic acid to anesthetized adult male and female rats. The rats were killed after 2, 5, 10, 15 or 30 min and the brains removed, weighed, homogenized and analysed for radiolabeled reaction products. Venous blood and expired air was also analysed for radioactivity.

Reliability: (2) valid with restrictions
 Well documented scientific reference

Flag: Critical study for SIDS endpoint

16-AUG-2005

(47)

In Vitro/in vivo: In vivo
Type: Metabolism

Remark: DMM is likely to be metabolized by unspecific (serine-) esterases of different tissues, in particular in the liver to the mono ester and finally to malonic acid and the corresponding alcohol, methanol. This is corroborated by the findings of the abiotic hydrolysis, in particular at alkaline pH that can be regarded as qualitatively similar to the hydrolysis catalyzed by unspecific esterases (Jacobi and Hoffmann, 1989). The hydrolysis products are likely to be metabolized via physiological pathways as the tricarboxylic acid cycle because they are part of the normal intermediate metabolism (WHO, 2000).

21-JUL-2005

(39) (72)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Value: = 5331 mg/kg bw

Method: other: no data
GLP: no data
Test substance: no data

Reliability: (4) not assignable
Secondary Reference only.

04-AUG-2004

(51)

Type: LD50
Species: rat
Value: ca. 4700 mg/kg bw

Method: other: Acute Oral Toxicity
Year: 1978
GLP: no data
Test substance: no data

Reliability: (4) not assignable
Data from handbook

04-AUG-2004

(63)

Type: LD50
Species: rat
Value: = 4577 - 6164 mg/kg bw

Method: other: Acute Oral Toxicity
Year: 1976
GLP: no data
Test substance: no data

Remark: No details reported.
Reliability: (4) not assignable
Secondary Reference only.

04-AUG-2004

(49)

Type: LD50

6. REFERENCES

ID: 108-59-8

DATE: 21.01.2005

Species: rat
Strain: other: Bor: WISW (SPF Cpb)
Sex: male/female
No. of Animals: 10
Doses: 2000 mg/kg bw
Value: > 2000 mg/kg bw

Method: other: OECD Guide-line 401 of 1987
Year: 1987
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: both female and male
 Limittest

Result: MORTALITY:
 No mortality occurred during the study.
 CLINICAL SIGNS: None. No influence on body weight gain.
 NECROPSY FINDINGS: No indications of substance related organ changes were observed.

Test condition: TEST ORGANISMS:
 - Source: Fa. Winkelmann, Borchon
 - Age: 6 - 8 weeks
 - Weight at study initiation: 128.6 +/- 20 %
 ADMINISTRATION:
 - Doses: 2000 mg/kg bw
 - Doses per time period: once
 - Volume administered or concentration: 1.74 cm³/kg bw
 - Post dose observation period: 2 weeks
 EXAMINATIONS:
 Clinical symptoms were recorded 0.5, 1, 2, 3, 4, 5, and 6 hours after administration of the test substance and once daily for the following two weeks. Body weight was determined at the day of administration of the test substance, on days 7 and 14. After 14 days the animals were killed by CO₂ inhalation, sectioned and investigated for any macroscopic organ changes.

Reliability: (1) valid without restriction
 Guideline study, GLP

Flag: Material Safety Dataset, Critical study for SIDS endpoint
 01-DEC-2004 (31)

5.1.2 Acute Inhalation Toxicity5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rat
Strain: other: Bor: WISW (SPF Cpb)
Sex: male/female
No. of Animals: 10
Doses: 2000 mg/kg bw
Value: > 2000 mg/kg bw

Method: OECD Guide-line 402 "Acute dermal Toxicity"
Year: 1987
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

6. REFERENCES

ID: 108-59-8

DATE: 21.01.2005

Remark:	both female and male	
Result:	MORTALITY: No mortality was observed during the study. CLINICAL SIGNS: No substance related clinical signs were observed. No local effects were observed at the application site. No substance related effects on body weight or body weight gain were reported. NECROPSY FINDINGS: No indications for any substance related organ effects were observed. No changes of the skin at the application site were found.	
Test condition:	TEST ORGANISMS: - Source: Fa. Winkelmann, Borchen - Weight at study initiation: 200 - 300 g ADMINISTRATION: - Area covered: intact shorn backside skin (10% of body surface) - Occlusion: semioclusive - Vehicle: none - Concentration in vehicle: undiluted test substance - Total volume applied: 1.74 cm ³ /kg - Doses: 2000 mg/kg bw - Removal of test substance: 24 hours after application, the skin was washed with warm water EXAMINATIONS: Clinical symptoms were recorded 0.5, 1, 2, 3, 4, 5, and 6 h after application of the test substance and once daily in the following two weeks. The application area was examined for substance related local effects. Body weights were recorded at days 0 (day of application of the test substance), 7 and 14. After the 14 day observation period all animals were killed by inhalation of CO ₂ , sectioned and investigated for substance related macroscopic organ changes.	
Reliability:	(1) valid without restriction Guideline study, GLP	
Flag: 04-AUG-2004	Material Safety Dataset, Critical study for SIDS endpoint	(28)
Type: Species: Value:	LD50 rabbit > 5000 mg/kg bw	
Method: GLP: Test substance:	other: no data no data no data	
Reliability: 12-AUG-2004	(4) not assignable	(51)
Type: Species: Value:	LD50 rabbit > 5000 mg/kg bw	
Method: Year: GLP: Test substance:	other: Acute Dermal Toxicity 1976 no data no data	
Reliability: 20-AUG-2004	(4) not assignable No details reported.	(49)

5.1.4 Acute Toxicity, other Routes

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Exposure: Occlusive
Exposure Time: 24 hour(s)
Result: irritating

Method: other: Skin Irritation
Year: 1976
GLP: no data
Test substance: no data

Remark: Dimethyl malonate applied full strength to intact or abraded rabbit skin for 24 h under occlusion was irritating. No further information available. Not classifiable according to current EEC directives.

Reliability: (4) not assignable
Secondary Reference only.

04-AUG-2004

(49)

Species: rabbit
Concentration: undiluted
Exposure: Semioclusive
Exposure Time: 4 hour(s)
No. of Animals: 3
Result: not irritating
EC classificat.: not irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Primary irritation score (24, 48, 72 hours)
AVERAGE SCORE
- Erythema: 3/3 animals: 0
- Edema: 3/3 animals: 0
Slight erythema (grade 1 was observed in all 3 animals 30 to 60 min after removal of the patch.
OTHER EFFECTS: No substance related clinical signs or macroscopic organ findings at necropsy were reported.

Test condition: TEST ANIMALS:
- Strain: Small white russian, Chbb: HM, SPF
- Sex: one male, 2 females
- Source: Fa. Dr. Karl Thomae GmbH, Biberach
- Weight at study initiation: 2 - 3 kg
- Number of animals: 3
ADMINISTRATION/EXPOSURE
- Preparation of test substance: undiluted test substance
- Area of exposure:
shorn skin of the dorsal area of the body, 6 cm²
- Occlusion: semiocclusive
- Vehicle: none
- Total volume applied: 0.5 cm³

- Postexposure period: 72 hours
 - Removal of test substance: remove by washing with warm water after 4 h.

EXAMINATIONS

- Scoring system: According to OECD No. 404
 - Examination time points: 30 to 60 min, 24 h, 48 h, 72 h.

Reliability: (1) valid without restriction
 Guideline study, GLP

Flag: Material Safety Dataset, Critical study for SIDS endpoint
 12-AUG-2004 (29)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 24 hour(s)
Comment: rinsed after (see exposure time)
No. of Animals: 3
Result: slightly irritating
EC classificat.: not irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: AVERAGE SCORE
 Primary irritation score (24, 48, 72 hours)
 Iris: animal 1: 1.0 animal 2: 0.33
 animal 3: 0.67
 Cornea: animal 1: 1.67 animal 2: 1.33
 animal 3: 1.0
 Conj. redness: 3/3 animals: 2.0
 Conj. chemosis: animal 1: 1.67 animal 2+3: 1.33
 Mean scores:
 Conjunctivae redness: 2.00
 Conjunctivae chemosis: 1.44
 Iris inflammatory changes: 0.67
 Corneal opacity: 1.33

DESCRIPTION OF LESIONS:

1 h p.a.: Medium grade erythema of the conjunctivae and slight corneal opacity as well as excretion of a white exsudate from the treated eyes was observed in all 3 animals. 2 animals had additionally a very slight edema, one a marked edema.

24 h p.a.: All 3 animals showed medium grade erythema and marked edema of the conjunctivae, indications of an irritation of the iris with intact pupil reaction. The cornea was slightly opaque (in 2 animals the whole area was affected, in 1 animal about half of the area). Exsudation was still observed in one animal.

48 h p.a.: The medium grade erythema was unchanged in all animals, while edema was reduced in 2 animals. 2 animals still showed iridal irritation while it was reduced in one animal. In 2 animals corneal opacity had increased slightly. Exsudation was still observed in one animal.

72 h p.a.: The medium grade erythema was unchanged in all animals, while edema was reversed. Iridal irritation was still observed in one animal. Slight corneal opacity was seen in 2

animals (covering one and 3 quarts of the surface respectively) while in 1 animal corneal opacity wa still unchanged.
6 d p.a.: Corneal and iris reactions were reversed completely in all animals, very slight conjunctival irritation and chemosis was observed in 2 animals.
8 d p.a.: All effects were reversible by day 8.
REVERSIBILITY: Effects were reversible within 8 days.

Test condition:

TEST ANIMALS:

- Strain: Small white Russian, Chbb: HM, SPF
- Sex: male
- Source: Fa. Dr. Karl Thomae GmbH, Biberach
- Weight at study initiation: 2 - 3 kg
- Number of animals: 3
- Control: untreated second eye of the animals

ADMINISTRATION/EXPOSURE

- Preparation of test substance: undiluted test substance
- Amount of substance instilled: 0.1 cm³
- Vehicle: none
- Postexposure period: 8 days p.a.

EXAMINATIONS

- Ophthalmoscopic examination: Fluorescein-test prior to the administration of the test substance to ensure intactness of cornea and 24 h, 48 h, 72 h, 6 d p.a.
- Scoring system: According to OECD No. 405.
- Observation period: 8 days

Reliability:

(1) valid without restriction

Guideline study, GLP

Flag:

Material Safety Dataset, Critical study for SIDS endpoint

04-AUG-2004

(27)

5.3 Sensitization

Type: Buehler Test
Species: guinea pig
Concentration 1st: Induction 100 %
2nd: Induction 100 %
3rd: Challenge 100 %

No. of Animals: 10
Result: not sensitizing
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
Year: 1981
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: no reaction observed in any of 10 animals

Result: RESULTS OF PILOT STUDY: No skin irritation was observed at any of the tested concentrations after 6, 24, and 48 h p.a.

RESULTS OF TEST

- Sensitization reaction: None of the test animals and non e of the control animals showed a positive skin reaction 24, 48, or 72 h after the challenge application. Under the conditions of the test the substance is not skin sensitizing.
- Clinical signs: None observed, body weight development during the study was normal.

Test condition: TEST ANIMALS: Guinea pigs
- Strain: Dunkin Hartley, Pirbright White Bor:DHPW (SPF)
- Sex: female
- Source: Winkelmann, Borchon
- Age: juvenile adult rats
- Weight at study initiation: 376-458 g
- Number of animals: 10
- Controls: 20
ADMINISTRATION/EXPOSURE
- Study type: Bühler test
- Preparation of test substance for induction: udiluted during all 3 induction applications.
- Induction schedule: 6 h occlusive applications on day 0, day 7 and day 14
- Challenge schedule: day 28, 6 h occlusive
- Concentrations used for challenge: undiltued test substance
EXAMINATIONS
- Grading system: according to OECD 406
- Pilot study: yes
- Positive control: regular assessment of the reliability and sensitivity of the test system with standard allergens.
- Deviations from guideline: although a negative result was obtained, the test group consisted only of 10 animals. (The guideline recommends the use of 20 test animals in this case).

Reliability: (2) valid with restrictions
Guideline study, GLP
Flag: Material Safety Dataset, Critical study for SIDS endpoint
30-NOV-2004 (34)

Type: other: Maximization test
Species: human
Result: not sensitizing

Method: other: Maximization Test According to Kligman, 1966 and Kligman and Eppstein, 1975
Year: 1975
GLP: no
Test substance: no data

Remark: A maximization test was employed and carried out on 25 volunteers.
Tested at a concentration of 8 % in petrolatum, dimethyl malonate produced no sensitization in the maximization test on human subjects.

Reliability: (2) valid with restrictions
Well documented secientific reference
Flag: Critical study for SIDS endpoint
30-NOV-2004 (44) (45) (46)

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: gavage
Exposure period: 39 to 51 days (from 14 days before mating to day 3 of lactation)
Frequency of treatment: daily, 7 days per week
Post exposure period: 14 days

Doses: 0, 100, 300, 1000 mg/kg bw per day
Control Group: yes, concurrent vehicle
NOAEL: = 300 mg/kg bw
LOAEL: = 1000 mg/kg bw

Method: OECD combined study TG422
Year: 2004
GLP: yes
Test substance: other TS: Dimethyl malonate

Method: OECD Combined Repeated Dose and Reproduction/Developmental Screening Test.

Result: Mortality: No mortality was observed in any of the dose groups.

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

- Clinical signs:
 No test item related clinical signs were observed throughout the test and recovery period in any of the dose groups.

- FOB:
 No treatment related changes were observed.

- Body weight gain:
 No treatment related effects on body weight and body weight gain were observed.

- Food consumption:
 No treatment related effects were observed.

- Clinical chemistry:
 No treatment related effects were observed.

- Haematology:
 No treatment related changes were observed.

- Organ weights:
 No treatment related effects were observed.

- Gross pathology:
 No treatment related effects were observed.

- Histopathology:
 1000 mg/kg bw: Livers of males and females showed a significantly increased incidence of hepatocellular hypertrophy. The change was considered reversible as the incidence was not significantly increased in the high dose recovery animals.

300 and 100 mg/kg bw: No treatment related changes of the liver were observed.

All other histopathological findings were not considered treatment related.

STATISTICAL RESULTS: Significantly increased hepatocellular hypertrophy in the high dose group only.

Test condition: TEST ORGANISMS

- HSDCpb-WU rats
 - Age at start of treatment: 11-12 weeks
 - Weight at study initiation: males: 377-379 g, females: 210-219 g
 - Number of animals: Main group: 10 m, 10 f, recovery groups: 5 m, 5 f

ADMINISTRATION / EXPOSURE

- Duration of test/exposure:
 Males: Test groups: 39 days, male recovery group: 39 exposure days, 45 test days.
 Females: Treatment groups: 51 +- 7 days, recovery group: 39 exposure days, 45 test days.

- Type of exposure: Oral gavage
- Post exposure period: 14 days
- Vehicle: Double distilled water
- Concentration in vehicle: 10 mg/ml, 30 mg/ml, 100 mg/ml
- Total volume applied: 10 ml/kg bw
- Doses: 100, 300, 1000 mg/kg bw

Treatment:

Male rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period and approximately 2 weeks post mating.

Female rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period, pregnancy and up to lactation day 4.

For the high dose recovery group animals (males and females) the test item was administered once daily, 7 days per week by gavage throughout the treatment period.

CLINICAL OBSERVATIONS AND FREQUENCY:

- Daily observations for appearance, behaviour, clinical signs and preterminal deaths. Females were observed for signs of difficult and prolonged parturition.

- Twice daily: morbidity and mortality.

- Detailed clinical observations: once before exposure and at least once per week thereafter. Signs included were changes in skin, fur, eyes, mucous membranes, occurrence of secretions or excretions and autonomic activity, changes in gait, posture, response to handling, behavioural changes, difficult or prolonged parturition.

Functional observation battery (FOB): Examinations were performed in randomly selected 5 animals of each group at the end of the dosing period for males and during the lactation period for females and included homecage observations, handling observations, open field tests, sensory observations, neuromuscular observations and physiological observations (body temperature).

Body weights were recorded at the beginning of the study, at least weekly thereafter and at termination. All dams were weighed on gestation days 0, 7, 14 and 20 and lactation days 0 and 4.

Food consumption was recorded weekly.

The fertility index for males and females was determined.

LITTER DATA: All pups from each litter were examined for any external deformities, litter size and sex distribution was determined. Pup weights were recorded on day 0 and 4. All pups were examined for malformations and subject to gross pathological examination. Pup survival index up to lactation day 4 was determined.

HAEMATOLOGY/CLINICAL CHEMISTRY: Standard haematological and clinical chemistry parameters were determined at the end of the pre-mating period and the recovery period in 5 randomly selected males and females of each group.

ORGAN WEIGHTS: Organ weights of liver, adrenals, kidneys, thymus spleen, brain and heart were determined of 5 males and females of each group. Testes and epididymis weights of all adult males of each group were also determined.

GROSS PATHOLOGY.

All adult animals and pups were examined for any structural abnormalities and pathological changes.

HISTOPATHOLOGY:

The following tissues of 5 males and females of the control and high dose group as well as all animals of the recovery and recovery control groups were examined microscopically: all gross lesions, brain, spinal cord, gastrointestinal tract, liver, kidney, adrenals, spleen, heart, thymus, thyroid, trachea, lungs, testes (fixed in Bouins fluid), epididymes (fixed in Bouins fluid), ovaries, uterus, seminal vesicles, coagulating glands, prostate, urinary bladder, axillary lymph nodes, mesenteric lymph nodes, sciatic nerve, femur with marrow, bone marrow smear. Stages of spermatogenesis and interstitial testicular structure in male gonads were determined additionally. Livers of 5 males and females in the mid and low dose groups and testes of 5 males of the mid and low dose groups were also examined.

STATISTICAL ANALYSIS:

Dunnett's t-Test: body weight, body weight change, food intake, haematology, clinical chemistry, organ weight, FOB, gestation length, litter size, No. corpora lutea, No. implantation.

Z-Test/Student's t-Test: Mating performance, conception rate, fertility index, gestation index, live birth index, viability index, sex ratio, pup survival data, No. littered, No. dead pups, No. live pups, Pup survival data, Pre- and Post-implantation loss. Histopathological data.

t-Test/ANOVA: dose correlation

Test substance: Dimethyl malonate, purity: 99.8%.

Reliability: (1) valid without restriction

Guideline study, GLP

Flag: Material Safety Dataset, Critical study for SIDS endpoint

11-AUG-2004

(13)

5.5 Genetic Toxicity 'in Vitro'

Type: Cytogenetic assay
System of testing: Human peripheral lymphocytes
Concentration: 312.5, 625, 1250, 2500, 5000 µg/ml medium
Cytotoxic Concentration: 5000 µg/ml
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 473
Year: 2003
GLP: yes
Test substance: other TS: Dimethyl malonate

Result: GENOTOXIC EFFECTS:
 - With metabolic activation:
 The mean incidence of chromosomal aberrations excluding gaps at concentrations from 625 to 5000 µg/ml ranged from 1.5% to 3.5% and was comparable to control rates and within the historical control range of 0 to 5%. There was no dose related increase in chromosomal aberrations. No polyploidy was noted.
 - Without metabolic activation: The mean incidence of

chromosomal aberrations excluding gaps at concentrations from 625 to 5000 µg/ml ranged from 1.0% to 3% and was comparable to control rates and within the historical control range of 0 to 5%. There was no dose related increase in chromosomal aberrations. No polyploidy was noted.

All positive and negative controls gave the expected results that were within the ranges of the laboratory and consistent with those reported in the literature.

MITOTIC INDEX:

Pretest:

Without S9 mix, 24 h exposure:

At concentrations up to 250 µg/ml \geq 1.

1000 µg/ml: 0.44

2500 µg/ml: 0.72

5000 µg/ml: 0

With S9, 4 h exposure:

At concentrations up to 1000 µg/ml: 0.72 to 1.0 (not concentration dependent)

2500 µg/ml: 0.44

5000 µg/ml: 0

Main experiment:

Without S9, 4 h exposure: not significantly reduced (0.88 to 1.0)

Without S9, 24 h exposure: \geq 1 up to 625 µg/ml.

1250 µg/ml: 0.79

2500 µg/ml: 0.54

With S9:

Not significantly reduced up to 1250 µg/ml: (0.92 to 1.22)

2500 µg/ml 0.72 and 0.87

5000 µg/ml: 0 in both experiments

CYTOTOXIC CONCENTRATION:

- With metabolic activation: Pretest and main test: 5000 µg/ml

- Without metabolic activation: Pretest and main test: 5000 µg/ml after 24 h.

STATISTICAL RESULTS: The incidence of chromosomal aberrations (excluding gaps) was not significantly different from the controls with and without metabolic activations at all tested dose levels using Fisher exact test ($P \leq 0.05$). The positive controls induced a statistically significant increase in chromosomal aberrations excluding gaps.

Test condition:

Cell culture:

Human peripheral blood was obtained by venipuncture from healthy donors without medication and collected in heparinised vessels. 0.5 ml samples of whole blood were added to tubes containing 5 ml of complete culture medium and incubated at 37 °C with occasional shaking.

Solvent: DMSO

Negative control: Solvent: DMSO

Positive control: Mitomycin C in the absence of metabolic activation (0.1 and 0.2 µg/ml medium), cyclophosphamide in the presence of metabolic activation (10 to 20 µg/ml medium).

Metabolic activation system: Postmitochondrial (S9) fraction of rats treated with Arochlor 1254

Preliminary cytotoxicity test:

With concentrations from 10 to 5000 µg/ml medium with and without metabolic activation, 48 h after culture establishment, 24 h incubation. Examination of 1 slide per

culture, 1000 lymphocytes per culture. Calculation of mitotic index.

Main study:

Experiment 1:

The test item or test item plus S9 mix was added to the the cultures after 48 h of culture and incubated for 4 h at 37 °C.

After centrifugation and washing the resuspended cell pellet was incubated for futher 20 h in the dark. Colcemid was added to arrest cell division and the cells incubated for a further 2 h. The cells were harvested, fixed in freshly prepared methanol: glacial acetic acid (4: 1) and slides prepared.

Experiment 2: With S9: same procedure as experiment 1.

Without S9:

In the absence of S9 a continuous treatment for 24 h was performed. After centrifugation and washing the resuspended cell pellet was incubated for futher 20 h in the dark. Colcemid was added to arrest cell division and the cells incubated for a further 2 h. The cells were harvested, fixed in freshly prepared methanol: glacial acetic acid (4: 1) and slides prepared.

All cultures were run in duplicate using blood from a different donor.

Stain: Giemsa

For each treatment and culture 100 metaphases per plate were examined.

For the determination of cytotoxicity 1000 cells were scored and the mitotic index determined as percentage of cells in metaphase.

Statistical evaluation:

Fisher Exact Test.

Test substance:

Dimethyl malonate, purity: 99.8%.

Reliability:

(1) valid without restriction

Flag:

Material Safety Dataset, Critical study for SIDS endpoint

17-AUG-2004

(16)

Type:

Ames test

System of testing:

Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100

Concentration:

8 - 5000 ug/plate

Metabolic activation:

with and without

Result:

negative

Method:

Directive 84/449/EEC, B.14

Year:

1992

GLP:

yes

Test substance:

as prescribed by 1.1 - 1.4

Method:

Method:

Directive 84/449/EEC, B.14

Year: 1984

Result:

GENOTOXIC EFFECTS:

- With metabolic activation: negative

- Without metabolic activation: negative

PRECIPITATION CONCENTRATION: no precipitation occurred.

CYTOTOXIC CONCENTRATION:

- With metabolic activation: >= 1000 µg/plate

- Without metabolic activation: >= 1000 µg/plate

STATISTICAL RESULTS:

The test substance did not induce a statistically significant increase in the number of revertants in any of the strains tested, neither with nor without metabolic activation. The test substance was non-mutagenic under the conditions of this test.

Test condition:

SYSTEM OF TESTING

- Species/cell type: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
- Metabolic activation system: phenobarbiturate induced rat liver S9 fraction of male Wistar rats.
- Number of replicates: 2 independent assays, one plate incorporation, on pre-incubation test. 3 replicates per concentration.

- Positive control substances:

Without S9:

Strain	Substance
TA 98, 1538	Nitrofluorene
TA 100, 1535	Sodium azide
TA 1537	Aminoacridine

With S9: Cyclophosphamide (TA 100 only).

- Pre-incubation time: 30 min at 30 +/- 1°C

STATISTICAL METHODS:

Mean values and standard deviation were calculated with BIOSYS software.

Reliability:

(1) valid without restriction

Guideline study in accordance with testguidelines valid at the time of the study, GLP

Flag:

Material Safety Dataset, Critical study for SIDS endpoint

04-AUG-2004

(30)

5.6 Genetic Toxicity 'in Vivo'**5.7 Carcinogenicity****5.8.1 Toxicity to Fertility****Type:**

other: OECD Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Screening Test

Species:

rat

Sex:

male/female

Strain:

Wistar

Route of administration:

gavage

Exposure Period:

39 to 51 days (from 14 days before mating to day 3 of lactation)

Frequency of treatment:

daily, 7 days per week

Premating Exposure Period**male:**

2 weeks

female:

2 weeks

Duration of test:

Males: 39 days, females 51 +/- 7 days, recovery groups: 39 days

Doses:

100, 300, 1000 mg/kg bw

Control Group:

yes, concurrent vehicle

NOAEL Parental:

= 300 mg/kg bw

NOAEL F1 Offspring:

> 1000 mg/kg bw

Result:

No treatment related effects on fertility

Method: OECD combined repeated dose and reproductive/developmental toxicity screening test

Year: 2004

GLP: yes

Test substance: other TS: Dimethyl malonate

Method: OECD Combined Repeated Dose and Reproduction/Developmental Screening Test.

Result: For effects on parent animals: See section 5.4, repeated dose toxicity.

Reproductive results

- Fertility index:

Males, all dose groups: 100%

Females, all dose groups: 100%

- Duration of gestation:

Dose (mg/kg bw)	Duration (days) +- SD
0	22 +- 0.3
100	23 +- 0.5
300	23 +- 0.5
1000	22 +- 0.4

- Gestation index:

100 % in all dose groups.

- Parturition: 100 % in all dose groups

- Effects on sperm: No treatment related effects

- Number of implantations:

Dose (mg/kg bw)	No.	Percent
0	12.3	88.1
100	11.8	84.7
300	12.0	88.9
1000	11.6	88.6

- Number of corpora lutea:

Dose (mg/kg bw)	No.
0	14.0
100	13.9
300	13.5
1000	13.1

Percentage pre-implantation loss

Dose (mg/kg bw)	Percent
0	11.9
100	15.3
300	11.1
1000	11.4

Percentage post-implantation loss

Dose (mg/kg bw)	Percent
0	8.1
100	19.1
300	10.4
1000	15.1

Litter results:

- Number of pups born

Dose (mg/kg bw)	No.
0	103
100	76
300	86
1000	84

No of live litters

Dose (mg/kg bw)	No.
0	9
100	8

6. REFERENCES

ID: 108-59-8

DATE: 21.01.2005

300	8
1000	8
Mean litter size index	
Dose (mg/kg bw):	
0	11.4
100	9.5
300	10.8
1000	10.5
Mean viable litter size:	
Dose (mg/kg bw):	
0	11.3
100	9.5
300	10.8
1000	9.9
No. of pups alive on day 0	
Dose (mg/kg bw) No.	
0	102
100	76
300	86
1000	79
Live birth index:	
Dose (mg/kg bw)	
0	99
100	100
300	100
1000	94
Sex ratio at birth (no of males/total number born x 100)	
Dose (mg/kg bw)	
0	46.6
100	60.5
300	54.7
1000	46.4
24 hour survival: 100% all dose groups.	
No of pups alive on day 4 of lactation	
Dose (mg/kg bw) No.	
0	101
100	75
300	83
1000	78
Day 4 survival index:	
Dose (mg/kg bw):	
0	99.0
100	98.7
300	96.5
1000	98.7
Sex ratio day 4	
Dose (mg/kg bw):	
0	44.7
100	60.5
300	53.5
1000	41.7
No of pups dead or cannibalised up to day 4	
Dose (mg/kg bw):	
0	2
100	1
300	3
1000	6
Observations and necropsy findings on pups:	
No treatment related effects were observed.	
STATISTICAL RESULTS:	

Fertility indices for males and females were not statistically different from controls in all dose groups.

In the low dose group post implantation loss and consequently the percentage of live pups born was significantly reduced compared to controls ($P \leq 0.05$). These changes were considered incidental and not treatment related as the effects were not observed at the higher dose groups.

No statistical significant differences from controls were observed for the number of pregnancies, number littered, number of live litters, mean litter size, mean viable litter size, sex ratio at birth, number of pups dead at first observation or day 2 to 4, number of live pups on day 0,3 and 4 and the associated survival indices, external abnormalities of life and dead pups at all dose levels.

A significantly higher percentage of male rats in the low dose group on day 4 was considered incidental and not treatment related as a similar change was not found in the higher dose groups.

The mean number and the mean weight of male and female (and both sexes combined) pups during different intervals of the lactation period were not statistically significantly different from controls except from a significantly lower ($P \leq 0.05$) mean number of female pups on lactation day 4 in the low dose group which was considered incidental and not related to treatment.

Test condition:

TEST ORGANISMS

- HSDCpb-WU rats
- Age at start of treatment: 11-12 weeks
- Weight at study initiation: males: 377-379 g, females: 210-219 g
- Number of animals: Main group: 10 m, 10 f, recovery groups: 5 m, 5 f

ADMINISTRATION / EXPOSURE

- Duration of test/exposure:
Males: Test groups: 39 days, male recovery group: 39 exposure days, 45 test days.
Females: Treatment groups: 51 +- 7 days, recovery group: 39 exposure days, 45 test days.
- Type of exposure: Oral gavage
- Post exposure period: 14 days
- Vehicle: Double distilled water
- Concentration in vehicle: 10 mg/ml, 30 mg/ml, 100 mg/ml
- Total volume applied: 10 ml/kg bw
- Doses: 100, 300, 1000 mg/kg bw

Treatment:

Male rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period and approximately 2 weeks post mating.

Female rats: The test item was administered once daily, 7 days per week by gavage for 2 weeks prior to mating, during the mating period, pregnancy and up to lactation day 4.

For the high dose recovery group animals (males and females) the test item was administered once daily, 7 days per week by gavage throughout the treatment period.

MATING PROCEDURES:

Male/female ratio: 1:1 per cage. Cohabitation period until evidence of pregnancy (sperm in vaginal smear) was observed.

CLINICAL OBSERVATIONS AND FREQUENCY:

- Daily observations for appearance, behaviour, clinical signs and preterminal deaths. Females were observed for signs of

difficult and prolonged parturition.
- Twice daily: morbidity and mortality.
- Detailed clinical observations: once before exposure and at least once per week thereafter. Signs included were changes in skin, fur, eyes, mucous membranes, occurrence of secretions or excretions and autonomic activity, changes in gait, posture, response to handling, behavioural changes, difficult or prolonged parturition.

Functional observation battery (FOB): Examinations were performed in randomly selected 5 animals of each group at the end of the dosing period for males and during the lactation period for females and included homecage observations, handling observations, open field tests, sensory observations, neuromuscular observations and physiological observations (body temperature).

Body weights were recorded at the beginning of the study, at least weekly thereafter and at termination. All dams were weighed on gestation days 0, 7, 14 and 20 and lactation days 0 and 4.

Food consumption was recorded weekly.

The fertility index for males and females was determined.

LITTER DATA: All pups from each litter were examined for any external deformities, litter size and sex distribution was determined. Pup weights were recorded on day 0 and 4. All pups were examined for malformations and subject to gross pathological examination. Pup survival index up to lactation day 4 was determined.

HAEMATOLOGY/CLINICAL CHEMISTRY: Standard haematological and clinical chemistry parameters were determined at the end of the pre-mating period and the recovery period in 5 randomly selected males and females of each group.

ORGAN WEIGHTS: Organ weights of liver, adrenals, kidneys, thymus spleen, brain and heart were determined of 5 males and females of each group. Testes and epididymis weights of all adult males of each group were also determined.

GROSS PATHOLOGY.

All adult animals and pups were examined for any structural abnormalities and pathological changes.

HISTOPATHOLOGY:

The following tissues of 5 males and females of the control and high dose group as well as all animals of the recovery and recovery control groups were examined microscopically: all gross lesions, brain, spinal cord, gastrointestinal tract, liver, kidney, adrenals, spleen, heart, thymus, thyroid, trachea, lungs, testes (fixed in Bouins fluid), epididymes (fixed in Bouins fluid), ovaries, uterus, seminal vesicles, coagulating glands, prostate, urinary bladder, axillary lymph nodes, mesenteric lymph nodes, sciatic nerve, femur with marrow, bone marrow smear. Stages of spermatogenesis and interstitial testicular structure in male gonads were determined additionally. Livers of 5 males and females in the mid and low dose groups and testes of 5 males of the mid and low dose groups were also examined.

STATISTICAL ANALYSIS:

Dunnett's t-Test: body weight, body weight change, food intake, haematology, clinical chemistry, organ weight, FOB, gestation length, litter size, No. corpora lutea, No. implantation.

Z-Test/Student's t-Test: Mating performance, conception rate, fertility index, gestation index, live birth index, viability index, sex ratio, pup survival data, No. littered, No. dead pups, No. live pups, Pup survival data, Pre- and Post-implantation loss. Histopathological data.

t-Test/ANOVA: dose correlation

Test substance: Dimethyl malonate, purity: 99.8%.

Reliability: (1) valid without restriction

Flag: Material Safety Dataset, Critical study for SIDS endpoint

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5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: gavage
Exposure period: 39 to 51 days (from 14 days before mating to day 3 of lactation)
Frequency of treatment: daily, 7 days per week
Duration of test: Males: 39 days, females 51 +- 7 days, recovery groups: 39 days
Doses: 100, 300, 1000 mg/kg bw
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: = 300 mg/kg bw
NOAEL Teratogenicity: >= 1000 mg/kg bw

Method: OECD Combined Repeated Dose and Reproduction/Developmental Screening Test.

Result: For effects on parent animals: See section 5.4, repeated dose toxicity.

Reproductive results

- Fertility index:

Males, all dose groups: 100%

Females, all dose groups: 100%

- Duration of gestation:

Dose (mg/kg bw)	Duration (days) +- SD
0	22 +- 0.3
100	23 +- 0.5
300	23 +- 0.5
1000	22 +- 0.4

- Gestation index:

100 % in all dose groups.

- Parturition: 100 % in all dose groups

- Effects on sperm: No treatment related effects

- Number of implantations:

Dose (mg/kg bw)	No.	Percent
0	12.3	88.1
100	11.8	84.7
300	12.0	88.9
1000	11.6	88.6

- Number of corpora lutea:

Dose (mg/kg bw)	No.
0	14.0
100	13.9
300	13.5
1000	13.1
Percentage pre-implantation loss	
Dose (mg/kg bw)	Percent
0	11.9
100	15.3
300	11.1
1000	11.4
Percentage post-implantation loss	
Dose (mg/kg bw)	Percent
0	8.1
100	19.1
300	10.4
1000	15.1
Litter results:	
- Number of pups born	
Dose (mg/kg bw)	No.
0	103
100	76
300	86
1000	84
No of live litters	
Dose (mg/kg bw)	No.
0	9
100	8
300	8
1000	8
Mean litter size index	
Dose (mg/kg bw):	
0	11.4
100	9.5
300	10.8
1000	10.5
Mean viable litter size:	
Dose (mg/kg bw):	
0	11.3
100	9.5
300	10.8
1000	9.9
No. of pups alive on day 0	
Dose (mg/kg bw)	No.
0	102
100	76
300	86
1000	79
Live birth index:	
Dose (mg/kg bw)	
0	99
100	100
300	100
1000	94
Sex ratio at birth (no of males/total number born x 100)	
Dose (mg/kg bw)	
0	46.6
100	60.5
300	54.7

1000 46.4
 24 hour survival: 100% all dose groups.
 No of pups alive on day 4 of lactation

Dose (mg/kg bw)	No.
0	101
100	75
300	83
1000	78

Day 4 survival index:
 Dose (mg/kg bw):

0	99.0
100	98.7
300	96.5
1000	98.7

Sex ratio day 4
 Dose (mg/kg bw):

0	44.7
100	60.5
300	53.5
1000	41.7

No of pups dead or cannibalised up to day 4
 Dose (mg/kg bw):

0	2
100	1
300	3
1000	6

Observations and necropsy findings on pups:
 No treatment related effects were observed.

STATISTICAL RESULTS:

Fertility indices for males and females were not statistically different from controls in all dose groups.

In the low dose group post implantation loss and consequently the percentage of live pups born was significantly reduced compared to controls ($P \leq 0.05$). These changes were considered incidental and not treatment related as the effects were not observed at the higher dose groups.

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- Concentration in vehicle: 10 mg/ml, 30 mg/ml, 100 mg/ml

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t-Test/ANOVA: dose correlation

Dimethyl malonate, purity: 99.8%.

Test substance:

Reliability:

Flag:

11-AUG-2004

(1) valid without restriction

Material Safety Dataset, Critical study for SIDS endpoint

(13)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

5.11 Additional Remarks

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