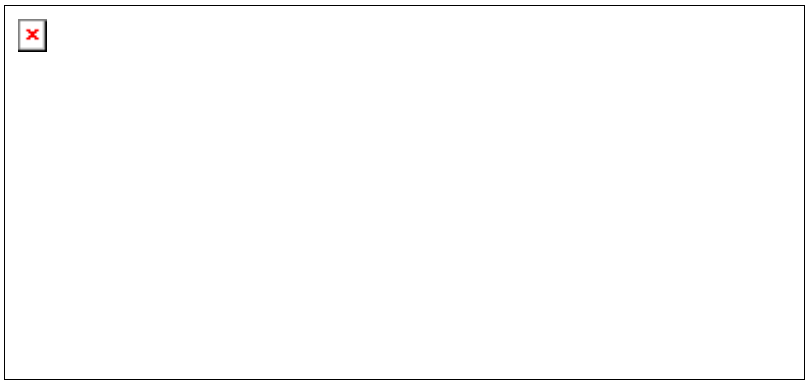

Printing Date 2010-01-06 07:56:33 CET

Restriction of specific regulatory purposes

EU: REACH, other:

Confidentiality

Name	166412-78-8_master_1,2-Cyclohexandicarbonsäurediisononylester (IUC4 DSN 322)
Legal entity owner	BASF SE / Ludwigshafen am Rhein / Germany

Substance: 166412-78-8_master_1,2-Cyclohexandicarbonsäurediisononylester (IUC4 DSN 322)

UUID IUC4-d38fc98e-d204-3a91-94e8-5213de8883c0
Dossier UUID 0
Author otterr / BASF SE / Ludwigshafen am Rhein / Germany
Date 2009-11-03 17:55:51 CET
Remarks Created endpoint study record 7.3 Irritation / corrosion

0 Related Information
0.1 Templates
0.2 Categories
0.3 Mixtures
1 General Information
1.1 Identification
Substance identification

Chemical name 166412-78-8_master_1,2-Cyclohexandicarbonsäurediisononylester (IUC4 DSN 322)

EU: REACH

Legal entity BASF SE / Ludwigshafen am Rhein / Germany

EU: REACH

Role in the supply chain

EU: REACH

Role: (X) Manufacturer ( ) Importer ( ) Only representative ( ) Downstream user

Reference substance

Reference substance 1,2-Cyclohexandicarbonsäurediisononylester / 166412-78-8

EC number **EC name**

CAS number **CAS name**
166412-78-8

IUPAC name

Type of substance

Composition other: Existing Chemical

Trade names

EU: REACH

Name 1,2-Cyclohexandicarbonsaeurediisononylester

Name 1,2-Cyclohexanedicarboxylic acid, diisononylester

Name DINCH

Name 1,2 Cyclohexanedicarboxylic acid, bis (isononyl)ester

Name Di(isononyl), Cyclohexan-1,2-dicarbonsaeureester

Contact person

Person flags **EU: REACH**

1.2 Composition

2 Classification and Labelling

2.1 GHS

Classification and Labelling according to GHS

EU: REACH

General information

Name 1,2-Cyclohexanedicarboxylic acid, diisononyl ester

(X) Not classified

Implementation EU

Classification

Physical hazards

	Hazard statement	Reason for no classification
Explosives		conclusive but not sufficient for classification
Flammable gases		data lacking
Flammable aerosols		data lacking
Oxidizing gases		data lacking
Gases under pressure		data lacking
Flammable liquids		conclusive but not sufficient for classification
Flammable solids		data lacking
Self-reactive substances and mixtures		data lacking
Pyrophoric liquids		conclusive but not sufficient for classification
Pyrophoric solids		data lacking
Self heating substances and mixtures		data lacking
Substances and mixtures which in contact with water emits flammable gases		conclusive but not sufficient for

	classification
Oxidising liquids	conclusive but not sufficient for classification
Oxidising solids	data lacking
Organic peroxides	data lacking
Substance and mixtures corrosive to metals	data lacking

Health hazards

	Hazard statement	Reason for no classification
Acute toxicity - oral		conclusive but not sufficient for classification
Acute toxicity - dermal		conclusive but not sufficient for classification
Acute toxicity - inhalation		data lacking
Skin corrosion/irritation		conclusive but not sufficient for classification
Serious damage/ eye irritation		conclusive but not sufficient for classification
Respiratory sensitization		data lacking
Skin sensitization		conclusive but not sufficient for classification
Aspiration hazard		data lacking

Germ cell mutagenicity

	Hazard statement	Reason for no classification
Germ cell mutagenicity		conclusive but not sufficient for classification
Route of exposure		

Carcinogenicity

	Hazard statement	Reason for no classification
Carcinogenicity		conclusive but not sufficient for classification
Route of exposure		

	Hazard statement	Reason for no classification

Reproductive toxicity	conclusive but not sufficient for classification
Specific effect	
Route of exposure	
Effects on or via lactation	conclusive but not sufficient for classification

Specific target organ toxicity - single

	Hazard statement	Reason for no classification
Specific target organ toxicity - single		conclusive but not sufficient for classification
Affected organs		
Route of exposure		

Specific target organ toxicity - repeated

	Hazard statement	Reason for no classification
Specific target organ toxicity - repeated		conclusive but not sufficient for classification
Affected organs		
Route of exposure		

Environmental hazards

	Hazard statement	Reason for no classification
Hazardous to the aquatic environment		conclusive but not sufficient for classification

Additional hazard classes

Specific concentration limits

Notes

2.2 DSD - DPD

Classification and Labelling according 67/548/EEC (DSD)

EU: REACH

General information

Name 1,2-Cyclohexanedicarboxylic acid, diisononyl ester

(X) Not classified

Status 67/548/EEC self classification

Remarks

Classification

	Classification	Reason for no classification
Explosiveness		conclusive but not sufficient for classification
Oxidising properties		conclusive but not sufficient for classification
Flammability		conclusive but not sufficient for classification
Thermal stability		conclusive but not sufficient for classification
Acute toxicity		conclusive but not sufficient for classification
Acute toxicity - irreversible damage after single exposure		conclusive but not sufficient for classification
Repeated dose toxicity		conclusive but not sufficient for classification
Irritation / Corrosion		conclusive but not sufficient for classification
Sensitisation		conclusive but not sufficient for classification
Carcinogenicity		conclusive but not sufficient for classification

Mutagenicity - Genetic Toxicity	conclusive but not sufficient for classification
Toxicity to reproduction - fertility	conclusive but not sufficient for classification
Toxicity to reproduction - development	conclusive but not sufficient for classification
Toxicity to reproduction - breastfed babies	conclusive but not sufficient for classification
Environment	conclusive but not sufficient for classification

Notes

7 Toxicological information
7.1 Toxicokinetics, metabolism and distribution
7.1.1 Basic toxicokinetics
Endpoint study record: Key.BASFAG48CO107/01181.Basic toxicokinetics

UUID	IUC5-4f5995e7-a66b-4ff1-9065-610b417df9e6
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:00 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2005
Title	1,2-Cyclohexanodicarboxylic acid, diisononyl ester: Liver enzyme induction study in Wistar rats; Administration in the diet over 2 weeks.		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	48CO107/01181
Owner company	BASF SE		
Company study no.		**Report date**	2005-10-04

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Objective of study

other: Liver enzyme induction

Test guideline

Qualifier	no guideline available
Guideline	
Deviations	

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Radiolabelling

no

Test materials

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Test substance No.: 01/0107-1
- Synonym: DINCH

- Production-/filling date: May 08, 2000
- Physical state: Liquid/clear-colourless
- Analytical purity: 99.7 area-% (report no. 04L00301)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: CrlGlxBrlHan:WI
- Source: Charles River, Sulzfeld, Germany
- Age at study initiation: 6 weeks
- Weight at study initiation: mean 165.4 g (males), 134.3 g (females)
- Housing: singly in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area about 800 cm2)
- Individual metabolism cages: no
- Diet (e.g. ad libitum): maintenance diet for mice/rats “GLP”, meal from Provimi KLIBA SA, Kaiseraugst / Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: 8 (males) and 9 (females) days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): once before the start of the study
- Mixing appropriate amounts with (Type of food): The test substance was weighed out and thoroughly mixed with a small amount of food. Then corresponding amounts of food were added to this premix in order to obtain the desired concentration, and mixing was carried out for about 3 minutes in a Hobart-mixing bowl and 10 minutes in a Ruberg EM 100 laboratory mixer

HOMOGENEITY AND STABILITY OF TEST MATERIAL:
The stability of the test substance in the diet was demonstrated over a period of 50 days at room temperature in a previous study

Duration and frequency of treatment / exposure

daily for 14 days

Doses / concentrations

Males: 15000 ppm/1418 mg/kg bw/day
Females: 15000 ppm/1568 mg/kg bw/day

No. of animals per sex per dose

5

Control animals

yes, plain diet

Details on dosing and sampling

PHARMACOKINETIC STUDY
- Tissues and body fluids sampled: liver
- Time and frequency of sampling: at the end of study
- Method: All surviving animals were sacrificed without fasting period. In order to investigate the phase I and phase II liver enzymes activity, all animals were killed by cervical dislocation and decapitation after the administration period. The liver was taken from the carcass. Liver homogenates and microsomes were prepared according to standard operation procedures. The assays were performed under internal laboratory quality control conditions with positive controls. S9 -fraction from Aroclor 1254 treated rats served as positive control to verify the correctness of the tests.

Statistics

Welch-Test (two sided) for the hypothesis of equal means for food consumption, body weight and body weight change; WILCOXON-test (onesided) for the hypothesis of equal medians for the enzyme concentrations

Any other information on materials and methods incl. tables

OBSERVED PARAMETERS FOR THE DETECTION OF POSSIBLE ENZYME INDUCTION:

Cytochrome P450 (Cyt.P450), Ethoxyresorufln-0-deethylase (EROD), Pentoxyresorufin-O-depentylase (PROD), Benzoxyresorufln-0-debenzy[ase (BROD), 4-Methylumbelliferone-glucuronyltransferase (MUF-GT), 4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT)

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule for examinations of morbidity and mortality: 2/workday; 1/weekend day

BODY WEIGHT: Yes

- Time schedule for examinations: weekly

FOOD CONSUMPTION:

- Food consumption for each animal determined and mean daily diet consumption
calculated as g food/kg body weight/day: Yes

WATER CONSUMPTION: Yes

- Time schedule for examinations: 1/d

SACRIFICE AND PATHOLOGY: No

Results and discussions

Metabolite characterisation studies

Metabolites identified

not measured

Remarks on results including tables and figures

Results:

MORTALITY:

No animal died during the study.

CLINICAL OBSERVATIONS:

No abnormal clinical signs were detected.

FOOD & WATER CONSUMPTION:

No treatment-related finding were observed.

BODY WEIGHT:

No treatment-related finding were observed.

SUBSTANCE INTAKE:

The mean daily test substance intake over the entire study period was 1418mg/kg bw/dfor males and 1568 mg/kg bw/d for females.BIOANALYTICS:

Increase by factor

Enzyme	males	females
Cyt P450	2.2	2.2
EROD	2.7	1.6
PROD	30	43
BROD	11	24
MUF-GT	3.3	2.4
HOBI-GT	7.2	2.7

All findings are considered to be treatment related.

Overall remarks, attachments

Overall remarks

Regarding the parameters determined for liver enzyme induction, marked increases for the total Cyt.P450 content in liver, the activities in the EROD-, PROD- and BROD-assay as well as for the phase 2-liver-enzymes MUF-GT and HOBI-GT were detected. For the phase 1-enzymes, the strongest induction effects were determined for the Pentoxyresorufin-O-depentylase and the Benzoxyresorufin-O-debenzylase, indicating an induction comparable to the Phenobarbital-type. For the phase 2- enzymes, HOBI-GT was more affected by the induction of 1,2-cyclohexanedicarboxylic acid, diisononyl ester than MUF-GT.

In conclusion, these findings indicate that 1,2-Cyclohexanedicarboxylic acid, diisononyl ester is an enzyme inductor of phase I- and phase II-liver-enzymes in male and female Wistar rats.

Endpoint study record: Key.BASFAG02B0608/016021.Basic toxicokinetics

UUID	IUC5-ec852219-508d-46cd-afeb-ef2e6881949a
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:02 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASFG AG	**Year**	2003
Title	14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester – Study of the Biokinetics in Rats		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	02B0608/016021
Owner company	BASF SE		
Company study no.		**Report date**	2003-10-15

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Objective of study

absorption

distribution

excretion

toxicokinetics

Test guideline

Qualifier	according to
Guideline	OECD Guideline 417 (Toxicokinetics)
Deviations	no
Qualifier	according to
Guideline	EU Method B.36 (Toxicokinetics)
Deviations	no
Qualifier	according to
Guideline	EPA OPPTS 870.7485 (Metabolism and Pharmacokinetics)
Deviations	no
Qualifier	according to
Guideline	other guideline: Japan/MAFF: Guidelines on the Compiling of Test Results on Toxicity; Tests on In Vivo Fate in Animals, 2001
Deviations	no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Radiolabelling

yes 1,2-Cyclohexanedicarboxylic acid, diisononyl ester

Test materials

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Test substance no.: 01/0107-1
- Physical state: Clear, colorless liquid
- Analytical purity: 99.6 % (sum of isomers)
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Storage condition of test material: ambient
- Stability under test conditions: The stability in the vehicle was confirmed in all experiments.

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Wistar: CrlGlxBrlHan:Wi
- Source: Charles River Laboratories, Sulzfeld (FRG)
- Age at study initiation: at least 7 weeks
- Housing: in type III Macrolon cages
- Individual metabolism cages: yes
- Diet (e.g. ad libitum): Kliba lab diet for rat-mouse-hamster either pelleted (e.g. during acclimatization) or granulated (e.g. in metabolism cages); origin: Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): Tap water; ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: 0.5 % Carboxymethylcellulose in double distilled water containing 1 % Cremophor EL (BASF AG)

Details on exposure

PREPARATION OF DOSING SOLUTIONS:
In order to achieve the required specific activity, respective amounts of unradiolabeled material were added to an aliquot of the stock solution of the radiolabeled material in toluene. The organic solvent was evaporated to dryness at 30°C under vacuum. The residue was suspended in 0.5 % Carboxymethylcellulose in double distilled water containing 1 % Cremophor EL (BASF AG) and filled up to the final volume in order to achieve the required concentration. Prior to administration the preparation was sonicated and stirred to produce a homogeneous suspension.

HOMOGENEITY AND STABILITY OF TEST MATERIAL:
Samples were taken to check the amount of radioactivity in the preparation and to demonstrate the stability, homogeneity and the correctness of the concentration of the test substance in the preparation

Duration and frequency of treatment / exposure

- Pretets: 48 h
- Blood/Plasma level: up to 168 h
- Balance/Excretion: up to 168 h
- Tissue distribution: up to 28 h
- Excretion via bile: up to 48 h

Doses / concentrations

- Pretest: 1000 mg/kg bw
- Blood/Plasma level: 50, 300, 1000 mg/kg bw
- Balance/Excretion: 3, 20, 1000 mg/kg bw
- Tissue distribution: 20, 1000 mg/kg bw

- Excretion via bile: 20, 1000 mg/kg bw

No. of animals per sex per dose

- Pretest: 2
- Blood/Plasma level: 4
- Balance/Excretion: 4
- Tissue distribution: 12
- Excretion via bile: 12

Control animals

no

Details on study design

- Dose selection rationale: using the results from previously performed studies on the subacute and subchronic toxicity

Details on dosing and sampling

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)
- Tissues and body fluids sampled: urine, faeces, blood, plasma, bile, Heart, liver, spleen, bone, skin, lung, ovaries, carcass, muscle, kidney, testes, brain, pancreas, uterus, adipose tissue, stomach & stomach contents, thyroid gland, adrenal glands, blood cells and plasma, gut and gut contents, bone marrow
- Time and frequency of sampling: after 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168 hours for blood/plasma levels; after 6, 12 and 24 hours and subsequently in time intervals of usually 24 hours up to 168 for balance/excretion; after 1 h, 8 h, 21 h, 28 h and 1 h, 4 h, 9 h, 16 h for tissue distribution;

Any other information on materials and methods incl. tables

- Preparation of samples and measurement of radioactivity:

Aliquots of liquid samples (plasma, urine, CO2-trap fluid and cage wash) were mixed
with scintillation cocktail (Hionic Fluor, Packard) and analyzed for radioactivity without any additional treatment. The femur was solubilized in 4 N HCl. After addition of scintillation cocktail radioactivity was measured. Feces, contents of gut and stomach were suspended in distilled water. The carcass was homogenized with distilled water using a WARING Blendor. Aliquots of these suspensions were dried by lyophilization in a freeze dryer (LYOVAC GT 3). Aliquots of the remaining powder and of the homogenates of the other tissues were solubilized in SOLUENE (Packard). In order to bleach these samples isopropanol and H2O2-solution were added. The samples were left for 24 hours at room temperature. After addition of scintillation cocktail the samples were counted for 10 minutes in a liquid scintillation counter (LSC; Wallac type 1409) and the disintegration rate corrected by the respective background.

Results and discussions

Preliminary studies

Two male and two female rats treated orally by gavage with 1000 mg/kg bw did not show clinical signs within a 48 h test period which would have required a change of oral dose.

Pharmacokinetic studies

Distribution in tissues

- 1000 mg/kg bw:
At 1 hour after administration to male and female rats highest tissue concentrations were found in the GI tract and in liver ranging from 1297.90 to 19375.66 µg Eq/g and from 49.54 to 76.40 µg Eq/g, respectively. Lowest concentrations were measured in brain and bone (0.80 - 1.19 µg Eq/g) of males and in muscles and adipose tissue (2.69 - 4.04 µg Eq/g) of females. A similar distribution of radioactivity was observed at 8 hours post dosing. In both sexes, radioactivity concentrations in organs and tissues excluding the GI tract declined rapidly and continuously and went parallel to the concentration in plasma during the following 20 hours. At 28 hours after dosing, concentrations in organs/tissues (excluding GI tract) of males were generally highest in bone marrow, pancreas, and liver (5.27 – 6.20 µg Eq/g) and lowest in testes, spleen, muscle, brain and bone (0.32 – 0.78 µg Eq/g); concentrations in female organs/tissues were highest in liver (14.53 µg Eq/g) and lowest in brain (0.48 µg Eq/g). Overall, tissue concentrations declined rapidly after having reached the peak level after 1 and 8 hours post dosing in males and females, respectively. Initial half-lives of radioactivity concentration in plasma and kidney were calculated to be about 3 and 7 hours and 5 and 5.5 hours in males and females, respectively. Initial half-lives of radioactivity concentration in liver and adipose tissue were calculated to be about 5 and 10 hours and 9 and 20 hours in males and females, respectively.
In order to calculate terminal tissue half-lives, the data determined in tissue distribution experiments were supplemented with tissue radioactivity concentrations from balance / excretion experiments, determined 168 h after administration of the respective single oral dose. The concentrations in adipose tissue and blood cells were lower after 28 h as compared to the respective concentrations observed after 168 hours, probably due to the fact that the data originate from two different subsets of animals with different dose preparations and different specific radioactivity. Due to this combination of data from different experiments, terminal half-lives have to be taken cautiously.
Terminal half-lives of radioactivity concentration in plasma, kidney and liver were calculated to be about 60, 65 and 74 hours and 34, 40 and 63 hours in males and females, respectively. A terminal half-life of radioactivity concentration in adipose tissue could not be calculated in female rats since the concentration after 28 hours was lower than that after 168 hours. Although the radioactivity concentration in adipose tissue of male rats was also lower after 28 hours than after 168 hours, a terminal half-life of about 98 hours was calculated within the time period of 21 to 28 hours post-dosing. This terminal half-life is therefore not meaningful. Overall, these initial and terminal half-lives do not indicate a potential for accumulation.

- 20 mg/kg bw:
At 1 hour after administration to male and female rats highest tissue concentrations were found in the GI tract and in liver ranging from 52.19 to 279.21 µg Eq/g and from 9.18 to 12.14 µg Eq/g, respectively. In males, lowest concentrations were measured in brain, muscle, testes, adipose tissue, and bone (0.21 – 0.48 µg Eq/g) at 1 hour after administration. In females, lowest concentrations were measured in brain, muscle, and bone (0.44 – 0.63 µg Eq/g) at 1 hour after administration.
Radioactivity concentrations in organs and tissues continuously declined in both sexes during the following 15 hours and - excluding the GI tract - went parallel to the concentrations in plasma. At 16 hours after dosing, concentrations in organs/tissues (except for the GI tract) were highest in liver (1.34 – 2.38 µg Eq/g) and adrenal glands (1.31 – 3.69 µg Eq/g) and lowest in brain and bone (0.01 - 0.08 µg Eq/g).
Overall, tissue concentrations declined rapidly after having reached the peak level after 1 hour (or 4 hours in case of adipose tissue) post-dosing in both sexes. Initial half-lives of radioactivity concentration of both sexes in plasma and kidney were calculated to be approximately 3 hours and 4.5 hours, respectively. Initial half-lives of radioactivity concentration in liver and adipose tissue were calculated to be about 6 and 8 hours for males and females, respectively. As reported for the high dose, terminal half-lives of radioactivity concentration of the low dose were also calculated by using the data from tissue distribution experiments supplemented with tissue radioactivity concentrations from

balance / excretion experiments, determined 168 h post-dosing. Due to this combination of data from different experiments, terminal half-lives have to be taken cautiously.

Excretion

- BALANCE AND EXCRETION PATTERN OF 14C-1,2-CYCLOHEXANEDICARBOXYLIC ACID, DIISONONYL ESTER:
Mean total recoveries of radioactivity were found to be 92.01 % in males and 99.42 % in females after a single administration of 1000 mg/kg bw. In the CO2-traps used to investigate radioactivity present as CO2 in exhaled air of two males during 48 hours after dosing, no radioactivity was detected. Within 48 hours after single oral administration of 1000 mg/kg bw to male and female rats 4.66 % and 4.47 % of the administered radioactivity were found in urine, respectively.
Total excretion of radioactivity via urine after 168 hours was 5.37 % for males and 5.30 % for females. During the first 48 hours after administration, 83.87 % and 92.78 % of the administered radioactivity were excreted via feces by males and females, respectively. After 168 hours the total amount of radioactivity excreted via feces was found to be 86.41 % for males and 93.55 % for females. These excretion data from urine and feces indicate rapid excretion of the absorbed material.
Together with cage wash, the total amount of excreted radioactivity was found to be 91.91 % of the administered radioactivity in males and 99.26% in females reflecting more than 99.8 % of the recovered radioactivity.
168 hours after dosing, small amounts of remaining radioactivity were found in liver (0.01 %), skin (0.03 %) and carcass (0.06 - 0.08 %). Mean concentrations of radioactivity were generally below 10 µg Eq/g in all organs and tissues.
Mean total recoveries of radioactivity were found to be 93.10 % in males and 92.60 % in females after a single administration of 20 mg/kg bw. In the CO2-traps used to investigate radioactivity present as CO2 in exhaled air of two males during 48 hours after dosing, radioactivity was not detected.
Within 48 hours after single oral administration of 20 mg/kg bw to male and female rats 28.31 % and 29.47 % of the administered radioactivity were found in urine, respectively.
Total excretion of radioactivity via urine after 168 hours was 29.90 % for males and 32.03 % for females.
During the first 48 hours after administration, 58.19 % and 57.31 % of the administered radioactivity were excreted via feces by males and females, respectively. After 168 hours the total amount of radioactivity excreted via feces was found to be 62.57 % for males and 58.93 % for females. These data on the time course of excretion of urine and feces indicate rapid excretion of the absorbed material.
Together with cage wash, the total amount of excreted radioactivity was found to be 92.80 % of the administered radioactivity in males and 92.12 % in females reflecting more than 99.4 % of the recovered radioactivity.
168 hours after dosing, small amounts of remaining radioactivity were found in liver (0.03 - 0.05 %), skin (0.08 - 0.12 %) and carcass (0.17 - 0.27 %). Mean concentrations of radioactivity were equal to or below 0.81 µg Eq/g (0.81 ppm) in all organs and tissues.

The repeated oral administration with 14 x non-radiolabeled and 1 x radiolabeled of 1000 mg/kg bw test substance, led to a found mean total recoveries of radioactivity 94.56 % in males and 100.58 % in females.
Within 48 hours after single oral administration of 1000 mg/kg bw to male and female rats 3.06 % and 3.67 % of the administered radioactivity were found in urine, respectively. Total excretion of radioactivity via urine after 168 hours was 3.47 % of dose for males and 4.22 % of dose for females. During the first 48 hours after administration, 88.99 % and 92.70 % of the administered radioactivity were excreted via feces by males and females, respectively. After 168 hours the total amount of radioactivity excreted via feces was found to be 90.58 % for males and 96.12 % for females. These excretion data from urine and feces indicate rapid excretion of the absorbed material with excretion being almost complete already after 48 hours. Together with cage wash, the total amount of excreted radioactivity was found to be 94.42 % of the administered radioactivity in males and 100.39 % in females reflecting more than 99.8 % of the recovered radioactivity.

- EXCRETION OF 14C-1,2-CYCLOHEXANEDICARBOXYLIC ACID, DIISONONYL ESTER VIA BILE:
Within 48 hours after administration 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester at a dose level of 1000 mg/kg bw, excretion via bile was found to be 0.53 and 0.49 % of the administered radioactivity in males and females, respectively. Biliary excretion of males was at maximum within the first 3 hours post dosing and declined gradually thereafter for the next 45 hours. In females, biliary excretion was at maximum within the first 3 hours post dosing and declined continuously during the next 15 hours to 0.01 % of the radioactive dose administered before it stopped after 39 hours.
Within 48 hours after administration 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester at a dose level of 20 mg/kg bw, excretion via bile was found to be 5.93 % of the administered radioactivity in males and 12.60 % in females. Biliary excretion of males was at maximum within the first 3 hours after dosing and declined slowly but constantly thereafter for the next 45 hours. In females, biliary excretion was at maximum within the first 6 hours after dosing and continuously declined thereafter for the next 42 hours.

Metabolite characterisation studies

Metabolites identified

not measured

Remarks on results including tables and figures

Pharmacokinetic parameters of radioactivity in plasma after single oral administration of 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester to male and female rats at dose levels of 1000, 300 and 50 mg/kg bw:

Sex	dose (mg/kg bw)	Cmax (µg Eq/g)	Tmax (h)	initial half life (h)	terminal half life (h)	AUC (µg Eq+h/g)
male	1000	24.73	1	9.5	52.2	402.1
	300	17.90	2	11.9	48.9	322.9
	50	11.26	1	4.8	34.6	83.0
female	1000	27.93	1	7.7	39.3	402.6
	300	19.56	1	11.3	38.3	327.8
	50	13.92	1	4.4	28.2	83.8

Comparison of excretion pattern after administration of 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester at different dose levels and application sites (% of the radioactivity administered):

	Dose (mg/kg bw)	1000	20	1000
	Application mode	single	single	repeated
	Urine 0-48 h	4.66	28.31	3.06

Males	Feces 0-48 h	83.87	58.19	88.99
	Subtotal	88.53	86.5	92.05
	Bile 0-48 h	0.53	5.93	-
Females	Urine 0-48 h	4.47	29.57	3.67
	Feces 0-48 h	92.78	57.31	92.7
	Subtotal	97.25	86.88	96.37
	Bile 0-48 h	0.49	12.6	-

Overall remarks, attachments

Overall remarks

After single oral administration, 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester was rapidly absorbed from the gastrointestinal tract. Absorption was incomplete at both dose levels amounting to about 5 – 6 % at the high dose and 40 - 49 % at the low dose.

After absorption, radioactive material was distributed in all organs and tissues. The excretion of radioactivity was rapid and occurred mainly via the feces. Biliary excretion amounted to about 0.5 % of the applied dose at the high dose and to about 6 - 13 % at the low dose level. Comparison of the bile excretion data indicated saturation of biliary excretion with increasing dose.
These data indicate saturation of gastrointestinal absorption of 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester with increasing dose. Plasma kinetic data confirmed this conclusion

Applicant's summary and conclusion

Interpretation of results

no bioaccumulation potential based on study results

Endpoint study record: BASFAG02B0608/016021.Basic toxicokinetics.amendment

UUID	IUC5-4b6e19ea-99ce-4c93-b25d-91f808c895f1
Dossier UUID	0
Author	henscho / BASF SE / Ludwigshafen am Rhein / Germany
Date	2009-09-23 15:46:16 CEST
Remarks	

Administrative Data

Purpose flag	(X) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Comparable to guideline study

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2005
Title	Amendment No. 1 to the Report - 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester - Study of the Biokinetics in Rats		
Bibliographic source	Unpublished data		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	02B0608/016021
Owner company	BASF SE		
Company study no.		**Report date**	2005-03-21

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Objective of study

absorption

distribution

excretion

toxicokinetics

Test guideline

Qualifier no guideline followed

Guideline

Deviations

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Radiolabelling

yes 14C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester

Test materials

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Test substance no.: 01/0107-1
- Physical state: Clear, colorless liquid
- Analytical purity: 99.6 % (sum of isomers)
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Storage condition of test material: ambient
- Stability under test conditions: The stability in the vehicle was confirmed in all experiments.

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Wistar: CrlGlxBrlHan:Wi
- Source: Charles River Laboratories, Sulzfeld (FRG)
- Age at study initiation: at least 7 weeks
- Housing: in type III Macrolon cages
- Individual metabolism cages: yes
- Diet (e.g. ad libitum): Kliba lab diet for rat-mouse-hamster either pelleted (e.g. during acclimatization) or granulated (e.g. in metabolism cages); origin: Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): Tap water; ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

intraveneous

Vehicle

other: plasma from untreated rats

Details on exposure

PREPARATION OF DOSING SOLUTIONS:
In order to achieve the required specific activity, respective amounts of unradiolabeled material were added to an aliquot of the stock solution of the radiolabeled material in toluene. The organic solvent was evaporated to dryness at 30°C under vacuum. The residue was suspended in plasma from untreated rats and filled up to the final volume in order to achieve the required concentration. Prior to administration the preparation was sonicated and stirred to produce a homogeneous preparation.

HOMOGENEITY AND STABILITY OF TEST MATERIAL:
Samples were taken to check the amount of radioactivity in the preparation and to demonstrate stability, homogeneity and the correctness of the concentration of the test substance in the preparation.

Duration and frequency of treatment / exposure

one application

Doses / concentrations

3 mg/kg bw (1 ml/kg bw)

No. of animals per sex per dose

4

Control animals

no

Details on study design

- Dose selection rationale: In agreement with the sponsor, a dose level of 3 mg/kg bw was selected for the balance experiment with intravenous application.

Details on dosing and sampling

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)
- Tissues and body fluids sampled : urine, faeces, cage washes, bile, heart, liver, spleen, bone, skin, lung, ovaries, carcass, muscle, kidney, testes, brain, pancreas, uterus, adipose tissue, stomach & stomach contents, thyroid gland, adrenal glands, blood cells and plasma, gut and gut contents, bone marrow
- Time and frequency of sampling: collecting excreta after 6, 12 and 24 hours and subsequently in time intervals of usually 24 hours up to 168 hours; at 168 hours post-dosing, animals were sacrificed and tissues were collected

Results and discussions

Pharmacokinetic studies

Distribution in tissues

At 168 hours after dosing, small amounts of remaining radioactivity were found in liver (0.55-0.66 %), skin (0.53-0.79 %) and the remaining carcass (1.42-2.07 %). Mean concentrations of radioactivity were very low and below 1 µg Eq/g in all organs and tissues.

Excretion

Mean total recoveries of radioactivity were found to be 95.25 % in males and 93.12 % in females.
Within 48 hours after single intravenous application of 3 mg/kg bw to male and female rats 37.74% and 33.70 % of the administered radioactivity were found in urine, respectively. Total excretion of radioactivity via urine after 168 hours was 43.45 % for males and 44.80 % for females. During the first 48 hours after administration, 38.11 % and 31.38 % of the administered radioactivity were excreted via feces by males and females, respectively. After 168 hours the total amount of radioactivity excreted via feces was found to be 48.06 % for males and 42.92 % for females. These excretion data from urine and feces indicate rapid excretion of the absorbed material. Together with cage wash, the total amount of excreted radioactivity was found to be 91.89 % of the administered radioactivity in males and 89.03% in females reflecting more than 95 % of the recovered radioactivity.

Metabolite characterisation studies

Metabolites identified

not measured

Remarks on results including tables and figures

Overall remarks, attachments

Overall remarks

In conclusion, equal amounts of radioactivity were excreted via urine and feces, i.e.bile, after single intravenous administration. No sex-specific difference in the excretion pattern was observed. The time course of the amount of radioactivity found in urine indicates enterohepatic circulation.

Applicant's summary and conclusion

Interpretation of results

low bioaccumulation potential based on study results

Endpoint study record: Key.BASFAG197662.Basic toxicokinetics

UUID	IUC5-7a1935ae-47bf-4810-b65b-c86867f678e8
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:04 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2005
Title	FINAL REPORT- Investigations on the Metabolism of 14C-1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) in Rats		
Bibliographic source	Unpublished report		
Testing laboratory	Ecology and Environmental Analytics, BASF AG	**Report no.**	197662
Owner company	BASF SE		
Company study no.		**Report date**	2005-03-16

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Objective of study

metabolism

Test guideline

Qualifier	according to

Guideline OECD Guideline 417 (Toxicokinetics)

Deviations no

Qualifier according to

Guideline EPA OPPTS 870.7485 (Metabolism and Pharmacokinetics)

Deviations no

Qualifier according to

Guideline EU Method B.36 (Toxicokinetics)

Deviations no

Qualifier according to

Guideline other guideline: MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan)

Deviations no

GLP compliance

yes (incl. certificate) Ecology and Environmental Analytics, BASF AG

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Radiolabelling

yes Ecology and Environmental Analytics, BASF AG

Test materials

Details on test material

- Name of test material (as cited in study report): 1,2-cyclohexane dicarboxylic acid diisononyl ester [cyclohexane-U-14C]
- Batch No.: 772-1101
- Analytical purity: >99% (by GC)
- Radiochemical purity (if radiolabelling): > 99% (by radio-HPLC), >98% (by radio-TLC)
- Specific activity (if radiolabelling): 2.7 MBq/mg a.i.; 162 000 dpm/µg
- Locations of the label (if radiolabelling): ring

- Name of test material (as cited in study report): 1,2-cyclohexanedicarboxylic acid diisononyl ester
- Test Substance No.: 01/0107-1
- Analytical purity: 01/0107-1
- Chemical Purity: 99.6% (sum of isomers)
- Lot/batch No.: 46-0959 (Partie 33A/0)

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Wistar: CrlGlxBrlHan:Wi
- Source: Charles River Laboratories, Sulzfeld (FRG)
- Age at study initiation: at least 7 weeks
- Housing: in type III Macrolon cages
- Individual metabolism cages: yes
- Diet (e.g. ad libitum): Kliba lab diet for rat-mouse-hamster either pelleted (e.g. during acclimatization) or granulated (e.g. in metabolism cages); origin: Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): Tap water; ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

other: oral: gavage and intraveneous

Vehicle

other: 0.5% aqueous solution of carboxymethylcellulose containing 1% Cremophor (orally) and plasma of untreated rats (intraveneous)

Details on exposure

PREPARATION OF DOSING SOLUTIONS:
To achieve the required specific activity, radiolabelled test item was diluted with unlabelled material. The organic solvent was evaporated and the residue was suspended in a 0.5% aqueous solution of carboxymethylcellulose containing 1% Cremophor.

For the intravenous administration, the residue after evaporation of the organic solvent was suspended in plasma from untreated rats and diluted.

Duration and frequency of treatment / exposure

- single oral dose of 20 or 1000 mg/kg bw
- repeated dose dose of 1000 mg/kg bw/day for 14 days
- single intravenous dose of 3 mg/kg bw

Doses / concentrations

- oral: 20, 1000 mg/kg bw
- intraveneous: 3 mg/kg bw

No. of animals per sex per dose

4

Control animals

no

Details on dosing and sampling

METABOLITE CHARACTERISATION STUDIES

- Tissues and body fluids sampled: urine, faeces, tissues, bile
- Time and frequency of sampling: feces and urine were collected after 6, 12, and 24 h and thereafter in intervals of 24 h for up to 168 h; bile was collected in time intervals of 3 h for up to 48 h
- From how many animals: (samples pooled or not): urine, feces, and bile samples of the respective time interval of interest were pooled over the individual animals per sex.
- Method type(s) for identification: radio-HPLC, LC-MS, LC-MS/MS, MS/MS, LC-NMR

Results and discussions

Metabolite characterisation studies

Metabolites identified

yes

Details on metabolites

- Metabolite pattern in urine:
The radioactivity detected in urine represented between 0.6% and 4.0% of an orally administered dose. One predominant metabolite, cyclohexanedicarboxylic acid (51-75% of radioactivity present in urine) was identified in the urine of animals after single or repeated oral treatment. Two to five minor metabolites were also detected, although none exceeded 1% of the dose in the investigated fractions. These were tentatively identified as sulfate-conjugated oxidative metabolites of the cyclohexanedicarboxylic acid monoester.
After intravenous administration, the urinary metabolite patterns were very similar to those following an oral dose, with the cyclohexane dicarboxylic acid accounting for 16-17% of the dose (between 0-24 hours). The detected minor metabolites were qualitatively similar to those detected after oral administration.
- Metabolite pattern in faeces:
The unabsorbed, unchanged test substance accounted for 84-100% of the radioactivity in faeces extracts after oral administration (24-76% of the administered radioactive dose at the investigated time points), reflecting its low oral bioavailability. A small amount of cyclohexane dicarboxylic acid monoisononyl ester was detected in the faeces extracts of low dose animals.
Metabolite patterns in faeces after intravenous administration were different compared to those after oral administration. No unmetabolised test substance was detected, although numerous metabolites were detected. These included cyclohexanedicarboxylic acid monoisononyl ester, which accounted for approximately 3% of the administered dose over 12-48 hours. The residual metabolites were characterised as comprising oxidation/hydroxylation products of the monoester.
- Metabolite pattern in bile:
The LC-MS/MS data of bile identified two to four groups of peaks. The most prominent metabolite was identified as the glucuronic acid conjugate of the monoisononyl ester, which represented 54-65% of the radioactivity in the bile (3.75/7.60% of the dose for females/males). Small amounts of the monoisononyl ester were also detected. The third metabolic fraction was characterised to contain degradation products of the monoisononyl ester (with or without further conjugation) and other derivatives/conjugates that may lack both isononyl groups

Remarks on results including tables and figures

Composition of radioactivity in urine and feces after oral administration of 14C diisononyl ester (% of dose):

	Single application			
	20 mg/kg bw		1000 mg/kg bw	
Metabolite	Urine (12 - 24 h)	Feces (12 - 24 h)	Urine (12 - 24 h)	Feces (12 - 2

Identity / Designation	male	female	male	female	male	female	male	fem
Cyclohexane dicarboxylic acid	3.95	3.66	nd	nd	0.90	0.65	nd	n
DINCH	nd	nd	34.69	24.31	nd	nd	62.68	n
MINCH	nd	nd	3.00	4.78	nd	nd	nd	n
Total identified	3.95	3.66	37.69	29.09	0.90	0.65	62.68	n
Characterized as sulfate conjugated oxidative degradation products of MINCH by LC-MS/MS	1.70	2.69	nd	nd	0.33	0.62	nd	n
Total identified and characterized	5.65	6.35	37.69	29.09	1.23	1.27	62.68	n

nd: not detected

np: not performed (no investigation, since no differences between sexes were obser

Composition of radioactivity in bile after single oral administration of 14C-1,2-c diisononyl ester (in % of dose):

Metabolite Identity / Designation	20 mg/kg bw		1000 mg/kg bw	
	Bile (0 - 12 h)		Bile (0 - 12 h)	
	male	female	male	female
DINCH	0.44	0.92	nd	nd
MINCH	3.75	7.6	0.27	0.23
Total identified	4.19	8.52	0.27	0.23
2-4 peaks, between 0.07 and 3.3% dose, partially convertible by glucuronidase/arylsulfatase	1.71	5.65	0.15	0.15
Total identified and characterized	5.9	14.17	0.42	0.38

nd: not detected

Overall remarks, attachments

Overall remarks

After oral dosing, 14C-1,2-cyclohexanedicarboxylic acid diisononyl ester (DINCH) showed a dose-dependent bioavailability in the range of 5-49%. Absorbed material is effectively excreted in urine, feces, and bile.

The following metabolic pathway was observed in this study. Starting with partial hydrolysis of DINCH to yield the monoisononyl ester (MINCH), two main metabolic transformations were seen: one is the direct glucuronidation as well as oxidation/hydroxylation reactions of theand subsequent conjugation. The other metabolic route is the hydrolysis of the remaining ester bond to yield free cyclohexane dicarboxylic acid, leading to the acid being the predominant metabolite in urine. Upon intravenous administration, the same metabolictransformations were observed. Furthermore, it was seen that after intravenous administrationthe absorbed material was efficiently and completely converted to polar metabolites since no unmetabolized DINCH was detected anymore. The metabolic pathway was found to be independent from dose levels and sex.

Applicant's summary and conclusion

Interpretation of results

low bioaccumulation potential based on study results

7.2 Acute Toxicity
7.2.1 Acute toxicity: oral
Endpoint study record: Key.BASFAG10A0223/991059.Acute toxicity: oral

UUID	IUC5-f8658d0f-44a7-43d0-8577-72501f0cd4c1
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:06 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	1999
Title	1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester Acute oral toxicity in rats.		
Bibliographic source	Unpublished data		
Testing laboratory	Department of Toxicology, BASF AG	**Report no.**	10A0223/991059
Owner company	BASF SE		
Company study no.		**Report date**	1999-11-03

Data access

data submitter is data owner

Materials and methods

Test type

acute toxic class method

Limit test

yes

Test guideline

Qualifier according to

Guideline OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)

Deviations no

Qualifier according to

Guideline EPA OPPTS 870.1100 (Acute Oral Toxicity)

Deviations no

Qualifier according to

Guideline EU Method B.1 tris (Acute Oral Toxicity - Acute Toxic Class Method)

Deviations no

GLP compliance

yes Department of Toxicology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid,di (isononyl)ester
- Substance number: 99/223-1
- Batch number: R 5116/1 (i 23725)
- Date of manufacturing: 26-May-1999
- Physical state: Liquid, colorless, clear
- Analytical purity: 99.7 g/100 g (GC)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Boehringer Ingelheim Pharma KG
- Age at study initiation: Young adult animals
- Weight at study initiation: mean 170 g (males), 166 g (females)
- Fasting period before study: at least 16 h
- Housing: single housing in stainless steel wire mesh cages, type DK-III(Becker & Co., Castrop-Rauxel, FRG)
- Diet (e.g. ad libitum): Kliba-Labordiaet, Provimi Kliba SA, Kaiseraugst,Switzerland; ad libitum

- Water (e.g. ad libitum): Tap water; ad libitum
- Acclimation period: at least 1 week

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 -24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: gavage

Vehicle

olive oil

Details on oral exposure

VEHICLE
- Concentration in vehicle: 50%
- Amount of vehicle (if gavage): 10 ml/kg bw
- Justification for choice of vehicle: Good solubility in olive oil

CLASS METHOD (if applicable)
- Rationale for the selection of the starting dose: Based on the physical and chemical characteristics of the test substance and thecomposition no pronounced acute oral toxicity was expected. Therefore a dose of 5000 mg/kg body weight has been chosen in a first step with 3 male animals. Because no mortality occurred, 5000 mg/kg body weight have been tested in a second step with animals of the other sex (3 female rats).

Doses

5000 mg/kg bw

No. of animals per sex per dose

3

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: shortly before application (day 0), weekly thereafter and at the endof the study (before fasting period); additionally animals that died or were sacrificed moribund. A check for any dead or moribund animal wasmade twice each workday and once on saturdays,sundays and on public holidays
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight

Any other information on materials and methods incl. tables

Results and discussions

Effect levels

Sex	male/female
Endpoint	LD50
Effect level	> 5000 mg/kg bw

95% CL

Remarks

Mortality

No mortality

Clinical signs

No abnormalities observed.

Body weight

Day 0: mean 170 g (males), 166 g (females)
Day 7: mean 233 g (males), 200 g (females)
Day 13: mean 304 g (males), 216 g (females)

Gross pathology

No abnormalities found.

Remarks on results including tables and figures

Overall remarks, attachments

Overall remarks

Under the conditions of this study the median lethal dose of 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester after oral application was found to be greater than 5000 mg/kg body weight for the male and female animals.

Applicant's summary and conclusion

Interpretation of results

practically nontoxic

Criteria used for interpretation of results

EU

7.2.3 Acute toxicity: dermal

Endpoint study record: Key.BASFAG11A0223/991060.Acute toxicity: dermal

UUID	IUC5-68b62a1a-8812-49a2-87b1-238bb6227837
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:08 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	1999
Title	1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester Acute dermal toxicity in rats		
Bibliographic source	Unpublished data		
Testing laboratory	Department of Toxicology, BASF AG	**Report no.**	11A0223/991060
Owner company	BASF SE		
Company study no.		**Report date**	1999-11-03

Data access

data submitter is data owner

Materials and methods

Test type

standard acute method

Limit test

yes

Test guideline

Qualifier according to

Guideline OECD Guideline 402 (Acute Dermal Toxicity)

Deviations no

Qualifier according to

Guideline EU Method B.3 (Acute Toxicity (Dermal))

Deviations no

Qualifier according to

Guideline EPA OPPTS 870.1200 (Acute Dermal Toxicity)

Deviations no

GLP compliance

yes Department of Toxicology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid,di (isononyl)ester
- Substance number: 99/223-1
- Date of manufacturing: 26-May-1999
- Physical state: Liquid, colorless, clear
- Analytical purity: 99.7 g/100 g (GC)
- Lot/batch No.: R 5116/1 (1 23725)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Boehringer Ingelheim Pharma KG
- Age at study initiation: Young adult animals
- Weight at study initiation: Animals of comparable weight (150g - 300g +/- 20% of the mean weight).
- Fasting period before study: at least 16 h
- Housing: single housing in stainless steel wire mesh cages, type DK-III(Becker & Co., Castrop-Rauxel, FRG)
- Diet (e.g. ad libitum): Kliba-Labordiaet, Provimi Kliba SA, Kaiseraugst,Switzerland; ad

libitum
- Water (e.g. ad libitum): Tap water; ad libitum
- Acclimation period: at least 1 week

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 -24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Type of coverage

semiocclusive

Vehicle

unchanged (no vehicle)

Details on dermal exposure

TEST SITE
- Area of exposure: about 50 cm2 on dorsal and dorsolateral parts of the trunk; clipped epidermis
- % coverage: at least 10 % of the body surface area
- Type of wrap if used: four layers absorbent gauze, Ph. Eur. LohmannGmbH & Co. KG and Fixomull stretch (adhesivefleece), Beiersdorf AG

REMOVAL OF TEST SUBSTANCE
- Washing (if done): warm water
- Time after start of exposure: 24 h

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 2.03 ml/kg bw
- Concentration (if solution): 100%

Duration of exposure

24 hours

Doses

2000 mg/kg

No. of animals per sex per dose

5

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: Recording of signs and symptoms several times onthe day of administration, at least once eachworkday for the individual animals; individual body weights shortly before application (day 0), weekly thereafter and at the end of the study (before fasting period); additionally animals that died or were sacrificed mori-bund.
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight

Statistics

The binominal test (Snedecor G.W., Cochran W.G. (1989), Statisticalmethods, 8th ed., Iowa State University Press/Ames) was used.

Any other information on materials and methods incl. tables

Results and discussions

Effect levels

Sex	male/female
Endpoint	LD50
Effect level	> 2000 mg/kg bw
95% CL	
Remarks	

Mortality

No mortality

Clinical signs

No abnormailites observed

Body weight

Day 0: mean 275 g (males), 217 g (females)
Day 7: mean 295 g (males), 221 g (females)
Day 14: mean 317 g (males), 228 g (females)

Gross pathology

No abnormalities were noted

Other findings

LOCAL EFFECTS: observed only on day 1

- very slight erythema: 4 males, 2 females
- Well defined erythema: 1 male, 3 females

Remarks on results including tables and figures

Overall remarks, attachments

Overall remarks

Under the conditions of this study the acute dermal median lethal dose (LD50) of 1,2-Cyclohexane dicarboxylic adid, di(isononyl)ester was found to be greater than 2000 mg/kg body weight for the male and female animals.

Applicant's summary and conclusion

Interpretation of results

practically nontoxic

Criteria used for interpretation of results

EU

7.3 Irritation / corrosion

Endpoint summary: Irritation / corrosion

UUID	IUC5-45614c73-402a-4474-ae07-89cb8149a58e
Dossier UUID	0
Author	otterr / BASF SE / Ludwigshafen am Rhein / Germany
Date	2009-11-03 19:09:43 CET
Remarks	

Administrative Data

Key parameter (optional)

Skin irritation / corrosion

not irritating

Eye irritation

not irritating

Discussion

The test substance used in the study 18H0010/042033 is respresentative for the material coming from the production plant. The mean score 24 h - 72 h was 1.8; only one out of 3 animals had a score of 2. Another study was undertaken with material coming from a pilot plant. In this study, 18H0223/992125, there were 2 out of 3 animals tested with a score of 2, the mean score was 1,4 and would not have been sufficient for classification as irritant. However, taken both studies together, the mean score and the number of animals 3/6 do not justify classification. Therefore, the test substance is not irritating to the skin according to Directive 67/548/EEC and Regulation EC (No)1272/2008

Justification for classification or non-classification

7.3.1 Skin irritation / corrosion

Endpoint study record: Key.BASFAG18H0010/042033.Skin irritation / corrosion

UUID	IUC5-3b5518b4-192f-469c-95d6-153799260fb6
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:10 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2004
Title	Hexamoll DINCH - Acute dermal irritation/corrosion in rabbits		
Bibliographic source	Unpublished data		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	18H0010/042033
Owner company	BASF SE		
Company study no.		**Report date**	2004-07-21

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	according to
Guideline	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)
Deviations	no

Qualifier	according to
Guideline	EU Method B.4 (Acute Toxicity: Dermal Irritation / Corrosion)
Deviations	no
Qualifier	according to
Guideline	EPA OPPTS 870.2500 (Acute Dermal Irritation)
Deviations	no
Qualifier	according to
Guideline	other guideline: Japan MAFF Testing Guideline of 12 Nousan No. 8147, November 2
Deviations	no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test material equivalent to submission substance identity

yes

Test materials

Test material identity

Identifier	CAS number
Identity	166412-78-8

Details on test material

- Name of test material (as cited in study report): Hexamoll* DINCH
- Test substance No.: 04/0010-2
- Date of production: December 2003
- Physical state: Liquid / colorless
- Analytical purity: 99.94 area-%
- pH-value: 5.0 (undiluted test substance)
- Lot/batch No.: PBG: 10244996
- Storage condition of test material: Room temperature

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: New Zealand White A1077 INRA (SPF)
- Source: Centre Lago S.A., 01540 Vonnas, France
- Age at study initiation: About 6 months
- Weight at study initiation: 3.48 kg – 3.67 kg
- Housing: single housing in stainless steel wire mesh cages with grating, floor area: 3000 cm²
- Diet (e.g. ad libitum): Kliba-Labordiät (Kaninchen & Meerschweinchenhaltung “GLP”), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland (about 130 g/animal per day); ad libitum
- Water (e.g. ad libitum): Tap water; ad libitum
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Test system

Type of coverage

semiocclusive

Preparation of test site

other: clipping of the fur at least 24 hours before application

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 0.5 ml
- Concentration (if solution): 100%

Duration of treatment / exposure

4 h

Observation period

14 days

Number of animals

3

Control animals

other: untreated skin sites of the same animal

Details on study design

TEST SITE
- Area of exposure: dorsolateral part of the trunk
- Type of wrap if used: 2.5 cm x 2.5 cm test patch (Idealbinde, Pfälzische Verbandstoff-Fabrik, Kaiserslautern) and Fixomull® stretch (adhesive fleece), Beiersdorf AG

REMOVAL OF TEST SUBSTANCE
- Washing (if done): with Lutrol and Lutrol/ water (1 : 1).
- Time after start of exposure: 4 h

SCORING SYSTEM:
The evaluation of skin reactions is performed according to the quoted guidelines:
- Erythema and eschar formation
0 = No erythema
1 = Very slight erythema (barely perceptible)
2 = Well defined erythema
3 = Moderate to severe erythema
4 = Severe erythema (beet redness) to eschar formation preventing grading of erythema

- Edema formation
0 = No edema
1 = Very slight edema (barely perceptible)
2 = Slight edema (edges of area well defined by definite raising)
3 = Moderate edema (raised approx. 1 mm)
4 = Severe edema (raised more than 1 mm and extending beyond area of exposure)

Any other information on materials and methods incl. tables

Results and discussions

Irritation / corrosion results

Irritation parameter	erythema score
Basis	mean
Time point	24, 48, 72 h
Score	1.8
Max. score	4
Reversibility	fully reversible
Remarks	

Irritation parameter	edema score
Basis	mean
Time point	24, 48, 72 h
Score	0
Max. score	4
Reversibility	other: no effects
Remarks	

Remarks on results including tables and figures

Readings:

Readings	Animal	Erythema	Edema	Additional findings
0 h	1	2	0	
	2	2	0	
	3	2	0	
1 h	1	2	0	
	2	2	0	
	3	2	0	

24 h	1	2	0	
	2	2	0	
	3	2	0	
48 h	1	2	0	
	2	2	0	
	3	2	0	
72 h	1	1	0	
	2	1	0	
	3	2	0	
7 d	1	0	0	SD
	2	0	0	SD
	3	1	0	
14 d	3	0	0	
mean 24 - 72 h	1	1.7	0.0	
	2	1.7	0.0	
	3	2.0	0.0	
mean		1.8	0.0	

SD: Study discontinued because the animal was free of findings

Overall remarks, attachments

Overall remarks

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

7.3.2 Eye irritation

Endpoint study record: Key.BASFAG11H0223/992126.Eye irritation

UUID	IUC5-16192ac0-f705-49a5-a16f-2b9ea3cec452
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:11 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (GLP)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	1999
Title	1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester - Acute eye irritation in rabbits.		
Bibliographic source	Unpublished data		
Testing laboratory	Department of Toxicology, BASF AG	**Report no.**	11H0223/992126
Owner company	BASF SE		
Company study no.		**Report date**	1999-11-03

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	according to
Guideline	OECD Guideline 405 (Acute Eye Irritation / Corrosion)
Deviations	

no

Qualifier according to

Guideline EU Method B.5 (Acute Toxicity: Eye Irritation / Corrosion)

Deviations no

Qualifier according to

Guideline EPA OPPTS 870.2400 (Acute Eye Irritation)

Deviations no

GLP compliance

yes Department of Toxicology, BASF AG

Test material equivalent to submission substance identity

yes

Test materials

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid,di (isononyl)ester
- Substance number: 99/223-1
- Date of manufacturing: 26-May-1999
- Physical state: Liquid, colorless, clear
- Analytical purity: 99.7 g/100 g (GC)
- Lot/batch No.: R 5116/1 (# 23725)
- Storage condition of test material: Room temperature

Test animals

Species

rabbit

Strain

Himalayan

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: Himalayan, Chbb: HM (outbred strain)
- Source: Boehringer Ingelheim Pharma KG
- Age at study initiation: young adults
- Weight at study initiation: 2.45 - 3.10 kg
- Housing: single housing in stainless steel wire mesh cages with grating, floor area: 3000 cm2
- Diet (e.g. ad libitum): Kliba-Labordiaet, Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum.
- Water (e.g. ad libitum): tap water; ad libitum
- Acclimation period: at least 1 week

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24

- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Test system

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 0.1 ml
- Concentration (if solution): 100%

Duration of treatment / exposure

24 h

Observation period

72 h

Number of animals

3

Control animals

other: untreated eye of the same animal

Details on study design

REMOVAL OF TEST SUBSTANCE
- Washing (if done): tap water
- Time after start of exposure: about 24 h after instillation

SCORING SYSTEM:
- Cornea
0 = No opacity
1 = Scattered or diffuse area, details of iris clearly visible
2 = Easily discernible translucent areas, details of iris slightly obscured
3 = Opalescent areas, no details of iris visible, size of pupil barely discernible
4 = Opaque, iris invisible

- Area of cornea involved
1 = One quarter (or less) but not zero
2 = Greater than one quarter, but less than half
3 = Greater than half, but less than three quarters
4 = Greater than three quarters, up to whole area

- Iris
0 = Normal
1 = Markedly deepened rugae, congestion, swelling, moderate circumcornealhyperaemia, or injection, any of these or combination of any thereof,iris still reacting to light (sluggish reaction is positive)
2 = No reaction to light, haemorrhage, gross destruction (any or all of these)

- Conjunctivae
(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
0 = Vessels normal
1 = Vessels definitely injected above normal
2 = More diffuse, deeper crimson red, individual vessels not easily discernible

3 = Diffuse beefy red

(B) Chemosis
0 = No swelling
1 = Any swelling above normal (includes nictitatinq membrane)
2 = Obvious swelling with partial eversion of lids
3 = Swelling with lids about half closed
4 = Swelling with lids about half closed to completely closed

(C) Discharge
0 = No discharge
1 = Any amount different from normal (does not include small amounts observed in inner canthus of normal animals
2 = Discharge with moistening of the lids and hairs just adjacent to lids
3 = Discharge with moistening of the lids and hairs, and considerable area around the eye

Any other information on materials and methods incl. tables

A check for any dead or moribund animal was made twice each workday and once on saturdays, sundays and on public holidays.

Results and discussions

Overall irritation / corrosion results

Irritation parameter	cornea score
Basis	mean
Time point	24, 48, 72 h
0	
Max. score	4
Reversibility	other: no effects
Remarks	
Irritation parameter	iris score
Basis	mean
Time point	24, 48m 72 h
0	
Max. score	2
Reversibility	other: no effects
Remarks	
Irritation parameter	conjunctivae score
Basis	mean
Time point	24, 48, 72 h
0.3	
Max. score	3

Reversibility fully reversible within: 48 h

Remarks

Irritation parameter chemosis score

Basis mean

Time point 24, 48, 72 h

0

Max. score 4

Reversibility other: no effects

Remarks

Remarks on results including tables and figures

Animal 1: male, 3.10 kg

Animal 2: male, 2.54 kg

Animal 3: male, 2.54 kg

Readings:

Readings	Animal	Cornea		Iris	Conjunctival		
		opacity	area		Erythema	Chemosis	Dis
1h	1	0	0	0	2	0	
	2	0	0	0	1	0	
	3	0	0	0	1	0	
24 h	1	0	0	0	1	0	
	2	0	0	0	1	0	
	3	0	0	0	1	0	
	1	0	0	0	0	0	

48 h	2	0	0	0	0	0	
	3	0	0	0	0	0	
72 h	1	0	0	0	0	0	
	2	0	0	0	0	0	
	3	0	0	0	0	0	
Mean 24 - 72 h	1	0	0	0	0.3	0	
	2	0	0	0	0.3	0	
	3	0	0	0	0.3	0	
mean	1	**0.0**	0.0	**0.0**	**0.3**	**0.0**	

Overall remarks, attachments

Overall remarks

The average score (24 to 72 hours) for irritation was calculated to be 0.0 for corneal opacity, iris and chemosis and 0.3 for conjunctival redness. The findings were reversible in all animals within 48 hours after application; thus the study was terminated after 72 hours. Under the test conditions chosen and considering the described findings 1,2 -Cyclohexane dicarboxylic acid, di(isononyl)ester does not give indication of an irritant property to the eye.

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

7.4 Sensitisation
7.4.1 Skin sensitisation
Endpoint study record: Key.BASFAG.30H0223/992127.Skin sensitisation

UUID	IUC5-0c6fd20e-3702-4677-b5a6-8330c6c91a89
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:13 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	1999
Title	1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester - Maximization Test in guinea pigs.		
Bibliographic source	Unpublished data		
Testing laboratory	Department of Toxicology, BASF AG	**Report no.**	30H0223/992127
Owner company	BASF SE		
Company study no.		**Report date**	1999-11-03

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Type of study

Guinea pig maximization test

Test guideline

Qualifier	according to
Guideline	OECD Guideline 406 (Skin Sensitisation)
Deviations	no
Qualifier	according to
Guideline	EU Method B.6 (Skin Sensitisation)
Deviations	no

GLP compliance

yes Department of Toxicology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester
- Physical state: liquid, colorless, clear
- Analytical purity: 99.7g /100g (GC)
- Lot/batch No.: R 5116/1 (#23725)
- Storage condition of test material: room temperature

Test animals

Species

guinea pig

Strain

other: Hsd Poc : DH (SPF)

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Harlan Winkelmann GmbH
- Age at study initiation: young adult animals
- Weight at study initiation: 269 - 375g
- Housing: 5 per cage (stainless steel wire mesh cages with plastic-coated grating, floor area 40 cm x 51 cm)
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21 - 25
- Humidity (%): 30 - 70

- Photoperiod (hrs dark / hrs light): 12/12

Test system

Traditional sensitisation test

Route of induction exposure

intradermal

Route of challenge exposure

other: intradermal and percutaneous

Vehicle

olive oil

Concentration

Intradermal pretest: test substance in 5% olive oil or Freund's adjuvant (Freund's adjuvant / 0.9% aqueous NaCl (1:1))
Percutaneous pretest: 1st: undiluted and 50%, 2nd: 50% and 25%
1st application: Induction 5 % in olive oil or in Freund's adjuvant / 0.9% aqueous NaCl-solution (1:1) intradermal
2nd application: Percutaneous induction: test substance undiluted
3rd application: Challenge 50 % in olive oil occlusive epicutaneous

No. of animals per dose

Intradermal pretest: 2 animals
Percutaneous pretest: 6 animals

Main test: 5 control and 10 test

Details on study design (Traditional tests)

RANGE FINDING TESTS: Intradermal and Percutaneous Pretests

MAIN STUDY
A. INDUCTION EXPOSURE
- No. of exposures: 6 intradermal injections, 1 week later precutaneous induction
- Exposure period: 24 hour after intradermal injection and 48 hour after precutaneous induction
- Test groups: 10 animals
- Control group: 5 animals
- Site: shoulder
- Concentrations: 5% test substance in interdermal test; undiluted for precutaneous induction

B. CHALLENGE EXPOSURE
- No. of exposures: 1
- Day(s) of challenge: 2 days
- Exposure period: 24 hours
- Test groups: 10 animals with 0.5 ml test substance
- Control group: 5 animals
- Site: intact flank
- Concentrations: 50% test substance in olive oil
- Evaluation (hr after challenge): 24 and 48 hours

Challenge controls

1st control group: right flank anterior: Test substance 50% olive oil; left flank anterior: olive oil
2nd control goup: left flank anterior: olive oil

Positive control substance(s)

no A positive control (reliability check) with a known sensitizer is not included in this study. However, a separate study is performed twice a year in the laboratory. The positive control with Alpha-Hexylcinnamaldehyde techn. 85% worked out well.

LLNA

Any other information on materials and methods incl. tables

Results and discussion

Positive control results

The positive control with Alpha-Hexylcinnamaldehyde techn. 85% showed that the test system was able to detect sensitizing compounds under the laboratory conditions chosen.

Traditional sensitisation test

Results of test (except LLNA)

Reading	1st reading
Hours after challenge	24
Group	negative control
Dose level	50% in olive oil
No. with + reactions	0
Total no. in group	5
Clinical observations	no abnormality detected
Reading	2nd reading
Hours after challenge	48
Group	negative control
Dose level	50% in olive oil
No. with + reactions	0
Total no. in group	5
Clinical observations	no abnormality detected
Reading	1st reading
Hours	24

after challenge	
Group	negative control
Dose level	olive oil
No. with + reactions	0
Total no. in group	5
Clinical observations	no abnormality detected
Reading	2nd reading
Hours after challenge	48
Group	negative control
Dose level	olive oil
No. with + reactions	0
Total no. in group	5
Clinical observations	no abnormality detected
Reading	1st reading
Hours after challenge	24
Group	test group
Dose level	50% in olive oil
No. with + reactions	0
Total no. in group	10
Clinical observations	no abnormality detected
Reading	2nd reading
Hours after challenge	48
Group	test group
Dose level	50% in olive oil
No. with +	0

reactions	
Total no. in group	10
Clinical observations	no abnormality detected
Reading	1st reading
Hours after challenge	24
Group	test group
Dose level	olive oil
No. with + reactions	0
Total no. in group	10
Clinical observations	no abnormality detected
Reading	2nd reading
Hours after challenge	48
Group	test group
Dose level	olive oil
No. with + reactions	0
Total no. in group	10
Clinical observations	no abnormality detected

LLNA

Remarks on results including tables and figures

The intradermal induction with 5% test substance preparations caused moderate and confluent to intense erythema and swelling in all test group animals. After the percutaneous induction with the undiluted test substance incrustation, partially open (caused by the intradermal induction) could be observed in addition to moderate and confluent erythema and swelling in all test group animals. After the challenge with a 50% test substance preparation in olive oil no skin reactions could be observed neither in control group 1 nor in the test group 24 and 48 hours after removal of the patches. Olive oil, which was applied as a vehicle control to all animals, did not cause any skin reactions.

Since no borderline results were observed, a 2nd challenge was not performed.

The expected body weight gain was generally observed in the course of the study.

Number of animals with skin finding after the challenge		
	Animals with skin findings	
Treatment	**TS 50% in olive oil**	**Olive oil**
Control group 1	0/5	0/5
Control group 2*	-	0/5
Test group	0/10	0/10

*: control group 2 that had been intended for a potential 2nd challenge was not treated with the test substance, since a 2nd challenge was not necessary on the basis of the unambiguous results of the 1st challenge.

The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch (challenge) is taken into account for the determination of the sensitization rate.

The evaluation "sensitizing" results if at least 30 per cent of the test animals exhibit skin reactions in this adjuvant test.

Overall remarks, attachments

Overall remarks

Based on the evaluation criteria the results of this study show that 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester does not have a sensitizing effect on the skin of the guinea pig in the Maximization test under the test conditions chosen

Applicant's summary and conclusion

Interpretation of results

not sensitising

Executive summary

.

7.5 Repeated dose toxicity

7.5.1 Repeated dose toxicity: oral

Endpoint study record: Key.BASFAG50S0107/01009.Repeated dose toxicity: oral 90 d

UUID	IUC5-d281c68f-abad-40f1-bce2-255dfa7e5b74
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:15 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2002
Title	1,2 Cyclohexane dicarboxylic acid, di(isononyl)ester - Subchronic oral toxicity study in Wistar rats Administration in the diet for 3 months		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	50S0107/01009
Owner company	BASF SE		
Company study no.		**Report date**	2002-01-28

Data access

data submitter is data owner

Materials and methods

Test type

subchronic

Limit test

no

Test guideline

Qualifier	according to
Guideline	OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)
Deviations	no
Qualifier	according to
Guideline	EU Method B.26 (Sub-Chronic Oral Toxicity Test: Repeated Dose 90-Day Oral Toxicity Study in Rodents)
Deviations	no
Qualifier	according to
Guideline	other guideline: U.S. Food and Drug Administration: Draft "Redbook II";Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food; Subchronic Toxicity Tests with Rodents and Non Rodents, pp. 106 - 108 (1993);
Deviations	no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2 Cyclohexane dicarboxylic acid, di (isononyl)ester
- Test substance No.: 01/0107-1
- Bottling date: May 08, 2000
- Physical state: Liquid / colourless-clear
- Analytical purity: 99.6% (analytical report Proj. No. 01L00125)
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: wistar, CrlGlxBrlHan:WI

- Source: Charles River Sulzfeld, Germany
- Age at study initiation: 35 - 37 days
- Weight at study initiation: mean 150.5 g (males), 122.4 g (females)
- Housing: singly in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area about 800 cm2)
- Diet (e.g. ad libitum): ground Kliba maintenance diet rat/mouse/hamster, meal (Provimi Kliba SA, Kaiseraugst, Switzerland); ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on oral exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): weekly
- Mixing appropriate amounts with (Type of food): The test substance was weighed out and thoroughly mixed with a small amount of food. Then corresponding amounts of food, depending on the dose group, were added to this premix in order to obtain the desired concentration, and mixing was carried out for 10 minutes in a Ruberg (EM 100) laboratory mixer.

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The stability of the test substance in the diet over a period of up to 21 days at room temperature was confirmed during the study by GC.
Homogeneity analyses of the test substance preparations were performed in samples of the high and low concentrations at the start of the administration period. These samples also served for concentration control analyses. Additional concentration control analyses were performed with a sample drawn from the mid concentrations at the start of the administration period, as well as with samples from all concentrations after about 10 weeks.

Duration of treatment / exposure

90 days

Frequency of treatment

daily

Doses/concentrations

1500, 4500, 15000 ppm

Basis nominal in diet

107.1, 325.7, 1102.9 mg/kg bw/day for males

Basis actual ingested

128.2, 389.4, 1311.8 mg/kg bw/day for females

Basis actual ingested

No. of animals per sex per dose

20

Control animals

yes, plain diet

Details on study design

- Dose selection rationale: In a test study (BASF Project No. 37S0223/99062), the test substance was administered at dose levels of 600, 3,000 and 15,000 ppm for 4 weeks. Food consumption, water consumption, body weight, clinical signs and macroscopic findings were recorded. No signs of toxicity were observed

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays
- Cage side observations checked in table were included.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: weekly

BODY WEIGHT: Yes
- Time schedule for examinations: on day 0 (start of administration period) and thereafter at weekly intervals

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: Yes

OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: prior to the start of the administration period and at the end of the stud
- Dose groups that were examined: all animals

HAEMATOLOGY: Yes
- Time schedule for collection of blood: prior to dosing, day 30, day 62, 62 and at the end of study
- Anaesthetic used for blood collection: No
- Animals fasted: No

- How many animals: 10/sex/dose
- Parameters examined: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood count, differential blood smears, clotting analysis (prothrombin time)

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: prior to dosing, day 30, day 62, 62 and at the end of study
- Animals fasted: No
- How many animals: 10/sex/dose
- Parameters examined: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-γ-glutamyltransferase, sodium, potassium, chloride, inorganic posphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, magnesium

URINALYSIS: Yes
- Time schedule for collection of urine: prior to dosing, day 29, day 61, day 86
- Metabolism cages used for collection of urine: Yes
- Animals fasted: No
- Parameters examined: volume, color, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity, sediment

NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations: 82/84 (males) and 83/85 (females) starting in the morning
- Dose groups that were examined: all
- Battery of functions tested: passive observations without disturbing the animals, followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests

HORMONES: Yes
- total triiodothyronine (T3) after 3 months
- total thyroxine (T4) after 1, 2 and 3 months
- thyroid stimulating hormone (TSH) after 1, 2 and 3 months

Sacrifice and pathology

GROSS PATHOLOGY: Yes:
- Determination of weights: anesthetized animals, liver, kidneys, adrenal glands, testes, epididymides, ovaries, uterus, spleen, brain, heart, thymus, thyroid glands (with parathyroid glands)

HISTOPATHOLOGY: Yes
- all gross lesions, salivary glands (Glandula mandibularis and Glandula sublingualis), esophagus, stomach (forestomach and glandular stomach), duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, brain, pituitary gland, sciatic nerve, spinal cord (cervical, thoracic and lumbar cord), eyes, adrenal glands, thyroid glands, parathyroid glands, trachea, lungs, pharynx, larynx, nose (nasal cavities), aorta, heart, bone marrow (femur), lymph nodes (mandibular and mesenteric), spleen, thymus, kidneys, urinary, bladder, ovaries, testes, oviducts/uterus/vagina, epididymides/prostate gland, seminal vesicles, female mammary gland, skin, skeletal muscle,sternum with marrow, femur with knee joint, extraorbital lacrimal glands, Zymbal glands

Statistics

- DUNNET's test (two sided): Food consumption, body weight, body weight change, food

efficiency
- KRUSKAL-WALLIS test (two-sided) folowed by Wilcoxon-test: Feces, rearing, grip strength, length forelimbs, grip strength, length hindlimbs, foot'splay test, motor activity, clinical pathology parameters except differential blood count, weight parameters
- FISHER's exact test: urinalysis, except volume, color, turbidity and specific gravity

Any other information on materials and methods incl. tables

Results and discussions

Effect levels

Endpoint	NOAEL
Effect level	107.1 mg/kg bw/day (nominal)
Sex	male
Basis for effect level / Remarks	urinalysis, kidney weights
Endpoint	NOAEL
Effect level	389.4 mg/kg bw/day (nominal)
Sex	female
Basis for effect level / Remarks	kidney weights

Observations

Details on results

CLINICAL SIGNS AND MORTALITY
No animal died during the study. No substance-related effects were found.

BODY WEIGHT AND WEIGHT GAIN
No substance-related effects were observed

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
Food consumption was significantly increased in females of group 2 on day 28 (+5.4%), on day 56 (+5.5%) and on day 77 (+6.5%). Due to the isolated occurrence and the lack of a dose-response relationship, this was clearly fortuitous and not related to treatment.

FOOD EFFICIENCY
No substance-related effects were observed.

OPHTHALMOSCOPIC EXAMINATION
No substance-related effects were obtained. All findings were spontaneous in nature and equally distributed between treated animals and controls.

HAEMATOLOGY
On day 62 statistically significantly prolonged prothrombin times were observed in the mid and high dose males. No corresponding findings were seen in the males on day 30 and on day 91 of the study and in the females throughout the study. Thus, the prolongation of prothrombin times on day 62 is considered to be incidental in nature and not of toxicological relevance. No treatment-related effects were seen in the other hematology parameters.

CLINICAL CHEMISTRY
Enzyme examinations revealed decreased aspartate aminotransferase activities in the mid and high dose males on day 91. These findings are within the range of historical control data (see Supplement) and are regarded not to be related to the test compound administered. The statistical significant differences between the control and treatment groups are caused by a high control value which is increased by chance. In the females slight, but statistically significant, increases in γ-glutamyltransferase activities were found in the serum of the high dose animals throughout the study. These findings are considered to be treatment-related. No toxicologically relevant changes were observed in the other serum enzyme activities.
Compound-related differences in blood chemistry parameters were not evident at any dose level in either males or females.

URINALYSIS
On day 29 increased blood was measured in the urine specimens of the mid and high dose males. An increased number of abnormal transitional epithelial cells was also detected in the urine sediments of the mid and high dose males throughout the study. However, on day 86 the increase in transitional epithelial cells in the urine sediments of the mid dose males was not statistically significantly different to the respective control

HORMONES
After 30, 62 and 91 days of test substance administration significantly increased thyroid stimulating hormone (TSH) concentrations were found in the serum of the high dose females. A tendency towards increased TSH concentrations were also observed in the serum of the other treated males and females. However, most of these findings were not statistically significantly different to the corresponding control and in the males the increases in TSH concentrations exhibited no dose-response relationship. Although the increases in serum TSH levels were not pronounced these findings are assessed as being treatment-related.

NEUROBEHAVIOUR
- Open field observations and reflex tests: All findings including alopecia or hyperactivity were assessed as being incidental, as they occurred in single animals, only, or were equally distributed between treated groups and controls
- Feces, Rearing, Grip strength, Landing foot-splay test: No substance-related effects were observed
- Motor activity measurement: Regarding the overall motor activity no statistically significant deviations were seen in treated males and females.
Comparing the single intervals with the control groups, there was a statistically significant increased motor activity in females of dose group 3, only during the first interval. Due to the isolated occurrence this was assessed as being incidental.

ABSOLUTE ORGAN WEIGHTS
15000 ppm:
- weight of the thyroid glands, was significantly increased in males (+20.8%)
- mean liver weight was slightly although significantly increased in females (+11.8%)
1500 ppm: mean weight of the thyroid glands was also slightly although significantly increased in males(+12.4%)

The other mean absolute weight parameters did not show significant differences when compared with the control group.

RELATIVE ORGAN WEIGHTS
15000 ppm:
- mean weight of the liver was significantly increased in males (+5.9%) and in females (+13.2%)
- mean kidney weight was significantly increased in males (+9.7%) and in females (+8.1%)

- mean weight of the testes was significantly increased (+4.2%), but with no dose response relationship
- mean weight of the spleen was significantly increased in males (9.3%)
- mean weight of the thyroid glands was significantly increased in males (+20%).
4500 ppm:
- mean weight of the liver was slightly although significantly increased in females (+5.7%)
- mean kidney weights were significantly increased in the mid dose males (+6.7%), but with no dose response relationship
- mean weight of the testes was significantly increased (+6.8%), but with no dose response relationship
mean spleen weight was also slightly although significantly increased in males (+6.8%).
1500 ppm:
-mean kidney weights were significantly increased in the low dose group in male (+8.4%), but with no dose response relationship
- mean weight of the testes was significantly increased (+5.6%), but with no dose response relationship
- mean weight of the thyroid glands was slightly although significantly decreased in females (-14.3%)

The other mean relative weight parameters did not show significant differences when compared with the control group.

GROSS PATHOLOGY
Only a few gross lesions were noted in the glandular stomach (erosion/ulcer), liver (red or yellow focus), kidneys (cyst, granular surface, pelvic dilation and/or cortical retraction), testes (reduced organ size, unilateral), epididymides (reduced organ size, unilateral or abscess) and skin (sparse hair).
With two exceptions (erosion/ulcer in the mucosa of the glandular stomach of three females of the mid dose group and cortical retraction in the kidneys of two low dose males), all of the gross lesions were single observations, randomly distributed over control and treatment groups

HISTOPATHOLOGY:
- Kidneys: In the kidneys of male rats, α2µ−globulin accumulation was detected in the epithelia (and tubular lumen) of the proximal tubules of the renal cortex of all but one control animal with graded severity ranged from minimal (grade 1) to severe (grade 4). In the low dose group, there was no substantial difference in the amount of α2µ−globulin accumulation as compared to the control group with the exception that all 20 low dose males revealed α2µ−globulin accumulation, whereas one control animal did not show a positive reaction. In the mid and high dose groups, more animals with higher grades of severity were affected with α2µ−globulin accumulation in a dose related fashion. Two animals of the high dose group even revealed a severe degree of α2µ−globulin accumulation. Morphologically, no other cellular alterations (cell sloughing, apoptosis, pyknosis or necrosis) were observed in the proximal tubules. The α2µ−globulin accumulation was therefore interpreted as a treatment related but not as an adverse effect. The increased mean relative kidney weights seen in all three dose groups could not be correlated with a meaningful histopathologic finding. They were, hence regarded fortuitous and unrelated to treatment since there was no dose response relationship and the mean control weight of this study was the third lowest in comparison to thehistorical control data of nine other 3-month studies with the same rat strain.
- Thyroid glands: minimal to slight (grade 2) hypertrophy/hyperplasia of the follicular epithelia was recorded from male and female rats in all dose groups including the control group. In males, hypertrophy/hyperplasia was noted twice in control rats, whereas the majority of the treated animals were so affected (14/20, 11/20, 16/20 in the low, mid or high dose groups, respectively). In female rats, minimal to slight hypertrophy/hyperplasia of the follicular epithelia was observed in individual animals of the control, low and mid dose groups (1/20, 1/20, 3/20 for control, low or mid dose groups, respectively) and in most of the high dose animals (15/20). Morphology did not highlight a histopathologic finding in the

low dose group female rats that may account for the recorded significant decrease of the mean relative thyroid gland weight.
- Testes: no morphologic correlate was obtained for the significantly increased mean relative weights of the testes, seen in all three dose groups. No indication of an obstructive process in the rete testis, in the area of the transition from rete testis to caput epididymis or in the epididymides themselves was detected. Therefore, a relationship to treatment was considered unlikely.
- Spleen: no microscopic finding was obtained in the high dose group that may account for the significantly increased mean relative spleen weights

All other microscopic findings recorded were either single observations, or they were recorded at a low incidence, or they occurred in control animals only, or at comparable incidence and graded severity in control and high dose males and/or females.

HISTORICAL CONTROL DATA (if applicable)
Summary of 9 repeated dose-toxicity studies:
- Terminal body weight: 305.1 - 392.8
- Relative kidney weight (%): 0.614 - 0.708

Remarks on results including tables and figures

Summary:

The following substance-related adverse effects were obtained:

- 15000 ppm (1102.9 mg/kg bw/day in males; 1311.8 mg/kg bw/day in females): incr
urine specimens of the males; significantly increased mean relative kidney weight (fe

- 4500 ppm (325.7 mg/kg bw/day in males; 389.4 mg/kg bw/day in females): increas
specimens of the males

- 1500 ppm (107.1 mg/kg bw/day in males; 128.2 mg/kg bw/day in females): no treat

Therefore, the no observed adverse effect level (NOAEL) under the conditions of this
bw/day), as well as 4500 ppm in female rats (389.4 mg/kg bw/day).

Mean body weight ± SD (g):

		Day 0	Day 14	Day 28	Day 42	Day 56	Da
Control	Males	150.0 ± 8.6	246.1 ± 12.0	301.5 ± 16.3	337.3 ± 22.4	364.1 ± 26.7	283 2
	Females	121.3 ± 8.3	164.4 ± 10.5	190.0 ± 13.3	205.4 ± 15.3	216.9 ± 16.9	225 1
1500 ppm	Males	152.0 ± 10.3	247.5 ± 13.6	304.3 ± 17.1	336.4 ± 18.9	362.9 ± 21.3	38 2
	Females	121.5 ± 8.7	165.0 ± 10.8	191.7 ± 11.7	207.2 ± 14.5	220.1 ± 13.0	226 1
4500 ppm	Males	149.4 ± 7.6	242.2 ± 12.1	296.1 ± 21.2	331.7 ± 23.8	356.6 ± 27.1	37 2
	Females	123.4 ± 7.7	167.6 ± 9.9	195.2 ± 12.6	211.2 ± 13.5	224.1 ± 16.3	229 1

15000 ppm	Males	149.0 ± 9.7	24.7 ± 12.9	294.5 ± 18.1	326.0 ± 23.6	352.0 ± 26.4	372 2
	Females	123.2 ± 9.3	163.9 ± 10.2	191.1 ± 12.2	204.4 ± 11.7	217.0 ± 11.3	223 1

Relative organ weights (%):

		Liver	Kidneys	Testes (m) / Ovaries (f)	Epididymis (m) / Uterus (f)	Heart	Sp
Control	Males	2.478	0.629	0.927	0.313	0.259	0
	Females	2.593	0.693	0.045	0.329	0.322	0
1500 ppm	Males	2.552	0.682**	0.979**	0.316	0.27	0
	Females	2.584	0.683	0.044	0.323	0.314	0
4500 ppm	Males	2.557	0.0671**	0.99*	0.313	0.268	0
	Females	2.741*	0.688	0.046	0.308	0.309	0
15000 ppm	Males	2.624**	0.69**	0.966**	0.316	0.269	0.
	Females	2.935**	0.749**	0.048	0.343	0.31	0

* p<0.05; ** p< 0.01

Overall remarks, attachments

Overall remarks

Endpoint study record: BASFAG37S0223/99062.Repeated dose toxicity: oral 28d

UUID	IUC5-697fd8ca-e609-43c0-b90c-113ce49486d1
Dossier UUID	0
Author	henscho / BASF SE / Ludwigshafen am Rhein / Germany
Date	2009-09-24 10:11:38 CEST
Remarks	

Administrative Data

Purpose flag	(X) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2000
Title	1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester Repeated dose oral toxicity study in Wistar rats Administration in the diet for 4 weeks and recovery period of 2 weeks		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	37S0223/99062
Owner company	BASF SE		
Company study no.		**Report date**	2000-05-09

Data access

data submitter is data owner

Materials and methods

Test type

subacute

Limit test

no

Test guideline

Qualifier	according to
Guideline	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)
Deviations	no

Qualifier according to

Guideline EU Method B.7 (Repeated Dose (28 Days) Toxicity (Oral))

Deviations no

Qualifier according to

Guideline other guideline: Japan/MHW: Guidelines for Toxicity Testing of Chemicals, twenty-eight-day repeated dose toxicity in mammalian species; MITI/MHW, 1987

Deviations no

GLP compliance

yes Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid, di (isononyl) ester
- Test substance No.: 99/223-1
- Date of production: May 26, 1999
- Physical state: Liquid/colorless-clear
- Analytical purity: 99.7% (method: gas chromatography)
- Lot/batch No.: R 5116/1 (#23725)
- Stability under test conditions: Proven by reanalysis (report of March 28, 2000)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: Wistar Crl: WI (Glx/BRL/HAN)BR
- Source: Charles River WIGA GmbH, Sulzfeld, Germany
- Age at study initiation: 33 - 35 days
- Weight at study initiation: mean 136.4 g (males), 117.2 g (females)
- Housing: single housing in in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, FRG (floor area about 800 cm2)
- Diet (e.g. ad libitum): ground, Conservation-feed for mouse/rat, 9433 LL Meal from the company Eberle Nafag AG Gossau, Switzerland; ad libitum
- Water (e.g. ad libitum): drinking wtare; ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on oral exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): once
- Mixing appropriate amounts with (Type of food): The test substance was weighed out and thoroughly mixed with a small amount of food. Then corresponding amounts of food, depending on the dose group, were added to this premix in order to obtain the desired concentration, and mixing was carried out for about 10 minutes in laboratory mixer.

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The stability of the test substance in diet was tested for a period of 10 days at room temperature with GC.
Homogeneity analyses of the test substance preparations were demonstrated in samples of the highest and lowest concentration at the start of the administration period. These samples also served for concentration control analyses. Additional concentration control analyses were performed with samples drawn from the mid concentration at the start of the administration period.

Duration of treatment / exposure

28 days

Frequency of treatment

daily

Doses/concentrations

600, 3000 and 15000 ppm

Basis nominal in diet

64, 318, 1585 mg/kg bw/d for males

Basis actual ingested

66, 342, 1674 mg/kg bw/d for females

Basis actual ingested

No. of animals per sex per dose

Control, 15000 ppm: 10 (5 used for recovery group)
600, 3000 ppm: 5

Control animals

yes, plain diet

Details on study design

- Dose selection rationale: In a test study (BASF, 1999), substance was administered to groups of 3 male and 3 female Wistar rats at dietary concentrations of 0, 5,000 and 15,000 ppm for 2 weeks. Food consumption, water consumption, body weight, clinical signs and macroscopic findings were recorded. No signs of toxicity were observed.
- Post-exposure recovery period: 14 days

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays
- Cage side observations checked in table were included.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: once before the administration (day -1), as well as on days 7, 14, 21 and 35

BODY WEIGHT: Yes
- Time schedule for examinations: on day 0 (start of administration period) and thereafter at weekly intervals

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day:Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: Yes

WATER CONSUMPTION: Yes
- Time schedule for examinations: daily

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: days 28, 42
- Anaesthetic used for blood collection: No
- Animals fasted: Yes
- How many animals: all
- Parameters examined: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood count, differential blood smears, clotting analyses (prothrombin time)

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: days 28, 42
- Animals fasted: Yes
- How many animals: all
- Parameters examined: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-γ-glutamyltransferase, cyanide-insensitive Palmitoyl-CoA-oxidation, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, magnesium

URINALYSIS: Yes
- Time schedule for collection of urine: days 25, 39
- Metabolism cages used for collection of urine: Yes
- Animals fasted: No
- Parameters examined: volume, color, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity, sediment

NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations: days 26 (males), 27 (females)
- Dose groups that were examined: all
- Battery of functions tested: open field observations, sensorimotor tests/reflexes, motor activity assessment

Sacrifice and pathology

GROSS PATHOLOGY: Yes:
- Determination of weights: anesthetized animals, liver, kidneys, adrenal glands, testes, epididymides, ovaries, spleen, brain, heart, thymus

HISTOPATHOLOGY: Yes
- brain, pituitary gland, thyroid glands/ parathyroid glands, thymus, trachea, lungs, heart, liver, spleen, kidneys, adrenal glands, testes/ ovaries, uterus/ vagina, epididymides/ prostate/ seminal vesicle, stomach (fore- and glandular stomach), duodenum/ jejunum/ ileum, cecum/ colon/ rectum, urinary bladder, lymph nodes (mandibular and mesenteric lymph node), sciatic nerve, bone marrow (femur), eyes, spinal cord (cervical, thoracic and lumbar cord)
- cytochemical presentation of peroxisomes in the liver

Statistics

- DUNNETT's test (two-sided): food consumption, body weight, body weight change, food efficiency
- KRUSKAL-WALLIS test (two-sided) followed by MANN-WHITNEY U-test (two-sided): Feces, rearing, grip strength forelimbs, grip strength hindlimbs,
landing footsplay test, motor activity, clinical pathology parameters, except differential blood count
- KRUSKALWALLIS test (two-sided) followed by WILCOXON test: weight parameters
- Welch t-test (two-sided): body weight, body weight change, food consumption, food efficiency
- FISHER's exact test: urinalysis, except volume, color, turbidity and specific gravity

Any other information on materials and methods incl. tables

Results and discussions

Effect levels

Endpoint NOAEL

Effect level	3000 ppm
Sex	male/female
Basis for effect level / Remarks	clinical chemistry; urinalysis
Endpoint	NOAEL
Effect level	342 mg/kg bw/day (actual dose received)
Sex	female
Basis for effect level / Remarks	clinical chemistry
Endpoint	NOAEL
Effect level	318 mg/kg bw/day (actual dose received)
Sex	male
Basis for effect level / Remarks	clinical chemistry, urinalysis

Observations

Details on results

CLINICAL SIGNS AND MORTALITY
No animal died during the study. No abnormal clinical signs were detected.

BODY WEIGHT AND WEIGHT GAIN
No substance-related findings were obtained.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
No substance-related findings were obtained.

FOOD EFFICIENCY
No substance-related findings were obtained.

HAEMATOLOGY
There are no treatment-related changes in the hematology parameters measured.

CLINICAL CHEMISTRY
At the end of the administration period slightly increased γ-glutamyltransferase activities were found in the sera of the females of high dose group. After cessation of test compound administration γ-glutamyltransferase activities in the high dose females returned to normal. No treatment-related changes were observed in the other enzyme determinations, particularly in the cyanide-insensitive palmitoyl-CoA-oxidation.
Blood chemistry examinations revealed significantly increased sodium concentrations in the males of mid and high dose groups and increased potassium levels in all treated males at the end of the administration period. In the females ofhigh dose group reduced total bilirubin concentrations were detected after 4 weeks of test substance administration. After withdrawal of the test compound all changes seen in blood chemistry parameters recovered within 2 weeks. No toxicologically relevant changes were seen in the other blood chemistry

parameters.

URINALYSIS
Male animals of test group 3 excreted increased numbers of degenerated epithelial cells. No treatment-related changes were observed in the other urine parameters. The increase in degenerated epithelial cells in the urine specimens of the males of the high dosegroup normalized within 2 weeks after withdrawal of the test compound.

NEUROBEHAVIOUR/FOB
As all findings were equally distributed between treated groups and control or as only single animals were affected, they were clearly incidental in nature

ABSOLUTE ORGAN WEIGHTS
There were no statistical significant weight changes

RELATIVE ORGAN WEIGHTS
A decrease of the heart weight was noted in the low, mid and high dose groups (approx. 8 %).

GROSS PATHOLOGY
Glandular stomach: The macroscopic finding ”Erosion/ ulcer” was recorded for two male animals of low dose group.
Ovaries: One ”Cyst” was noted unilaterally in one female animal of mid dose group.

HISTOPATHOLOGY
All lesions noted are seen to be incidental or spontaneous in origin and not related to treatment.

Remarks on results including tables and figures

Mean body weight ± SD (g):

		Day 0	Day 14	Day 27	Day 41
Control	Male	135.6 ± 7.5	213.8 ± 13.1	252.4 ± 20.4	296.5 ± 22.7
	Female	116.3 ± 6.4	148.2 ± 11.5	166.8 ± 17.2	188.3 ± 7.7
600 ppm	Male	136.7 ± 7.1	214.1 ± 5.7	255.6 ± 9.5	-
	Female	117.0 ± 7.6	155.0 ± 9.7	173.0 ± 10.9	-
3000 ppm	Male	136.8 ± 5.5	210.1 ± 5.1	243.2 ± 5.6	-
	Female	117.9 ± 6.2	152.8 ± 8.4	173.1 ± 8.7	-
15000 ppm	Male	136.6 ± 6.5	209.6 ± 9.3	249.1 ± 13.2	280.5 ± 13.0
	Female	117.5 ± 5.1	150.2 ± 5.3	168.1 ± 6.9	183.7 ± 7.8

Summary:

The following substance-related findings were observed:

15000 ppm (1585 mg/kg bw in males, 1674 mg/kg bw in females):

- increased γ-glutamyltransferase in the females

- increased numbers of degenerated epithelial cells in the urine of males

- decreased total bilirubin in the females

3000 ppm (318 mg/kg bw in males, 342 mg/kg bw in females):

- no substance related effects

600 ppm (64 mg/kg bw in males, 66 mg/kg bw in females)

- no substance related effects

Thus, the oral administration of the test compound at a concentration of 15000 ppm caused changes in clinical chemistry parameters associated with microsomal enzyme induction in the females and indications of mild impairment of renal function in the males.

There was no increase in cyanide-insensitive palmitoyl-CoA-oxidation or accumulation of liver peroxisomes.

The no observed adverse effect level (NOAEL) under the conditions of this study was 3000 ppm (318 mg/kg bw in males, 342 mg/kg bw in females).

Overall remarks, attachments

Overall remarks

7.6 Genetic toxicity

7.6.1 Genetic toxicity in vitro

Endpoint study record: Key.BASF.40M0223/994088.Genetic toxicity in vitro. Ames

UUID	IUC5-e5d1b70c-9aea-46ed-b662-37df81eb3814
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:17 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2000
Title	Salmonella typhimurium/ Escherichia coli Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester.		
Bibliographic source	Unpublished report		
Testing laboratory	Department of Toxicology, BASF AG	**Report no.**	40M0223/994088
Owner company	BASF SE		
Company study no.		**Report date**	2000-01-19

Data access

data submitter is data owner

Materials and methods

Type of genotoxicity

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	according to
Guideline	OECD Guideline 471 (Bacterial Reverse Mutation Assay)
Deviations	no
Qualifier	according to
Guideline	EU Method B.13/14 (Mutagenicity - Reverse Mutation Test Using Bacteria)
Deviations	no

GLP compliance

yes Department of Toxicology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester
- Physical state: colorless liquid
- Analytical purity: 99.7%
- Lot/batch No.: R5116/1 (# 23725)
- Stability under test conditions: verified
- Storage condition of test material: room temperature

Method

Target gene

- Salmonella typhimurium: his
- E. coli: trp

Species/strain

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	S-9 mix
Species/strain	E. coli WP2 uvr A

Details on mammalian cell lines (if applicable)	
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	S-9 mix

Test concentrations

20 µg - 5000 µg/plate (SPT);
4 µg - 2500 µg/plate (PIT)

Vehicle

- Vehicle(s)/solvent(s) used: acetone
- Justification for choice of solvent/vehicle: Due to the limited solubility of the test substance in water, acetone was selected as the vehicle, which had been demonstrated to be suitable in bacterial reverse mutation tests and for which historical control data are available.

Controls

Negative controls	no
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	other: 2-aminoanthracene
Remarks	with S-9 mix
Negative controls	no
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	other: N-methyl-N'-nitro-N-nitrosoguanidine
Remarks	without S-9 mix for TA1535 and TA100
Negative controls	no
Solvent / vehicle	yes

controls	
True negative controls	no
Positive controls	yes
Positive control substance	other: 4-nitro-o-phenylendiamine
Remarks	without S-9 mix for TA98
Negative controls	no
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	9-aminoacridine
Remarks	without S-9 mix for TA1537
Negative controls	no
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	4-nitroquinoline-N-oxide
Remarks	without S-9 mix for E . coli WP2 uvrA

Details on test system and conditions

METHOD OF APPLICATION: in agar (plate incorporation); preincubation;

DURATION
- Preincubation period: 20 min
- Exposure duration: 48 - 72 hours

DETERMINATION OF CYTOTOXICITY
- Method: relative total growth

Evaluation criteria

The test chemical is considered positive in this assay if the following criteria are met:
A dose-related and reproducible increase in the number of revertant colonies, i .e. about doubling of the spontaneous mutation rate in at least one tester strain either without S-9 mix or after adding a metabolizing system.
A test substance is generally considered nonmutagenic in this test if:
The number of revertants for all tester strains were within the historical negative control

range under all experimental conditions in two experiments carried out independently of each other.

Any other information on materials and methods incl. tables

1st Experiment

Strains: TA 1535, TA 100, TA 1537, TA 98, E . coli WP2 uvrA

Doses: 0; 20; 100; 500 ; 2,500 and 5,000 pg/plate

Vehicle: Acetone

Type of test: Standard plate test with and without S-9 mix

Number of plates: 3 test plates per dose or per control

2nd Experiment

Strains: TA 1535, TA 100, TA 1537, TA 98, E . coli WP2 uvrA

Doses: 0 ; 4; 20; 100 ; 500 and 2,500 pg/plate

Vehicle: Acetone

Type of test: Preincubation test with and without S-9 mix

Number of plates : 3 test plates per dose or per control

Results and discussions

Test results

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	other: weak bacteriotoxic effect
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes
Species/strain	E. coli WP2 uvr A
Metabolic activation	with and without
Test	all strains/cell types tested

system	
Genotoxicity	negative
Cytotoxicity	other: weak bacteriotoxic effect
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Remarks on results including tables and figures

Preincubation Test:

Dose µg/plate	metabolic activation	TA98			TA100	
		mean	SD	FAC	mean	SD
0	+	30	3	1.0	149	8
4	+	25	2	0.8	137	8
20	+	25	5	0.8	153	24
100	+	22	2	0.8	137	8
500	+	19	1	0.6	126	9
2500	+	16P	5	0.5	139P	13
2.5 µg 2-Aminoanthracene	+	511	11	17.2	634	55
0	-	27	4	1.0	132	10
4	-	24	4	0.9	124	5
20	-	18	6	0.7	129	11
100	-	18	2	0.7	148	21
500	-	18	3	0.7	137	7
2500	-	15P	5	0.6	145P	15

5 µg MNNG	-				1097	21
10 µg 4-Nitro-o-phenylendiamin	-	680	40	25.2		
5 µg 4-nitroquinoline-N-oxide	-					
100 µg 9-amninoacridine	-					

SD = standard deviation, FAC = factor, P = Precipitation

for E. coli: 60µg 2 -Aminoanthracene

Standard Incubation Test:

Dose µg/plate	**metabolic activation**	**TA98**			**TA100**	
		mean	**SD**	**FAC**	**mean**	**SD**
0	+	36	5	1.0	156	3
20	+	35	4	1.0	138	3
100	+	33	3	0.9	144	14
500	+	24	2	0.7	141	2
2500	+	24P	3	0.7	148P	2
5000	+	21P	2	0.6	139P	12
2.5 µg 2-Aminoanthracene	+	796	53	21.9	1496	250
0	-	27	1	1.0	136	17
20	-	20	3	0.8	126	10
100	-	22	7	0.8	116	7
500	-	18	5	0.7	108	6
2500	-	11P	2	0.4	109P	17
5000	-	11P	2	0.4	117P	15

5 µg MNNG	-				1692	129
10 µg 4-Nitro-o-phenylendiamin	-	825	75	30.5		
5 µg 4-nitroquinoline-N-oxide	-					
100 µg 9-amninoacridine	-					

SD = standard deviation, FAC = factor, P = Precipitation
for E. coli: 60µg 2 -Aminoanthracene

A slight decrease in the number of his+ revertants was observed in the SPT with the strains T factor decreased down to 0.4 at 2500 and 5000µg/plate for both strains. With S-9 mix the fac

Weak signs for bacteriotoxicity were ocassionally observed in the PIT depending on the strai

Solubility

Test substance precipitation was found from about 2500 µg/plate onward.

Overall remarks, attachments

Overall remarks

An increase in the number of his^+or trp^+revertants was not observed in the standard plate test or in the preincubation test either without S-9 mix or after the addition of a metabolizing system. According to the results of the present study, the test substance 1,2 - Cyclohexane dicarboxylic acid, di (iisononyl)ester is**not mutagenic**in the Salmonella typhimurium/Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Key.BASF.32M0223/999040.Genetic toxicity in vitro. Chromosome abberation

UUID	IUC5-06b09256-2edd-4fff-b70d-8bde0275ef89
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:19 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	RCC Cytocell Research GmbH	**Year**	2000
Title	In vitro Chromosome Aberration Assay in Chinese Hamster V79 Cells with 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester.		
Bibliographic source	BASF AG, unpublished report		
Testing laboratory	RCC Cytocell Research GmbH	**Report no.**	RCC - CCR Project 651400
Owner company	BASF SE		
Company study no.	32M0223/999040	**Report date**	2000-05-24

Data access

data submitter is data owner

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	according to
Guideline	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)
Deviations	no
Qualifier	according to
Guideline	EU Method B.10 (Mutagenicity - In Vitro Mammalian Chromosome Aberration Test)
Deviations	no

GLP compliance

yes (incl. certificate) RCC Cytocell Research GmbH

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester
- Molecular weight (if other than submission substance): 424
- Substance type: colourless
- Physical state: liquid
- Analytical purity: >99%
- Lot/batch No.: R5116/1 (#23725)
- Storage condition of test material: room temperature

Method

Target gene

not applicable

Species/strain

Species/strain	Chinese hamster lung fibroblasts (V79)
Details on mammalian cell lines (if applicable)	- Type and identity of media: MEM (Minimal Essential Medium; SEROMED; D-1 2247 Berlin) supplemented with 10 % fetal calf serum (FCS; PAA Laboratories GmbH, D-35091 Cölbe) - Properly maintained: yes - Periodically checked for Mycoplasma contamination: yes - Periodically checked for karyotype stability: yes
Additional strain characteristics	no data
Metabolic activation	with and without
Metabolic activation system	S-9 mix

Test concentrations

25, 50, 100, 200, 400, 1000 µg/ml

Vehicle

- Vehicle(s)/solvent(s) used: acetone
- Justification for choice of solvent/vehicle: The solvent was chosen to its solubility properties and its relative nontoxicity to the cell cultures .

Controls

Negative controls	yes
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	cyclophosphamide
Remarks	with S-9 mix
Negative controls	yes
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	ethylmethanesulphonate
Remarks	without S-9 mix

Details on test system and conditions

METHOD OF APPLICATION: in medium

DURATION
- Exposure duration: 4 or 18 hours
- Expression time (cells in growth medium): 18 or 28 hours
- Fixation time (harvest of cells): 18 or 28 hours

STAIN (for cytogenetic assays): Giemsa staining

NUMBER OF REPLICATIONS: two cultures (10 coordinate defined fields per culture)

DETERMINATION OF CYTOTOXICITY
- Method: relative total growth

OTHER EXAMINATIONS:
- Determination of polyploidy: yes
- Other: mitotic index, chromosome aberrations (gaps, chromatid type, chromosome type)

Evaluation criteria

A test article is classified as non-mutagenic if:
- the number of induced structural chromosome aberrations in all evaluated dose groups are in the range of our historical control data (0.0 - 4.0 % aberrant cells exclusive gaps).
and/or
- no significant increase of the number of structural chromosome aberrations is observed.

A test article is classified as mutagenic if:
- the number of induced structural chromosome aberrations are not in the range of our historical control data (0.0 - 4.0 % aberrant cells exclusive gaps) .
and
- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistics

Statistical significance was confirmed by means of the Fischer's exact test ($p < 0.05$). However, both biological and statistical significance should be considered together. If the criteria for the test article are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Any other information on materials and methods incl. tables

Evaluated experimental point after treatment with 1,2 -Cyclohexane dicarboxylic acid, di(isononyl)ester:

Exp.	Preparation interval	Exposure period	Concentrations in µg/ml			
			without S-9 mix			
I	18 h	4 h	25.0	50.0	100.0^{P}	200.0^{P}
III	18 h	18 h	25.0	50.0	100.0^{P}	1000.0^{P}
III	28 h	18 h	25.0	50.0	100.0^{P}	1000.0^{P}
			with S-9 mix			
I	18 h	4 h	25.0	50.0	100.0	200.0^{P}
II	18 h	4 h		100.0^{P}	200.0^{P}	400.0^{P}
III	28 h	4 h	25.0	50.0^{P}	100.0^{P}	200.0^{P}

P: precipitation observed

Acceptability of the Assay

The chromosome aberration assay performed in our laboratory is considered acceptable if it meets the following criteria:

a) The number of structural aberrations found in the negative and/or solvent controls falls within the range of our historical laboratory control data: 0.00 % - 4.00 %.

b) The positive control substances should produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratories historical control data (600 µg/ml EMS: range: 9 -39; 0.47 -0.93 µg/ml CPA range: 7.5 -49.5).

Results and discussions

Test results

Species/strain	Chinese hamster lung fibroblasts (V79)
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	yes from 1062.5 µg/ml onward
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS
- Effects of pH: no relevant influence of the test substance
- Effects of osmolality: no relevant influence of the test substance
- Precipitation: In the pre-test on toxicity, precipitation of the test substance, was observed at 132 .8 µg/ml and above .

RANGE-FINDING/SCREENING STUDIES: A pre-test on cell growth inhibition with 4 h and 24 h treatment was performed in order to determine the toxicity of the test substance. Cytotoxicity was determined using concentrations separated by no more than a factor of 2 - 3.33. The general experimental
conditions in this pre-test were the same as described below for the cytogenetic main experiment.
The following method was used:
In a quantitative assessment, exponentially growing cell cultures (seeding 39600 cells/slide, with regard to the culture time 48 h) were treated with the test article for simulating the conditions of the main experiment. A qualitative evaluation of cell number and cell morphology was made 4 h and 24 h after start of treatment. 24 h after start of treatment the cells were stained and in 10 coordinate defined fields of the slides (2 slides per treatment group) the cells were counted. The cell number of the treatment groups is given as % cells in relation to the control.

ADDITIONAL INFORMATION ON CYTOTOXICITY: In a range finding pre-test on toxicity cell numbers 24 h after start of treatment were scored as indicator for cytotoxicity. Concentrations between 33.2 and 4250 µg/ml were applied. In the absence and the presence of S9 mix after 4 h treatment no toxic effects could be observed. However, 24 h continuous treatment with 1062.5 µg/ml and above in the absence of S9 mix induced clearly reduced cell numbers.

Remarks on results including tables and figures

Summery of results of the chromosomal aberration study with 1,2 -Cycohexan

Exp.	Preparation interval	Test Substance Concentration in µg/ml	Polyploid cells in %	Cell number % of contro
Exposure period 4 h without S-9 mix				
I	**18 h**	negative control	4.1	n.t.
		acetone 0.5%	3.6	100
		EMS 1000µg/ml	4.0	n.t.
		25.0	3.8	98
		50.0	5.7	104
		100.0 (P)	3.5	76
		200.0 (P)	4.3	105
Exposure period 18 h without S-9 mix				
III	**18 h**	negative control	3.5	n.t.
		acetone 0.5%	2.1	100
		EMS 600µg/ml	2.3	n.t.
		25.0	3.8	86
		50.0	3.8	74
		100.0 (P)	2.2	66
		1000.0 (P)	3.7	47
III	**28 h**	negative control	4.6	n.t.
		acetone 0.5%	2.6	100
		EMS 600µg/ml	1.7	n.t.
		25.0	2.0	89
		50.0	3.4	107
		100.0 (P)	2.4	106

		1000.0 (P)	3.5	122
Exposure period 4 h with S-9 mix				
I	**18 h**	negative control	2.4	n.t.
		acetone 0.5%	4.9	100
		CPA 0.7 µg/ml	4.3	n.t.
		25.0	5.4	92
		50.0	3.7	91
		100.0	4.8	115
		200.0 (P)	4.5	113
II	**18 h**	negative control	3.4	n.t.
		acetone 0.5%	3.7	100
		CPA 0.7 µg/ml	3.1	n.t.
		100.0 (P)	4.5	106
		200.0 (P)	5.0	86
		400 (P)	4.8	87
III	**28 h**	negative control	3.1	n.t.
		acetone 0.5%	3.4	100
		CPA 0.7 µg/ml	4.2	n.t.
		25.0	5.7	85
		50.0 (P)	4.8	123
		100.0 (P)	4.5	115
		200.0 (P)	3.8	123

* = inclusive cells carrying exchanges; n.t. = not tested; (P) = precipitation occured; S
solvent control values; EMS = Ethylmethan sulfonate; CPA = Cyclophosphamide
In this study, in the absence of S9 mix, toxic effects indicated by reduced cell numbe
Cultures after 4 h treatment in experiment I and 18 h treatment with a recovery time
of S9 mix, toxic effects indicated by reduced mitotic indices were observed in experi
revealed neither reduced mitotic indices nor reduced cell numbers.
However, an apparent concentration related increase of aberrant cells exclusive gap
cells carrying exchanges after treatment with 200 µg/ml in experiment I in the preser
observation in experiment I is regarded as incidental and thus, as being biologically i
< 0.05) increase of the aberration rate (4 % excluding gaps) was observed after trea
(1.0% exclusive gaps). This response is, however, biologically irrelevant, since firstly

the test substance. Additionally, the observed 4.0 % is within our historical range.

Overall remarks, attachments

Overall remarks

Neither a significant nor a biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test article.

The test substance is considered to be**non-clastogenic**in this chromosome aberration assay.

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Key.BASF.50M0107/014031.Genetic toxicity in vitro.gene mutation

UUID	IUC5-5f25eace-9ba1-4c7d-8346-368654bb5bc6
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:21 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2001
Title	In Vitro Gene Mutantion Test With 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester In CHO Cells (HPRT Locus Assay)		
Bibliographic source	Unpublished data		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	50M0107/014031
Owner company	BASF SE		
Company study no.	50M0107/014031	**Report date**	2001-12-12

Data access

data submitter is data owner

Materials and methods

Type of genotoxicity

gene mutation

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier	according to
Guideline	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)
Deviations	no
Qualifier	according to
Guideline	EU Method B.17 (Mutagenicity - In Vitro Mammalian Cell Gene Mutation Test)
Deviations	no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2 Cyclohexane dicarboxylic acid, di (isononyl)ester
- Substance type: colourless
- Physical state: liquid
- Analytical purity: 99.6%
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Storage condition of test material: room temperature

Method

Target gene

hypoxanthine-guanine phosphoribosyl transferase (HGPRT)

Species/strain

Species/strain	Chinese hamster Ovary (CHO)
Details on mammalian cell lines (if applicable)	- Type and identity of media: Ham's F12 medium with glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS). During exposure to the test substance, Ham's F12 medium was used without FCS supplementation. In the case of continuous treatment Ham's F12 medium with FCS supplementation was used. Pretreatment medium (HAT medium): FCS-supplemented Ham's F12 medium with glutamine and hypoxanthine containing per ml: Hypoxanthine (13.6µg), Aminopterin (0.18µg) and Thymidine (3.88µg) Selection medium (TG medium): Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/ml All media were supplemented with 1% (v/v) penicillin/streptomycin (10,000 IU / 10,000 µg/ml) and 1 % (v/v) amphotericine B (250 µg/ml) - Properly maintained: yes - Periodically checked for Mycoplasma contamination: yes - Periodically "cleansed" against high spontaneous background: yes
Additional strain characteristics	other: substrain K1

Metabolic activation	with and without
Metabolic activation system	S-9 mix

Test concentrations

312.5; 625; 1250; 2500; 5000 µg/ml

Vehicle

- Vehicle(s)/solvent(s) used: acetone
- Justification for choice of solvent/vehicle: Due to the limited solubility of the test substance in water, acetone was selected as the vehicle, which had been demonstrated to be suitable in the CHO/HPRT test and for which historical data is available.

Controls

Negative controls	yes
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	ethylmethanesulphonate
Remarks	without S-9 mix
Negative controls	yes
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	3-methylcholanthrene
Remarks	with S-9 mix

Details on test system and conditions

METHOD OF APPLICATION: in medium

DURATION
- Preincubation period: Attachment: 500 000 cells per flask were incubated for about 24 hours
- Exposure duration: incubated for 4 hours with test substance and with or without S-9 mix in serum free medium; replacing the serum free medium with Ham's F12 medium + 10% FCS and incubation of further 17 to 24 hours
- Expression time (cells in growth medium): about 7 to 9 days (at the 4th passage cells were transferred to selection medium)
- Selection time (if incubation with a selection agent): 6x 300000 cells were seeded in selection medium and incubated for about 1 week

- Fixation time (start of exposure up to fixation or harvest of cells): after selection cells were fixed with methanol

SELECTION AGENT (mutation assays): 6-thioguanine
STAIN (for cytogenetic assays): Giemsa

NUMBER OF REPLICATIONS: 6

DETERMINATION OF CYTOTOXICITY
- Method: relative total growth

Evaluation criteria

- Increases of the corrected mutation frequencies above the concurrent negative control values and above 15 mutants per 106 clonable cells and/or the evidence of a doseresponse relationship in the increase in mutant frequencies .
- Evidence of reproducibility of any increase in mutant frequencies .
- A statistically significant increase in mutant frequencies and the evidence of a doseresponse relationship .

Isolated increases of mutant frequencies above 15 mutants per 1000000 clonable cells or isolated statistically significant increases without a dose-response- relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.
The test chemical is considered non-mutagenic according to the following criteria:
The corrected mutation frequency in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

Any other information on materials and methods incl. tables

Uncorrected mutant frequency (MF):

The uncorrected mutant frequency per10^6cells was calculated for each test group as follows:

$MF_{uncorr.}$ = (total number of mutant colonies / number of seeded cells) x 10^6

Corrected mutant frequency (MF):
The corrected mutant frequency / 10^6cells was calculated for each test group as follows:
$MF_{corr.}$ = ($MF_{uncorr.}$ / $CE_{2\ absolute}$) x 100

Cytotoxicity :
The cloning efficiency (CE, %) was calculated for each test group as follows :

$CE_{absolute}$ = (total number of colonies in the dose group / total number of seeded cells in the dose group) x 100
$CE_{relative}$ = (CE of the dose group / CE of the vehicle control) x 100

The numbers of colonies counted in the test groups are given as “absolute CE” values. The “relative CE” values are related to the corresponding vehicle control which is set 100%.

Cloning efficiency 1 (survival):
The CE_1 of the test substance after the exposure period was determined for each test group and given as absolute and relative cloning efficiencies.
Cloning efficiency 2 (viability):
The CE_2 of the test substance at the end of the expression period was determined for each test group and given as absolute and relative cloning efficiencies.

Results and discussions

Test results

Species/strain	Chinese hamster Ovary (CHO)
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	no
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Remarks on results including tables and figures

Summerized results:

			corrected mutant frequency (per 10^6cells)		relative cloning efficiancy (%)	
			Exp.1	Exp. 2	Exp. 1	Exp. 2
	conc (µg/ml)	S9 mix			CE_1(survival)	
solvent control	0	-	2.99	8.73	100.0	100.0
Ethylmethan sulfonate	300.0	-	112.46	163.48	97.4	87.0
1,2 Cycolohexane dicarboxylic acid	312.5	-	1.28	1.22	98.9	99.8
	625.0	-	0.35	1.94	81.6	93.1
	1250.0	-	2.37	0.00	50.2	98.0
	2500.0	-	1.63	1.83	46.9	93.0
	5000.0	-	0.00	3.64	43.0	70.8
solvent						

control	0	+	1.77	6.43	100.0	100.0
3-Methylcholanthrene	10	+	75.15	112.73	88.5	87.2
1,2 Cycolohexane dicarboxylic acid	312.5	+	1.21	6.47	88.1	77.8
	625.0	+	0.66	0.67	93.8	82.4
	1250.0	+	3.02	3.04	98.4	76.9
	2500.0	+	2.52	1.68	85.5	84.5
	5000.0	+	0.40	0.82	85.3	68.0

Cytotoxicity could not be detected up to the highest recommended test concentration which distinct test substance precipitation was observed.

On the basis of the results of the present study, the test substance did not cause any increase i frequencies either without S-9 mix or after adding a metabolizing system in two experiments independently of each other. Thus, under the experimental conditions of this assay, 1,2 Cyclc acid, di(isononyl)ester has no mutagenic activity in vitro in the CHO/HPRT forward mutatio

Overall remarks, attachments

Overall remarks

Applicant's summary and conclusion

Interpretation of results

negative

7.6.2 Genetic toxicity in vivo

Endpoint study record: Key.BASF.26M0107/019004.Genetic toxicity in vivo

UUID	IUC5-c72a7f4a-b40f-47bf-80da-11c6fc8d5e8b
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:23 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	RCC Cytotest Cell Research GmbH	**Year**	2001
Title	Micronucleus assay in bone marrow cells of the mouse with 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester		
Bibliographic source	Department of Product Safety. Unpublished data		
Testing laboratory	RCC Cytotest Cell Research GmbH	**Report no.**	RCC - CCR Project 698101
Owner company	BASF SE		
Company study no.	26M0107/019004	**Report date**	2001-09-25

Data access

data submitter is data owner

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier according to

Guideline OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)

Deviations yes

Principles of method if other than guideline

In the main experiment the treated animals were examined for acute signs of toxicity at 1h, 2-4h, 6 h and 24 h and not at 1h, 6 h and 24 h. This deviation, however, does not affect the validity of the study.

GLP compliance

yes (incl. certificate) RCC Cytotest Cell Research GmbH

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester
- Substance type: colourless
- Physical state: liquid
- Analytical purity: 99.6 %
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Storage condition of test material: room temperature

Test animals

Species

mouse

Strain

NMRI

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Source: RCC Ltd., Biotechnology and Animal Breeding Division; CH-4414 Füllinsdorf
- Age at study initiation: 8 - 10 weeks
- Weight at study initiation: mean weight 37.5 ± 2.9 g
- Assigned to test groups randomly: yes
- Fasting period before study: none
- Housing: single
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21 ± 4

- Humidity (%): 30 -70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

intraperitoneal

Vehicle(s)

- Vehicle(s)/solvent(s) used: olive oil
- Justification for choice of solvent/vehicle: The vehicle was chosen to its relative non-toxicity for the animals

Details on exposure

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 10 ml/kg bw

Duration of treatment / exposure

24, 48 h

Frequency of treatment

single administration

Post exposure period

The animals of the highest dose group were examined for acute toxic symptoms at intervals of around 1 h, 2-4h, 6 h and 24 h after administration of the test substance.

Doses / concentrations

500, 1000 and 2000 mg/kg bw

Basis other: nominal dose

No. of animals per sex per dose

5 male animals per dose

Control animals

yes, concurrent vehicle

Positive control(s)

cyclophosphamide
- Route of administration: intraperitoneal
- Doses: 40 mg/kg solved in deionised water

Examinations

Tissues and cell types examined

bone marrow

Details of tissue and slide preparation

CRITERIA FOR DOSE SELECTION: It is generally recommended to use the maximum tolerated dose or the highest dose that can be formulated and administered reproducibly or 2000 mg/kg as the upper limit for non-toxic test substances. The maximum tolerated dose level is determined to be the dose that causes toxic reactions without having major effects on survival within 48 hours. The volume to be administered should be compatible with physiological space available .

DETAILS OF SLIDE PREPARATION: The animals were sacrificed by cervical dislocation. The femora were removed, the epiphyses were cut off and the marrow was flushed out with fetal calf serum, using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the resuspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald / Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample .

METHOD OF ANALYSIS: Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. At least 2000 polychromatic erythrocytes (PCE) were analysed per animal for micronuclei. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in normochromatic erythrocytes per 2000 PCEs . The analysis- was performed with coded slides.

Evaluation criteria

The study was considered valid as the following criteria are met :
- the negative controls are in the range of our historical control data (0-30 - 1 .50 ‰ ; mean = 0 .86 ± 0.27 ‰ PCEs with micronuclei).
- the positive controls are in the range of our historical control data (10 .0 - 27.1 ‰; mean = 16 .53 ± 4.09 ‰ PCEs with micronuclei).
- at least 80 % of animals are evaluable

Any other information on materials and methods incl. tables

Six males were assigned to each test group. The animals were identified by their cage number as shown below in the table.

Test group	hours post treatment	
	24	48
Negative control	1-6	31-36
500 mg/kg test substance	7-12	-
1000 mg/kg test substance	13-18	-
2000 mg/kg test substance	19-24	-
Positive control	25-30	37-42

Results and discussions

Test results

Sex	male
Genotoxicity	negative
Toxicity	no effects
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Additional information on results

RESULTS OF RANGE-FINDING STUDY
- Dose range: 2000mg/kg bw.
- Clinical signs of toxicity in test animals: reduction of spontaneous activity until 30 hours post treatment

RESULTS OF DEFINITIVE STUDY
- Induction of micronuclei (for Micronucleus assay): no induction
- Ratio of PCE/NCE (for Micronucleus assay): see table
- Appropriateness of dose levels and route: valid
- Statistical evaluation: non-parametric mann Whitney test (see table)

Remarks on results including tables and figures

Summary of Micronucleus Test Results:

test group	dose mg/kg b.w.	sampling time (h)	PCEs with miconuclei (‰)	range	PCE/NCE mean
vehicle	0	24	0.50	0-3	2000/2057
test substance	500	24	0.20	0-1	2000/1953
test substance	1000	24	0.90	0-4	2000/1934
test substance	2000	24	0.80	0-4	2000/1775
positive control	40	24	19.80	32-58	2000/2036
vehicle	0	48	0.30	0-2	2000/1509
test substance	2000	48	0.80	1-4	2000/1630

(range: number of micronucleated cells per 2000 PCEs in one group of 5 animals)

Biometry:
Statistical significance at the five per cent level ($p<0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

Vehicle control versus test group	Significance	p
500 mg test substance/kg b.w.; 24 h	n.t.	-
1000 mg test substance/kg b.w.; 24 h	-	0.2778
2000 mg test substance/kg b.w.; 24 h	-	0.3254
40 mg postive control/kg b.w.; 24h	+	0.0040
2000 mg test substance/kg b.w.; 48 h	-	0.1786

- = not significant

+ = significant
n.t. = not tested, as the mean micronucleus frequency was not above the vehicle control value

Overall remarks, attachments

Overall remarks

The substance is considered to be non-clastogenic and non-aneugenic in this micronucleus assay.

Applicant's summary and conclusion

Interpretation of results

negative

7.7 Carcinogenicity

Endpoint study record: Key.BASFAG82S0107/01094.Carcinogenicity

UUID	IUC5-ed1c27f5-76b3-4987-8c95-2f0375857336
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:24 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2005
Title	1,2-Cyclohexane dicarboxylic acid, diisononyl ester - Combined Chronic Toxicity/Carcinogenicity study in CrlGlxBrlHan:Wl-rats; Administration in the diet up to 24 months		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	82S0107/01094
Owner company	BASF SE		
Company study no.		**Report date**	2005-12-21

Data access

data submitter is data owner

Materials and methods

Limit test

no

Test guideline

Qualifier	according to
Guideline	

OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)

Deviations no

Qualifier according to

Guideline EPA OPPTS 870.4300 (Combined Chronic Toxicity / Carcinogenicity)

Deviations no

Qualifier according to

Guideline EU Method B.33 (Combined Chronic Toxicity / Carcinogenicity Test)

Deviations no

Qualifier according to

Guideline other guideline: Japan/MAFF: Testing Guidelines for Toxicology Studies; Combined Chronic Toxicity/Oncogenicity Study, p. 42 - 44, 1985

Deviations no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Test substance No.: 01/0107-1
- Physical state: fluid / colorless-clear
- Analytical purity: 99.6 g / 100 g
- Purity test date: study no. 01L00125 from July 25, 2001
- Stability under test conditions: stable (study no. 04L00301 from February 22, 2005) at room temperature
- Storage condition of test material: room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: Wistar, CrlGlxBrlHan:WI
- Source: Charles River, Sulzfeld, Germany
- Age at study initiation: 33 -36 days

- Weight at study initiation: mean 154.3 g (males), 120.4 g (females)
- Fasting period before study:
- Housing: singly in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area about 800 cm²)
- Diet (e.g. ad libitum): ground Kliba maintenance diet rat/mouse meal (GLP), supplied by Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: at least one week

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): every 3 months
- Mixing appropriate amounts with (Type of food): For each concentration, the test substance was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer.
- Storage temperature of food: room temperature
- Adjustment: Dietary concentrations of 1,2-Cyclohexanedicarboxylic acid, diisononyl ester for each group and sex were adjusted weekly during the first 13 weeks and at 4 -week intervals thereafter, based on body weight and food consumption measurements from the previous week.

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Analysis was done by GC-analysis. At the start of the study, the homogeneity was demonstrated using each 3 samples of the highest and lowest doses. These samples also served for concentration control analyses. Additional concentration control analysis were performed with one sample of mid dose.
During the study, analyses of the test substance preparations with respect to concentration control and/or homogeneity were conducted after about 3, 6, 9, 12, 15, 18 and 21 months as well as towards the end of the study. Homogeneity was demonstrated using 3 samples of one concentration in uprising row, and concentration control analyses were performed with 1 sample. The homogeneity analyses also served as concentration controls for the respective concentration.The analyses from 3 months onwards were usually performed with samples taken at the end of the time period for which the respective test substance preparations were used. The samples were taken out of randomly selected reserve food boxes being stored in the animal room.

Duration of treatment / exposure

Main groups: 24 months
Satellite groups: 12 months

Frequency of treatment

daily

Post exposure period

a fasting period of at least 16 - 20 hours until sacrifice

Doses / concentrations

40, 200, 1000 mg/kg

Basis nominal in diet

No. of animals per sex per dose

Main groups: 50
Satellite groups: 10

Control animals

yes

Details on study design

- Dose selection rationale: as requested by the sposor 1000 mg/kg bw/d was selected high dose with expected toxic effects

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays
- Cage side observations checked in table were included.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: prior to the administration period and thereafter at weekly intervals

BODY WEIGHT: Yes
- Time schedule for examinations: on day 0 (start of the administration period), at weekly intervals during the first 13 weeks of the study, thereafter at 4-week intervals, and p rior to start of necropsy

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: No

FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: Yes

OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations and dose groups examined: One day prior to the start of the administration period the eyes of all animals were examined. On day 363 and 362, only animals of the dose group 3 (1000 mg/kg body weight/day) and controls (0 mg/kg body

weight/day) of the satellites were examined. On day 723 and 715 the eyes of all surviving animals were examined.

HAEMATOLOGY: Yes
- Time schedule for collection of blood: days 93-96, 181-182, 357-359
- Anaesthetic used for blood collection: No
- Animals fasted: Yes
- How many animals: 10/sex/dose
- Parameters examined: Leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood count, activated partial thromboplastin time, prothrombin time (Quick's test)

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: days 93-96, 181-182, 357-359
- Animals fasted: No
- How many animals: 10/dose group
- Parameters examined: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-g-glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, magnesium

URINALYSIS: Yes
- Time schedule for collection of urine: days 92-97, 184-185, 359-360
- Metabolism cages used for collection of urine: Yes
- Animals fasted: Yes
- Parameters examined: Volume, color, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity, sediment

NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes
- Wight parameters examined: Anesthetised animals, liver, kidneys, adrenal glands, testes, epididymides, ovaries, uterus, spleen, brain, heart, thyroid glands (with parathyroid glands), thymus (only in animals of the Satellite group)
HISTOPATHOLOGY: Yes
- all gross lesions, salivary glands (mandibular gland, sublingual gland), esophagus, stomach (fore- and glandular stomach), duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, brain, pituitary gland, sciatic nerve, spinal cord (cervical, thoracic and lumbar cord), eyes with optic nerve, adrenal glands, thyroid glands, parathyroid glands, trachea, lungs, pharynx, larynx, nasal cavity, aorta, heart, bone marrow (femur), lymph nodes (mesenteric and mandibular lymph node), spleen, thymus, kidneys, urinary, bladder, testes, ovaries, uterus, oviducts, vagina, rpididymides, prostate, seminal vesicle, female mammary gland, skin, skeletal muscle, sternum with marrow, femur with knee joint, zymbal gland, harderian gland, extraorbital lacrimal glands

Statistics

- DUNNETT's test (two-sided): body weight, body weight change, food consumption, food efficiency
- KRUSKAL-WALLIS test (two-sided) followed by Wilcoxon-test (two-sided): clinical pathology parameters, except differential blood count and reticulocytes, weight parameters
- FISHER's exact test: urinalysis, except volume, color, turbidity and specific gravity

Any other information on materials and methods incl. tables

Results and discussions

Effect levels

Endpoint	NOAEL
Effect type	carcinogenicity
Effect level	40 mg/kg bw/day
Sex	male
Basis for effect level / Remarks	increased weight and histopathological findings on thyroid glands; liver weight changes
Endpoint	NOAEL
Effect type	carcinogenicity
Effect level	200 mg/kg bw/day
Sex	female
Basis for effect level / Remarks	increased weight and histopathological findings on thyroid glands; urinalysis; liver weight changes

Observations

Details on results

CLINICAL SIGNS AND MORTALITY
- Mortality: the administration of the test substance in all dose groups did not adversely affect the mortality of the animals in both sexes (control: 17% of males, 27% of females; test substance: 12-22% of males, 18-28% of females)
- Clinical signs: All findings were assessed as being incidental and not related to treatment. Abnormal clinical signs were equally distributed between control and treated animals or occurred in single animals, only.

BODY WEIGHT AND WEIGHT GAIN
No significant influence on body weight could be detected. Body weight change in male animals of group 2 and female animals of group 3 was slight but statistically significant increased on days 7, 49, 77 and 364 in males, resp. days 119 till 203 in females. These deviations were measured in dose group 2 males and dose group 3 females and therefore not incidental and clearly not substance-related

FOOD CONSUMPTION AND COMPOUND INTAKE
Food consumption in both sexes was statistically significantly increased in groups 3 and 2, more pronounced in group 3, and up to +11.7% in females of group 3 on day 595. Because of no clear relationship to other clinical findings like body weight data and food efficiency and the negligible minor deviation, these findings were estimated as toxicologically irrelevant.

FOOD EFFICIENCY
On several occasions, food efficiency differed (increased values) statistically significantly in

all treatment groups from controls in males and females on some days of the study. These findings were spontaneous in nature and therefore not substance-related.

WATER CONSUMPTION
No substance-related o vert changes in water consumption were observed

OPHTHALMOSCOPIC EXAMINATION
No substance-related effects were obtained. All findings were spontaneous in nature and equally distributed between treated animals and controls.

HAEMATOLOGY
In the peripheral blood of all treated males mean corpuscular volume (MCV) was significantly reduced and mean corpuscular hemoglobin (MCH) was slightly, but statistically significantly decreased in the low and high dose males on day 182. On day 359 significant decreases in MCV and MCH were also found in the male animals of the low and high dose groups. Moreover, at this sampling interval red blood cell counts were increased in the circultion of the mid and high dose males. No treatment-related effects were seen in the red blood cell parameters of the females and in the white blood cells of both sexes. In the females platelets were significantly higher in the animals of the high dose group on day 357. No further effects were observed in the other hematology parameters examined in either sex. After careful consideration all changes seen in the red blood cell parameters of the low and mid dose males were assessed not to be treatment-related, because there was no dose-response relationship and the effects were not of sufficient magnitude to be regarded adverse. Furthermore, no similar response was seen in the treated females at either sampling interval.

CLINICAL CHEMISTRY
Increased alkaline phosphatase activities in the high dose males on day 359 and higher g-glutamyltransferase activities in the high dose females on days 181 and 357. Moreover, significantly decreased aspartate aminotransferase activities were recorded in the serum of all treated females on day 357. The statistically significant decreases in aspartate aminotransferase were originated in the statistical calculation. The activity in the control group was abnormally high. Due to this abnormal high control value all means of the treatment groups differed statistically significantly from the corresponding control. The increase in aspartate aminotransferase activity in the control group of the females was caused by 3 animals, whose sera were hemolytic. Therefore, the serum aspartate aminotransferase activities in the treatment groups were deemed not to be test compound-related. No changes were seen in the other enzyme parameters.
Blood chemistry examinations showed significantly decreased total bilirubin concentrations in the high dose males on days 182 and 359 and in the high dose females on days 97,181 and 357. The slight, but statistically significant fall in total bilirubin in the low and mid dose groups was assessed as not being test substance-related, because these findings were of minor degree and, thus, were considered to be of no toxicological or biological relevance. No treatment-related findings were observed in the other blood chemistry parameters fo r male or female rats.
URINALYSIS
In the urine sediments of the high dose males increased amounts of degenerated transitional epithelial cells and granulated and/or epithelial cell casts were seen on day 97. At the same time interval increased number of casts were also detected in the urine sediments of the mid dose males. No treatment-related changes occurred in the other urine parameters examined

ABSOLUTE ORGAN WEIGHTS
Increased mean absolute weights of the thyroid glands in males (dose group 2: +68.9 %, dose group 3: +52.4 %) and females (dose group 3: +70.4 %). Increased mean absolute liver weights of in males (dose group 2: +6.7 %, dose group 3: +6.8 %) and females (dose group 3: +13.8 %). The increased absolute kidney weights in males of group 2 (+4.5 %) and 3 (+3.1 %) are considered to be incidental. The decreased absolute uterus weights of

females of groups 2 ((-70.1 %) and 3 (-77.5 %) are due to the lesser number of tumors compared to the control group. All other mean absolute weight parameters did not show significant differences when compared with the control group.

RELATIVE ORGAN WEIGHTS
Increased mean relative weights of the thyroid glands in males (dose group 2: +71.4 %, dose group 3: +42.9 %) and females (dose group 3: +55.6 %). Increased mean absolute liver weights of in males (dose group 2: +4.5 %) and females (dose group 3: +14.6 %).

GROSS PATHOLOGY
In the satellite groups, all gross lesions occurred either singly or were biologically equally distributed over the control group and the treatment groups.
Main groups:
- Thyroid glands: In mid and top dose males, the number of animals with enlarged thyroid glands was increased (8 and 9 compared to 1 in control group). In females of the top dose group, the number of masses (4) was slightly increased.
- Mammary gland: The number of mid and top dose females with masses in the mammary gland was slightly increased (11 and 11 compared to 5 in control group)
- Liver: In treated females of the low, mid and high dose group, the number of foci in the liver was increased (10, 20,11 compared to 5 in control group)

HISTOPATHOLOGY: NON-NEOPLASTIC
In the satellite groups, some of the treated females showed altered colloid in the thyroid gland with most animals affected in the top dose group (8) compared to 0 in control group.
In the liver of male animals of the dose groups 2 and 3 there was a slight increase in numbers of foci of cellular alteration (both 7 compared to 0 in control group).
In the thyroid gland of male and female animals of the main top dose groups there was a higher incidence of follicular cell hyperplasia (15 and 14 compared to 8 and 3, respectively)
All other findings noted were either single observations or they were biologically equally distributed between control and treatment groups. All of them were considered to be incidental or spontaneous in origin and without any relation to treatment

HISTOPATHOLOGY: NEOPLASTIC (if applicable)
In the satellite groups, there were single neoplastic findings which were biologically equally distributed over the control group and the treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.
- Mammary gland: the macroscopically diagnosed masses in the mammary gland were mainly fibroadenomas. Females of the mid and top dose group had increased numbers of fibroadenomas (5 and 9 compared to 1 in control group).
- Pancreas: the number of islet cell adenomas was slightly increased in treated males of the low (5), mid (4) and high dose group (4) compared to control (1)

The numbers of animals with neoplasms, benign and malignant neoplasms and systemic neoplasms were comparable between control and top dose animals.The total numbers of primary, benign, malignant, and systemic neoplasms were comparable between control and top dose males and females. They were biologically equally distributed over the control and treatment groups.

Remarks on results including tables and figures

Absolute and relative organ weights:

Males:

Absolute weight	Relative weight (%)

					weight		
	control	40 mg/kg bw/d	200 mg/kg bw/d	1000 mg/kg bw/d	control	40 mg/kg bw/d	2 mg bv
terminal body weight (g)	475.408 ± 61.64	485.512 ± 56.36	483.795 ± 68.125	497.554 ± 43.51	100	100	1
adrenal glands (mg)	69.1 ± 20.426	71.927 ± 26.999	79.784 ± 51.942	67.953 ± 11.33	0.015 ± 0.007	0.015 ± 0.005	0.0 0.
brain (g)	2.177 ± 0.059	2.193 ± 0.138	2.188 ± 0.084	2.157 ± 0.072	0.466 ± 0.064	0.46 ± 0.088	0.4 0.
epididymis (g)	1.203 ± 0.181	1.22 ± 0.172	1.204 ± 0.2	1.164 ± 0.16	0.258 ± 0.053	0.254 ± 0.044	0.2 0
heart (g)	1.415 ± 0.167	1.409 ± 0.214	1.433 ± 0.236	1.409 ± 0.153	0.303 ± 0.061	0.292 ± 0.04	0.2 0.
kidneys (g)	3.245 ± 0.883	3.182 ± 0.477	3.391 ± 0.491*	3.347 ± 0.416*	0.703 ± 0.309	0.66 ± 0.1	0.7 0.
liver (g)	11.931 ± 2.025	12.02 ± 1.846	12.732 ± 2.204*	12.746 ± 1.485	2.527 ± 0.467	2.475 ± 0.236	2.6 0.3
spleen (g)	1.067 ± 0.303	1.162 ± 0.8	1.17 ± 0.474	1.082 ± 0.198	0.225 ± 0.06	0.241 ± 0.176	0.2 0.
testes (g)	3.83 ± 0.798	3.83 ± 0.749	3.749 ± 0.847	3.843 ± 0.871	0.814 ± 0.173	0.769 ± 0.154	0.7 0
thyroid glands (mg)	33.769 ± 15.889	33.146 ± 13.442	57.027 ± 85.246**	51.465 ± 45.139**	0.007 ± 0.003	0.007 ± 0.003	0.0 0.0

Females:

	Absolute weight				Relati (%)
	control	40 mg/kg bw/d	200 mg/kg bw/d	1000 mg/kg bw/d	con
terminal body weight (g)	292.027 ± 56.952	295.695 ± 49.782	293.821 ± 38.291	289.953 ± 56.473	10
adrenal glands (mg)	78.088 ± 16.86	73.25 ± 10.683	84.788 ± 52.936	71.553 ± 14.465	0.028 ±
brain (g)	1.967 ± 0.099	1.993 ± 0.081	1.993 ± 0.096	1.998 ± 0.084	0.695 ±
heart (g)	1.076 ± 0.184	1.076 ± 0.169	1.022 ± 0.143	1.031 ± 0.139	0.376 ±
kidneys (g)	2.263 ± 0.624	2.175 ± 0.276	2.224 ± 0.263	2.301 ± 0.263	0.794 ±
liver (g)	7.244 ± 1.229	7.17 ± 1.076	7.729 ± 1.378	8.246 ± 1.585**	2.506 ±
ovaries (mg)	2798.853 ± 15276.35	380.139 ± 1685.198	103.303 ± 80.728	110.921 ± 137.802	0.77 4.4
spleen (g)	0.902 ± 0.853	0.69 ± 0.126	0.765 ± 0.361	0.717 ± 0.257	0.317 ±

thyroid glands (mg)	25.265 ± 13.663	26.861 ± 14.155	28.788 ± 20.053	43.053 ± 42.863**	0.009 ±
uterus (g)	2.993 ± 6.868	2.119 ± 6.345	0.894 ± 0.734*	0.674 ± 0.288**	1.054

*p<0.05; **p<= 0.01

Histopathological findings:

		Males				Females			
		Control	40 mg/kg bw/d	200 mg/kg bw/d	1000 mg/kg bw/d	Control	40 mg/kg bw/d	200 mg/kg bw/d	100 mg/ bw/
Thyroid gland	enlarged	1	0	8	9	2	1	2	2
	mass	3	1	3	2	0	1	1	4
	altered colloid					0	5	3	8
Mammary gland						5	3	11	11
Uterine mass						11	5	9	3
Liver foci	12 months	3	5	7	7				
	24 months					5	10	20	11

Tumor incidences:

		Males				Females		
		Control	40 mg/kg bw/d	200 mg/kg bw/d	1000 mg/kg bw/d	Control	40 mg/kg bw/d	20 mg/ bw
Thyroid gland adenoma		3	5	11*	14**	1	3	3
Hyperplasia, follicular cell		8	6	9	15	3	4	5
Mammary glad	adenocarcinoma					3	1	5
	fibroadenoma					1	2	5
Pancreas, adenoma islet cell		1	5	4	4	0	0	0

*p<0.05; **p<= 0.01

Summary:

The following substance-related adverse findings were obtained:

1000 mg/kg body weight/day

· Increased platelets in females

· Increased number of females with altered colloid in the thyroid glands after 12 mon

· Increased mean absolute and relative weights of the thyroid glands in males and fe

· Increased number of follicular adenomas in the thyroid glands of males (14 versus
treatment

· Increased number of males and females with follicular cell hyperplasia in the thyroi
months of treatment

200 mg/kg body weight/day

· Increased mean absolute and relative weights of the thyroid glands in males

· Increased number of follicular adenomas in the thyroid glands of males (11 versus

40 mg/kg body weight/day

· No substance-related adverse findings obtained

In conclusion, the mortality was not adversely affected after 24 months administratio
malignant neoplasia in treated animals was recorded. The thyroid glands are conside
be a secondary effect due to liver enzyme induction. An increased number of follicula
both sexes treated with 1000 mg/kg as well as in males administered 200 mg/kg boc
adenomas in rats in terms of human risk assessment is low.

The no-observed-adverse-effect level (NOAEL) under the conditions of this study wa
200 mg/kg body weight/day in females.

Overall remarks, attachments

Overall remarks

7.8 Toxicity to reproduction

7.8.1 Toxicity to reproduction

Endpoint study record: Key.BASFAG70R0107/01021.Toxicity to reproduction

UUID	IUC5-18e0230c-2c4a-4aca-8bc7-7fe698998ce0
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:27 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2003
Title	1,2-Cyclohexanedicarboxylic acid, diisononyl ester Two-Generation Reproduction Toxicity Study in Wistar Rats Continuous Dietary Administration		
Bibliographic source	Unpublished data		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	70R0107/01021
Owner company	BASF SE		
Company study no.		**Report date**	2003-06-10

Data access

data submitter is data owner

Materials and methods

Test type

two-generation study

Limit test

no

Test guideline

Qualifier	according to
Guideline	OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)
Deviations	no
Qualifier	according to
Guideline	EPA OPPTS 870.3800 (Reproduction and Fertility Effects)
Deviations	no
Qualifier	according to
Guideline	EU Method B.35 (Two-Generation Reproduction Toxicity Test)
Deviations	no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Test substance No.: 01/0107-1
- Filling date: May 8, 2000
- Physical state: liquid, colorless-clear
- Analytical purity: 99.6 g/l 00 g (analytical report 01 L00125)
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Stability under test conditions: Proven by reanalysis after the in life phase of the study (analytical report 03L00107)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: Wistar, CrlGlxBrlHan:WI
- Source: Charles River Laboratories, Germany
- Age at study initiation: (P) 36 (± 1) days
- Weight at study initiation: (P) Males: 106.3 – 133.4 g; Females: 92.4 – 119.7 g

- Housing: during study individually in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, Germany (floor area of
about 800 cm2); overnight matings: male and female mating partners housed together in type DK III cages; gestation day 18 – lactation day 21: pregnant animals and their litters housed in Makrolon type M III cages.
- Diet (e.g. ad libitum): ground Kliba maintenance diet rat/mouse/hamster, meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: about 8 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): weekly for males during premating period, mating, gestation, lactation and post weaning periods; once for males during mating periods; weekly for females during premaing period, once during all other periods
- Mixing appropriate amounts with (Type of food): During the first week of premating period, F0 parental animals received dietary concentrations based on the body weight of randomization and historical feed consumption data. During the remainder of the premating period, dietary DINCH concentrations for each group and sex were adjusted weekly based on body weight and food consumption measurements from the previous week.
- Storage temperature of food: room temperature

Details on mating procedure

- M/F ratio per cage: 1/1
- Length of cohabitation: from about 4.00 p.m. until 7.00 - 9.00 a.m. of the following morning for max. two weeks
- Proof of pregnancy: sperm in vaginal smear referred to as day 0 of pregnancy
- Further matings after two unsuccessful attempts: no

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Analyses wer done by GC. Homogeneity and concentration control analyses had been carried out at the beginning and toward the end of the premating periods, as well as during the periods after weaning. At least one analysis of the test substance preparations of the female animals had been carried out during the gestation and lactation periods.

Duration of treatment / exposure

38 weeks

Frequency of treatment

daily

Details on study schedule

- F1 parental animals not mated until at least 73 days after selected from the F1 litters.
- Selection of parents from F1 generation when pups were 21 days of age.

Doses / concentrations

100, 300, 1000 mg/kg

Basis nominal in diet

No. of animals per sex per dose

25

Control animals

yes, plain diet

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: daily for clinically evident signs of toxicity and twice daily for mortality on working days or once daily (Saturday, Sunday or on public holidays).
- Cage side observations checked in table were included.

DETAILED CLINICAL OBSERVATIONS: No data

BODY WEIGHT: Yes
- Time schedule for examinations: In general, the body weight of the male and female parental animals was determined on the first day of the premating period and then once a week at the same time of the day (in the morning); however following exceptions are notable for the female animals: during each mating period the F0 and the F1 generation parental females were weighed on the day of positive evidence of sperm (day 0 p.c.) and on days 7, 14 and 20 post coitum; females showing no positive evidence of sperm in vaginal smears were not weighed during the mating interval; females with litter were weighed on the day after parturition (day 1 p.p.) and on days 4, 7, 14 and 21 post partum; females without litter were not weighed during the lactation phase; after weaning of the last F1 A pups the female F0 generation parental animals were weighed again once weekly (parallel to the male F0 generation parental animals) until the second mating interval (for F1 B).

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

HAEMATOLOGY: Yes
- Time schedule for collection of blood: shortly before terminal sacrifice
- Anaesthetic used for blood collection: No
- Animals fasted: Yes
- How many animals: 12/sex/dose
- Parameters examined: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood count, prothrombin time (Quick’s test)

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: shortly before terminal sacrifice
- Animals fasted: No
- How many animals: 21/dose group
- Parameters examined: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-g-glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, magnesium

URINALYSIS: Yes
- Time schedule for collection of urine: shortly before terminal sacrifice
- Metabolism cages used for collection of urine: Yes
- Animals fasted: Yes
- Parameters examined: Volume, color, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity, sediment

Estrous cyclicity (Parental animals)

Estrous cycle length and normality were evaluated daily for all F0 and F1 female parental rats for a minimum of 3 weeks prior to mating and were continued throughout the mating period until the female exhibited evidence of mating. Moreover, at necropsy a vaginal smear was examined to determine the stage of the estrous cycle for each F0 and F1 female with scheduled sacrifice.

Sperm parameters (Parental animals)

Parameters examined in F0 and F1 male parental generations:
sperm motility, sperm morphology, sperm head count (cauda epididymis), sperm head count (testis)

Litter observations

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: yes
- If yes, maximum of 8 pups/litter (4/sex/litter as nearly as possible); excess pups were killed and discarded.

PARAMETERS EXAMINED
The following parameters were examined in F1 / F2 offspring:
number and sex of pups, stillbirths, live births,postnatal mortality, presence of gross anomalies, weight gain, physical or behavioural abnormalities

GROSS EXAMINATION OF DEAD PUPS:
yes, for external and internal abnormalities; possible cause of death was not determined for pups born or found dead

Postmortem examinations (Parental animals)

SACRIFICE
- Male animals: All surviving animals after weaning of F1B litter
- Maternal animals: All surviving animals after weaning of F1B litter

GROSS NECROPSY
- Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.
- Weight determination: anesthetized parental animals, liver, kidneys, adrenal glands,

testes, epididymides (total), epididymides (cauda), prostate gland, seminal vesicles with coagulating glands (and their fluids), ovaries, uterus (with oviducts and cervix uteri), spleen, brain, pituitary gland, thyroid glands (with parathyroid glands

HISTOPATHOLOGY
The tissues indicated were prepared for microscopic examination:
vagina, cervix uteri, uterus, ovaries, oviducts, left testis, left epididymis, seminal vesicles, coagulating glands, prostate gland, pituitary gland, liver, kidneys, spleen, brain, adrenal glands, thyroid glands, with parathyroid glands, all gross lesions

Postmortem examinations (Offspring)

SACRIFICE
- The F1 offspring not selected as parental animals and all F2 offspring were sacrificed at 21 days of age.
- These animals were subjected to postmortem examinations macroscopically as follows: If there were notable findings or if abnormalities were found in the daily clinical observation of the animals after their delivery, the affected animals were, if it was deemed necessary, examined additionally using appropriate methods (e.g., skeletal staining according to a modified method of KIMMEL and TRAMMELL (Kimmel, C.A. and Trammell C., 1981) and/or further processing of head according to WILSON's method (Wilson, J.G. and Warkany, J., 1965)). The stained skeletons were evaluated under a stereomicroscope or a magnifying glass. All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation.

GROSS NECROPSY
- Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

ORGAN WEIGTHS
The tissues indicated were weighed:
brain, spleen and thymus of 1 pup/sex and litter from the F1 A, F1 B and F2 pups were weighed.

Statistics

- DUNNETT-test (two-sided): food consumption (parental animals), body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), estrous cycle duration, number of mating days, duration of gestation, number of pups delivered per litter, duration of sexual maturation (days to preputial separation, days to vaginal opening)
- FISHER'S EXACT test: male and female mating index, male and female fertility index, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, number of litters with affected pups at necropsy, sexual maturation data (preputial separation, vaginal opening), males with >4% abnormal sperm, urinalysis, except volume, color, turbidity and specific gravity
- WILCOXON-test (onesided): proportions of affected pups per litter with necropsy observations, total spermatids/g testis, total sperm/g cauda epididymides, % motility, follicles: primordial, growing, and primordial + growing
- KRUSKAL-WALLIS test (two-sided): pup organ weights (absolute and relative), clinical pathology parameters, except differential blood count, weight parameter

Reproductive indices

- Female mating index (%) = number of females mated / number of females placed with males * 100
- Female fertility index (%) = number of females pregnant / numbers of females mated * 100

- Gestation index (%) = numbers of females with live pups on the days of birth / number of females pregnant * 100

Offspring viability indices

- Live birth index (%) = number of liveborn pups at birth / total number of pups born * 100
- Postimplantation loss (%) = (number of implantations – number of pups delivered) / number of implantations * 100
- Viability index (%) = number of live pups on day 4 after birth / number of live pups on day of birth * 100
- Lactation index (%) = number of live pups on day 21 after birth / number of live pups on day 4 after birth * 100

Any other information on materials and methods incl. tables

Estrous cycle data were evaluated for F0 and F1 generation females over a three week period prior to mating until evidence of mating occurred. Moreover, the estrous stage of each female was determined on the day of scheduled sacrifice.

Sexual maturation (day of balanopreputial separation/vaginal opening) of all F1A pups selected to become F1 parental generation animals was determined. Blood and urine samples were taken from 12 F0 and F1 parental animals of each sex and test group, shortly before terminal sacrifice.

Remarks:

A technical error was found to have caused false positive vaginal smears for sperm in the F0 generation, which resulted in a high incidence of supposed male infertility of treated animals. This technical error related to contamination with rat sperm of the physiological saline solution used to prepare the vaginal smears. Therefore a second litter (i.e. F1B) was generated from the F0 generation.

Results and discussions

Effect levels

Endpoint	NOAEL general toxicity
Generation	P
Sex	male/female
Effect level	1000 mg/kg bw/day
Basis for effect level / Remarks	no substance-induced adverse effects
Endpoint	NOAEL general toxicity
Generation	F1
Sex	male/female
Effect level	100 mg/kg bw/day
Basis for effect level / Remarks	organ weights; histopathology

Endpoint	NOAEL developmental toxicity
Generation	other: F1 and F2
Sex	male/female
Effect level	1000 mg/kg bw/day
Basis for effect level / Remarks	no substance-induced adverse effects regarding development

Observations: parental animals

Details on results (parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)
- Mortality: Although Two female control animals died, there were no substance-related mortalities in any of the male and female F0 parental animals in any of the groups. There were no substance-related mortalities in any of the male and female F1 parental animals in any of the groups.
- Clinical signs: No clinical signs or changes of general behavior which might be attributed to the test substance administration were detected in male or female F0 generation parental animals. The 3 doses (100; 300 and 1,000 mg/kg body weight/day) administered in the diet did not lead to further disturbances of the general behavior in any of the F0 parental animals. Marginal clinical findings, which were considered to be spontaneous in nature, occurred in various animals of different dose groups without any dose-response relationship. No clinical signs or changes of general behavior which might be attributed to the test substance were detected in male or female F1 generation parental animals.

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)
- Food consumption: The few statistically significantly increased food consumption values of the mid and/or high dose F0 males (weeks 10-11, 13-14, 17-18, 22-24) and the mid and/or high dose females on different gestation days (F1 A and F1 B) are considered to be without any biological relevance and spontaneous in nature. Thus, the food consumption of the F0 parental male and female animals in the substancetreated groups (100; 300; 1,000 mg/kg body weight/day) was unaffected during the different study phases in comparison to the controls. This includes gestation and lactation periods of the F0 females for the F1 A and FIB litters. The food consumption of the F1 parental male and female animals in the substancetreated groups (100; 300; 1,000 mg/kg body weight/day) was unaffected during the different study phases in comparison to the controls

- Body weight: The observable differences in body weight data (significantly increased mean body weight gains of the low, mid and high dose F0 males during study weeks 17-18 and the statistically significantly reduced value of the high dose males during study weeks 18-19; statistically significantly increased mean body weight gains of the high dose females during gestation of the F1A and Fl B litters on days 0-7 p.c) reflect the usual biological variation in the strain of rats used for this study. Mean body weights and mean body weight gains of the F1 males of test groups 11, 12 and 13 (100; 300 and 1,000 mg/kg body weight/day) were unaffected by the test substance administration during the entire study period and substantially similar to the corresponding control values.

TEST SUBSTANCE INTAKE (PARENTAL ANIMALS)
The measured intakes of 1,2-Cyclohexanedicarboxylic acid, diisononyl ester by the different test groups (calculated on the basis of interpolated mean body weights) generally correlated well with the desired target doses

REPRODUCTIVE FUNCTION: ESTROUS CYCLE (PARENTAL ANIMALS)
The mean duration of a cycle varied between 3.9 and 4.1 days. From these results it can be concluded, that the administration of 1,2-Cyclohexanedicarboxylic acid, diisononyl ester

had no adverse effect on estrous cycle data of the F0 and F1 parental females.

REPRODUCTIVE FUNCTION: SPERM MEASURES (PARENTAL ANIMALS)
Sperm paremeters were not affected by the test substance at any dose level and was substantially similar to the control group.

REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS)
The male fertility index varied between 96% and 100% without showing any relation to dosing. Moreover, male mating and fertility indices values calculated for the F0 males after the second mating period (to generate Fl B pups) were fully within the range of the corresponding historical control values. The administration of 1,2-Cyclohexanedicarboxylic acid, diisononyl ester up to 1,000 mg/kg body weight/day did not adversely affect reproduction and delivery data of the F0 and F1generation parental females.

HEMATOLOGY
There were no treatment-related changes in the hematological parameters measured in F0 and F1 patental animals

CLINICAL CHEMISTRY
Slightly increased y-glutamyltransferase activities and decreased total bilirubin concentrations were found in the serum of the mid and high dose F0 females. Clinical chemistry examinations revealed significant, dose-dependent decreases in total bilirubin concentrations in the high dose F 1animals of either sex and in the mid dose F1 females. Moreover, slightly increased y-glutamyltransferase activities were measured in the serum of the mid and high dose F1 females. The other clinicochemical parameters were not affected.

URINALYSIS
There were no treatment-related changes in urinalyses of both sexes of F0 and F1 animals

ABSOLUTE ORGAN WEIGHTS (PARENTAL ANIMALS)
Significant increased weight of liver in mid and high dose F0 males (+7.0 % and +7.9 %) and females (5.7% and 15.4 %). Significant increased weight of kidneys in mid and high dose F0 males (+7.1 % and +9.0 %) and females (7.1% and 7.4 %).
Significant increased weight of liver high dose F1 females (12.3 %). Significant increased weight of kidneys in mid and high dose F1 males (+8.7 % and +8.2 %) and females (6.8% and 8.8 %). Significant increase of weight of the throid gland in high dose F1 females (+16.4 %).
The other mean absolute weight parameters of the parental animals of the F0 and F1 generation were incidental and not regarded as substance-related or did not show significant differences when compared with control group.

RELATIVE ORGAN WEIGHTS (PARENTAL ANIMALS)
Significant increased weight of liver in mid and high dose F0 males (+3.8 % and +8.8 %) and females (5.5% and 13.0 %). Significant increased weight of kidneys in mid and high dose F0 males (+2.9 % and +8.6 %) and females (6.9% and 5.1 %).
Significant increased weight of liver in mid and high dose F1 males (+5.0 % and +7.0 %) and females (7.7 % and 12.2 %). Significant increased weight of kidneys in low, mid and high dose F0 males (+4.6 %, +7.8 % and +12.8 %) and females (+5.2, 8.4 % and 8.6 %).
Significant increase of weight of the thyroid gland in high dose F1 females (+11.1 %).
The other mean absolute weight parameters of the parental animals of the F0 and F1 generation were incidetal and not regarded as substance-related or did not show significant differences when compared with control group.
GROSS PATHOLOGY (PARENTAL ANIMALS)
All observed gross lesions occurred only once per group, with no indication of a relationship to treatment.

HISTOPATHOLOGY (PARENTAL ANIMALS)
In F0 animals, all correlated microscopic findings and the few which were not met in the slides or remained without a correlate had anyway developed spontaneously and unrelated to treatment.
Minimal to slight vacuolization of the tubular epithelia was noted in all F1 animals of the high dose group. In the mid dose group, minimal or slight tubular vacuolization was noted in 9 (of 25) males, whereas in the control and in the low dose group each one animal showed (spontaneous) minimal tubular vacuolization.
In the thyroid glands, minimal to slight hypertrophy/hyperplasia of the follicular epithelia was recorded in 21 of 25 F1 female rats of the high dose group. In the mid dose group, minimal or slight hypertrophy/hyperplasia was recorded in 10 of 25 F1 females, whereas slight hypertrophy/hyperplasia was noted as a spontaneous finding in three control and in three low dose F1 female rats.
In the high dose group F1 animals, hypertrophy/hyperplasia of the follicular epithelium of the thyroid glands was very often (16 of 21 animals) associated with minimal or slight (multi)focal accumulation of a flaky colloid within the lumen of the follicles. In the mid dose group, minimal or slight amounts of flaky colloid were noted in 12 F1 females, which was in association with hypertrophy/hyperplasia of the follicular epithelia in 10 F1 animals.

Observations: offspring

Details on results (offspring)

VIABILITY (OFFSPRING)
The mean number of delivered F1A and F1 B pups/dam and the rate of liveborn and stillborn pups were not affected by the administration of the test substance. No substance-related differences exist between the control and the 100, 300 and 1000 mg/kg F1 A/ F1 B pups concerning viability and mortality.

CLINICAL SIGNS (OFFSPRING)
The scattered occurrence of clinical findings (sparse hair, hydrocephaly and bilateral anophthalmia) in single pups of different test groups including the controls of F1A, F1 B and F2 litters without a consistent pattern does not suggest any substancerelated origin.

BODY WEIGHT (OFFSPRING)
Mean body weights and body weight gains of the F1 A and F1B pups in all dose groups were not affected by the test substance administration.
The observable differences in F2 pup body weight data reflect the usual biological variation in the strain of rats used for this study.

SEXUAL MATURATION (OFFSPRING)
- Vaginal opening: The mean number of days to reach the criterion amounted to 31.5 / 31.9 / 31.8 / 31.7 days in test groups0, 100, 300 and 1000 mg/kg body weight/day, respectively. The observable differences between the control and the substance-treated groups are in the range of biological variation and do not show any relation to dosing.
- Preputial separation: The mean number of days to reach the criterion amounted to 43.3 / 42.6 / 42.9 / 43.1 in test groups 0, 100 300 and 1000
mg/kg body weight/day, respectively. The observable differences between the control and the substance-treated groups are in the range of biological variation and do not show any relation to dosing

ORGAN WEIGHTS (OFFSPRING)
The statistically significantly increased absolute and relative spleen weights of the mid (+21.7 % and 18.3 %) and high dose (13.8% and 11.5 %) male F1A pups are probably a consequence of the slightly higher pup body weights / body weight gains in these groups. The other mean relative pup organ weight parameters of the F1A and F1 B pup generations did not show statistically significant differences when compared with control group.
There were no treatment-related pup organ weight changes in the F2 pups. All differences observed reflect the normal biological variation in this strain of rats.

GROSS PATHOLOGY (OFFSPRING)
A few of the large number of F1 A, F1 B and F2 pups showed spontaneous findings at gross necropsy or when examined additionally using other appropriate methods. Findings which occurred were e.g. post mortem autolysis, partly cannibalized pups, anasarca, incisors sloped, cleft palate, hemorrhagic thymus, small thymus, empty stomach, dilated renal pelvis and/or ureter, hydroureter, distended bladder, hemorrhagic testis, brachydactyly, anencephaly, anophthalmia, hydrocephaly, diaphragmatic hernia, acaudate and kinked tail. These findings occurred without a clear relation to dosing and/or most of it can be found in the historical control data at comparable or even higher incidences.

OTHER FINDINGS (OFFSPRING)
The sex distribution and sex ratios of live F1 A and F1 B pups on the day of birth and on day 21 p.p. did not show any substantial differences between controls and treated groups; all differences observed are regarded to be spontaneous in nature.

Remarks on results including tables and figures

Mean test substance intake (mg/kg body weight/day):

		100 mg/kg bw/d	300 mg/kg bw/d	1000 mg/kg bw/d
F0 males		101.0	302.5	1007.5
F0 females (premating)		103.7	310.9	1073.9
F0 females (F1 A litters)	gestation period	101.4	308.3	1058.1
	lactation period	101.0	295.0	967.8
F0 females (F1 B litters)	gestation period	109.4	333.8	1100.7
	lactation period	98.8	283.0	999.3
F1 males		94.2	284.0	948.1
F1 females (premating)		97.1	292.4	973.2
F1 females (F1 A litters)	gestation period	101.4	301.2	1037.1
	lactation period	95.3	271.3	942.0

Male and female fertility indices for FO females (in %):

			control	100 mg/kg bw/d	30
F0	concerning F1 A litters	male	100	72	
		female	100	72	
	concerning F1 B litters	male	96	100	
		female	96	100	
F1	concerning F2 litters	male	96	96	
		female	96	96	

Changes of absolute and relative organ weights (%):

absolute organ weight changes

			100 mg/kg bw/d	300 mg/kg bw/d	1000 mg bw/d
F0	males	liver		+7.0*	+7.9**
		kidneys		+7.1*	+9.0*
		testes		+11.9**	
	females	liver		+5.7*	+15.4*
		kidneys		+7.1**	+7.4**
		adrenal gland	+7.8*		+14.4*
		pituitary gland	+16.8**		
F1	males	kidneys		+8.7**	+8.2*
		liver			
		testes		+5.1*	
		prostata gland		+13.1**	
	females	liver			+12.3*
		kidneys		+6.8*	+8.8**
		thyroid glands			+16.4*

*p <= 0.05; **p <= 0.01

Overall remarks, attachments

Overall remarks

Under the conditions of this study 1,2-Cyclohexanedicarboxylic acid, diisononyl ester had no adverse effects on fertility and reproductive performance of the F0 or F1 parental animals of both genders at 100; 300 and 1000 mg/kg body weight/day. Estrous cycle data, mating behavior, conception, gestation, parturition, lactation and weaning as well as sperm parameters, sexual organ weights and gross and histopathological findings of these organs (including differential ovarian follicle counts in the F1 females) were similar between the rats of the substance-treated groups and the corresponding controls. Nearly all of the 0, 100, 300 and 1000 mg/kg F0 and F1 parental animals proved to be fertile. The scattered occurrence of a few infertile F0 (1 high dose male/1 mid dose female) and F1 (one couple of the control, low and high dose group each) parental rats throughout the different dose groups including the controls does not suggest any relation to treatment. The clinical examinations of the F0 and F1 parental rats for general signs of toxicity failed to reveal substance-induced effects up to and including the dose of 1000 mg/kg body weight/day. Clinical pathology examinations of the parental rats revealed indications for hepatic enzyme induction (high dose F1 males; mid and high dose F0 and F1 females). Substance-induced weight increases were observed in kidneys, thyroid glands and liver of the F0 and/or F1 parental rats, which were partly correlated with associated microscopic findings (kidneys and thyroid glands) or findings from clinical pathology (liver enzyme induction). The slight vacuolization of the tubular epithelia of the kidneys in the mid and high dose group F1 generation males (300 and 1000 mg/kg body weight/day) and the minimal to slight hypertrophy/hyperplasia of the follicular epithelia of the thyroid glands (with or without the presence of flaky colloid) in the mid and high dose group F1 generation females (300 and 1000 mg/kg body weight/day) with the associated weight changes are regarded as signs of

substance-induced general toxicity. All other organ weight changes and the indications for hepatic enzyme induction, however, are considered to be of no toxicological relevance and not as adverse, but adaptive effects. There occurred no substance-induced signs of developmental toxicity in the progeny of the F0 and/or F1 parents, i.e. in the F1 A, F1 B and F2 pups. Pup mortality and survival rate, sex ratio, body weight data, clinical and necropsy findings, organ weights and sexual maturation data were unaffected by treatment.

Under the conditions of this two-generation reproduction study, the NOAEL for fertility and reproductive performance is 1000 mg/kg bw/day for F0 and F1 generation rats of both genders.
The NOAEL for general toxicity is 1000 mg/kg bw/day (F0 rats of both genders) and 100 mg/kg bw/day for the F1 male and female rats (based on tubular vacuolisation and flaky thyroid follicular colloid)
The NOAEL for developmental toxicity (growth and development of offspring) was 1000 mg/kg bw/day for the F1 and F2 pups

7.8.2 Developmental toxicity / teratogenicity

Endpoint study record: Key.BASFAG30R0223/99124.Developmental toxicity / teratogenicity

UUID	IUC5-8d09ae7d-27b8-4658-91ad-076b9ace6fb0
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:32 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2002
Title	1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (Gavage)		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	30R0223/99124
Owner company	BASF SE		
Company study no.		**Report date**	2002-01-25

Data access

data submitter is data owner

Materials and methods

Limit test

yes

Test guideline

Qualifier	according to

Guideline OECD Guideline 414 (Prenatal Developmental Toxicity Study)

Deviations no

Qualifier according to

Guideline EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)

Deviations no

Qualifier according to

Guideline other guideline: EC Commission Directive 87/302/EEC of Nov. 18, 1987; Part B: Methods for the determination of toxicity: Teratogenicity study (rodent and non-rodent); Official Journal of the European Communities; No. L 133, pp. 24 - 26 (1988)

Deviations no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid, di (isononyl) ester
- Test substance No.: 99/223-1
- Date of production: May 26, 1999
- Physical state: Liquid/colorless - clear
- Analytical purity: 99.7 g/100 g (analytical report, No. 99L00402)
- Lot/batch No.: R 5116/1 (#23725)
- Stability under test conditions: Proven by reanalysis after the in life phase of the study (analytical report, No. 01 L00342)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: Wistar, CrlGIxBrlHan:Wl
- Source: Charles River Laboratories, Germany
- Age at study initiation: 10 - 12 weeks
- Weight at study initiation: ca. 165 g
- Housing: singly from day 0 - 20 p.c. in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, FRG (height: 15 cm, length: 37,5 cm, width: 21 cm; floor area about 800 cm2).

- Diet (e.g. ad libitum): ground Kliba maintenance diet rat/mouse/hamster meal, supplied by PROVIMI KLIBA SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water of tap water quality; ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: gavage

Vehicle

olive oil

Details on exposure

PREPARATION OF DOSING SOLUTIONS:
The test substance solutions in olive oil Ph.Eur./DAB were prepared at the beginning of the administration period and thereafter at intervals of 3 - 4 days. For the preparation of the solutions, an appropriate amount of the test substance was weighed depending on the dose group, in a graduated measuring flask and subsequently dissolved in olive oil by intensive mixing.

VEHICLE
- Justification for use and choice of vehicle (if other than water): due to solubility in vehicle
- Concentration in vehicle: 4, 12, 24 g/100 ml
- Amount of vehicle (if gavage): 5 ml/kg bw
- Purity: olive oil Ph.Eur./DAB

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The test substance solutions were analyzed by GC. Analytical verifications of the stability of the test substance in olive oil Ph.Eur./DAB for a period of at least 4 days at room temperature were carried out before the study was initiated. Samples of the test substance solutions were sent to the analytical laboratory twice during the study period (at the beginning and towards the end) for verification of the concentrations.

Details on mating procedure

- Impregnation procedure: purchased timed pregnant
- Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0

Duration of treatment / exposure

day 6 through day 19 post coitum

Frequency of treatment

daily

Duration of test

20 days

Doses / concentrations

200, 600, 1200 mg/kg bw

Basis nominal conc.

No. of animals per sex per dose

25

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale: The following doses were chosen for the present full-scale toxicity study in Wistar rats, with 1,200 mg/kg body weight/day as a dose distinctly above the limit dose, which could induce some signs of maternal and/or developmental toxicity on the one hand or as another possible no observed adverse effect level on the other hand

Examinations

Maternal examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: A check was made twice a day on working days or once a day (Saturday, Sunday or on public holidays) (days 0 - 20 p.c.).
- Cage side observations checked in table were included.

DETAILED CLINICAL OBSERVATIONS: No

BODY WEIGHT: Yes
- Time schedule for examinations: on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Yes
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

POST-MORTEM EXAMINATIONS: Yes
- Sacrifice on gestation day 20p.c.
- Organs examined: uterus

Ovaries and uterine content

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes

Fetal examinations

- External examinations: Yes: all per litter
- Soft tissue examinations: Yes: all per litter
- Skeletal examinations: Yes: all per litter
- Head examinations: Yes: all per litter

Statistics

- DUNNETT-test (two-sided): Food consumption, body weight, body weight change, corrected body weight gain (net maternal body weight change), carcass weight, weight of

unopened
uterus, number of corpora lutea, number of implantations, number of resorptions, number of live fetuses, proportions of preimplantation loss, proportions of postimplantation loss, proportions of resorptions, proportion of live fetuses in each litter, litter mean fetal body weight, litter mean placental weight
- FISHER's EXACT test (onesided): Female mortality, females pregnant at terminal sacrifice, number of litters with fetal findings
- WILCOXON-test (one-sided): Proportions of fetuses with malformations, variations and/or unclassified observations in each litter

Indices

- conception rate (%) = number of pregnant animals / number of fertilized animals x 100
- preimplantation loss (%) = (number of corpora lutea - number of implantations) / number of corpora lute x 100
- postimplantation loss (%) = (number of implantations - number of live fetuses) / number of implantations x 100

Any other information on materials and methods incl. tables

Results and discussions

Effect levels

Endpoint	NOAEL
Effect type	maternal toxicity
Effect level	1200 mg/kg bw/day
Basis for effect level / Remarks	no adverse test substance-induced effects
Endpoint	NOAEL
Effect type	teratogenicity
Effect level	1200 mg/kg bw/day
Basis for effect level / Remarks	no adverse test substance-induced effects
Endpoint	NOAEL
Effect type	developmental toxicity
Effect level	1200 mg/kg bw/day
Basis for effect level / Remarks	no adverse test substance-induced effects

Maternal toxic effects

no effects

Details on maternal toxic effects

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)

No substance-related clinical signs nor any disturbances of the general behavior occurred in any animal during the whole administration period.
There were no substance-related or spontaneous mortalities in any of the groups.

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)
There were no statistically significant or biologically relevant differences between the controls and the substance-treated dams concerning mean body weights and body weight gains. No effect on food consumption was noted considering normal biological variation

UTERUS WEIGHT (PARENTAL ANIMALS)
The mean gravid uterus weights of the dams of test groups 1 - 3 (200; 600 or 1,200 mg/kg body weight/day) were not affected by the administration of the test substance. All differences were without any biological relevance and/or not dose-related.

CONCEPTION RATE (PARENTAL ANIMALS)
There were no substance-related and/or biologically relevant differences between the different test groups in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the postimplantation losses, the number of resorptions and viable fetuses. All differences observed are considered to reflect the normal range of fluctuations for animals of this strain and age.
GROSS PATHOLOGY (PARENTAL ANIMALS)
There were no substance-related observations at necropsy in any of the animals.

Embryotoxic / teratogenic effects

no effects

Details on embryotoxic / teratogenic effects

SEX DISTRIBUTION (FETUSES)
The sex distribution of the fetuses in test groups 1 - 3 (200; 600 and 1,200 mg/kg body weight/day) was comparable with that of the control fetuses. The differences observed in comparison to the control were without any biological relevance.

WEIGHT OF PLCENTAE
No effect on mean placental weights were noted considering normal biological variation

BODY WEIGHT (FETUSES)
The mean body weights of the fetuses of test group 1, 2 and 3 (200; 600 and 1,200 mg/kg body weight/day) were not statistically significantly different from the corresponding control values. The few minor differences were without any biological relevance and/or not dose-related

EXTERNAL EXAMINATION (FETUSES)
External malformations were observed at low incidences in fetuses of test groups 1 and 2 (200 and 600 mg/kg body weight/day), but not in fetuses of test group 0 and 3 (0 and 1,200 mg/kg body weight/day). No external variations and so-called unclassified observations were recorded for any
of the fetuses.

SOFT TISSUE EXAMINATIN (FETUSES)
One isolated soft tissue malformation which occurred was observed in high dose male and was considered to be incidental in nature.
Two soft tissue variations (uni- or bilateral dilation of the renal pelvis and/or ureter) were detected in each group including the control. The mean percentages of affected fetuses/litter with total soft tissue variations amounted to 4.0% (control), 6.5% (200 mg/kg body weight/day), 7.3% (600 mg/kg body weight/day) and 5.5% (1,200 mg/kg body weight/day).

No so-called unclassified soft tissue observation (like blood imbibition of kidney(s)) was recorded in any of the fetuses.

SKELETAL EXAMINATION (FETUSES)
Malformations of the fetal skeletons, skeletal variations and unclassified cartilage observations were observed at low incidences in single fetuses of test groups 0, 1 and 2 (0; 200 and 600 mg/kg body weight/day). All of the noted skeletal malformations appeared without any relation to dosing, without biologically relevant differences between the groups and/or can be found at a comparable frequency in the historical control data

Remarks on results including tables and figures

Summary of all classified fetal external, soft tissue and skeletal observations:

		control	200 mg/kg bw/d	600 mg/kg bw/d	1200 mg/kg bw/d
Total malformations	Litters evaluated (n)	24	20	22	22
	Fetuses Evaluated (n)	219	178	192	201
	Fetal incidences (%)	1.4	1.1	0.6	0.5
	litter incidences (%)	13	10	4.5	4.5
	affected fetuses /litter (%)	1.3 ± 3.38	1.1 ± 3.37	0.4 ± 1.94	0.6 ± 2.67
Total variations	Fetal incidences (%)	48	46	48	41
	litter incidences (%)	100	95	95	86
	affected fetuses /litter (%)	48.9 ± 14.54	46.4 ± 17.03	48.3 ± 17.49	38.7 ± 20.29

Overall remarks, attachments

Overall remarks

Based on these results, the no observed adverse effect level (NOAEL) for maternal and prenatal developmental toxicity is 1,200 mg/kg body weight/day, i.e. a dose, which is distinctly above the limit dose of 1,000 mg/kg body weight/day.

Endpoint study record: Key.BASF.42R0107/01135.Developmental toxicity / teratogenicity.rabbit

UUID	IUC5-8ce0d932-383b-4de5-92a1-e31d645a2317
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:34 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2004
Title	1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester - Prenatal Developmental Toxicity Study in Himalayan Rabbits Administration in the diet		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	42R0107/01135
Owner company	BASF SE		
Company study no.	42R0107/01135	**Report date**	2004-08-18

Data access

data submitter is data owner

Materials and methods

Limit test

no

Test guideline

Qualifier	according to
Guideline	OECD Guideline 414 (Prenatal Developmental Toxicity Study)

Deviations no

Qualifier according to

Guideline other guideline: EC Commission Directive 87/302/EEC of Nov. 18, 1987; Part B: Methods for the determination of toxicity: Teratogenicity study (rodent and non-rodent); Official Journal of the European Communities; No. L 133, pp. 24 - 26 (1988)*

Deviations no

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester
- Physical state: Liquid/colorless-clear
- Analytical purity: 99.6g/100g (analytical report 01L00125)
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Stability under test conditions: The stability under storage conditions was confirmed by reanalysis (analytical report 03L00107)
- Storage condition of test material: Room temperature
- Other: Homogeneity: Homogeneous

Test animals

Species

rabbit

Strain

Himalayan

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Germany
- Age at study initiation: 105 - 133 days old
- Weight at study initiation: 1973 – 2644 g
- Fasting period before study: none
- Housing: single
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

PREPARATION OF DOSING SOLUTIONS: For each concentration, the calculated amounts of the test substance were weighed out once before the start of the study

DIET PREPARATION
- Rate of preparation of diet (frequency): once
- Mixing appropriate amounts with (Type of food): Provimi Kliba SA mixed the respective amounts of test substance thoroughly with appropriate amounts of food (Kliba maintenance diet type 3418 for rabbits) in a “Diosna 2 – Mischer” for 15 minutes, each. The mixtures of test substance and food were pelleted in a pelleting machine using distilled water, thereafter put in a pellet dryer to reach 11.5% analytical moisture.
- Storage temperature of food: at or below 15°C

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

At the beginning of the study, the mean values of 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester in feed were found to be in the range of 89.3 - 95.3% of the nominal concentration. At the end of the study, the concentrations ranged from 86.0 to 97.5% of the nominal concentration. Although individual values are marginally less than 90% of nominal concentration, these analytical results are considered to show the correctness of the concentration of 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester in feed for rabbit / guinea pig. Due to the lack of proper references samples for recovery experiments, a recovery of 100% was assumed. However, a recovery of 100% is observed rarely. If a more realistic recovery rate of 95% was used, which is very high for such samples, all analytical values would have been more than 90% of the nominal value.

Details on mating procedure

- Impregnation procedure: artificial insemination
- Verification of same strain and source of both sexes: yes
- Proof of pregnancy: day of insemination was designated as day 0 (beginning of the study) and the following day as day 1 post insemination (p.i.)

Duration of treatment / exposure

treatment from day 6 through day 29 post insemination

Frequency of treatment

daily

Duration of test

29 days

Doses / concentrations

100, 300, 1000 mg/kg bw/d

Basis nominal in diet

102.2, 310.7, 1028.5 mg/kg bw/d

Basis analytical conc.

No. of animals per sex per dose

25 females per dose

Control animals

yes, plain diet

Further details on study design

- Dose selection rationale: The following doses were chosen for the present full-scale prenatal developmental toxicity study in Himalayan rabbits:
100 mg/kg bw/d: as the expected no adverse effect level
300 mg/kg bw/d: as intermediate dose level
1000 mg/kg bw/d: as the dose level which should induce some developmental and/or maternal toxicity but not death or severe suffering

Examinations

Maternal examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: Mortality was checked in the females twice a day on working days or once a day on Saturdays, Sundays or on public holidays (days 0 - 29 p.i.).

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: The animals were examined for clinical symptoms at least once a day, or more often when clinical signs of toxicity were elicited (days 0 - 29 p.i.)

BODY WEIGHT: Yes
- Time schedule for examinations: All animals were weighed on days 0, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 and 29 p.i.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Yes
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

POST-MORTEM EXAMINATIONS: Yes
- Sacrifice on day 29 post insemination
- Organs examined: Weight of the unopened uterus; Number of corpora lutea; Number and distribution of implantation sites; examination of the fetus

Ovaries and uterine content

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes
- Other: conception rate, preimplantation loss, postimplantation loss

Fetal examinations

- External examinations: Yes: all per litter
- Soft tissue examinations: Yes: all per litter
- Skeletal examinations: Yes: all per litter
- Head examinations: Yes: half per litter

Statistics

- Dunnett-Test (two-sided): food consumption, body weight, body weight change, corrected body weight gain (net maternal body weight change), carcass weight, weight of unopened uterus, number of corpora lutea, number of implantations, number of resorptions, number of live fetuses, proportion of preimplantation loss, proportions of postimplantation loss, proportions of resorptions, proportion of live fetuses in each litter, litter mean fetal body weight, litter mean placental weight
- Fischer's exact test (one-side): female mortality, females pregnant at terminal sacrifice, number of litters with fetal findings
- Wilcoxon-test (one-sided): proportions of fetuses with malformations, variations and/or unclassified observations in each litter

Indices

- conception rate (%) = (number of pregnant animals / number of fertilized animals) x 100
- preimplantation loss (%) = [(number of corpora lutea - number of implantations) / number of corpora lute)] x 100
- postimplantation loss (%) = [(number of implantations - number of live fetuses) / number of implantations] x 100

Any other information on materials and methods incl. tables

Results and discussions

Effect levels

Endpoint	NOAEL
Effect type	maternal toxicity
Effect level	1000 mg/kg bw/day
Basis for effect level / Remarks	no adverse test substance-induced effects
Endpoint	NOAEL
Effect type	teratogenicity
Effect level	1000 mg/kg bw/day
Basis for effect level / Remarks	no adverse test substance-induced effects
Endpoint	NOAEL
Effect type	developmental toxicity
Effect level	1000 mg/kg bw/day
Basis for effect level /	no adverse test substance-induced effects

Remarks

Maternal toxic effects

no effects

Details on maternal toxic effects

MORTALITY:
There were no substance-related mortalities in any of the groups.

CLINICAL SYMPTOMS:
Before treatment started (on day 4 p.i.) high dose rabbit No. 87 showed some blood in bedding.
Reduced defecation occurred in control rabbit No. 19 on day 22 p.i.. Additionally, one low dose female (No. 32) showed blood in bedding and was found dead on day 26 p.i.. This doe did not show any findings at necropsy, which could explain its sudden death. There were no abnormal clinical findings in the other does of the study.

FOOD CONSUPMTION:
The food consumption of the females of test groups 1, 2, and 3 (100; 300 and 1,000 mg/kg body weight/day) was comparable to that of the control group. It did not show any dose-related changes if normal biological variation is taken into account. This statement includes the statistically significantly increased food consumption of the rabbits of test groups 2 and 3 on days 25 – 27 and 28 – 29 or 23 – 29 p.i., respectively.

BODY WEIGHT:
Mean body weights of the females in test groups 1, 2, and 3 (100; 300 and 1,000 mg/kg body weight/day) did not show any statistically significant differences in comparison to the concurrent control group during the entire study period (days 0 - 29 p.i.).

UTERUS WEIGHT:
The mean gravid uterus weights of the animals of test groups 1, 2, and 3 (100; 300 or 1,000 mg/kg body weight/day) were not influenced by the administration of the test substance. The observed differences were neither statistically significant nor biologically relevant.

NECROSCOPY FINDINGS:
There were no substance-related observations at necropsy in any of the dams.

REPRODUCTION DATA:
The conception rate reached 96% in test groups 0 and 3 (control and 1,000 mg/kg body weight/day), 80% in test group 1 (100 mg/kg body weight/day) and 84% in test group 2 (300 mg/kg body weight/day). 19 – 24 rabbits/group had implantation sites at necropsy; thus, a sufficient number of females for the purpose of the study were available. There were no substance-related and/or biologically relevant differences between the different test groups in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the postimplantation losses, the number of resorptions and viable fetuses. All differences observed are considered to reflect the normal range of fluctuations for animals of this strain and age.

Embryotoxic / teratogenic effects

no effects

Details on embryotoxic / teratogenic effects

SEX DISTRIBUTIONS OF FETUSES:
The sex distribution of the fetuses in test groups 1 - 3 (100; 300 and 1,000 mg/kg body weight/day) was comparable with the sex ratio of the control fetuses. The differences observed in comparison to the control are without any biological relevance.

WEIGHT OF PLACENTAE:
The mean placental weights in the test groups 1 - 3 (100; 300 and 1,000 mg/kg body weight/day) were not influenced by the test substance administration. The observable differences between the groups did not show any relation to dosing and reflect the usual fluctuation for this parameter.

WEIGHT OF FETUSES:
The mean fetal weights of all test groups were not influenced by the test substance administration and were close to or even exceeded the corresponding control values. It is very likely, that the observed differences in fetal body weights were caused by the spontaneous fluctuations in the mean number of live fetuses/doe between the groups.

EXTERNAL EXAMINATION OF THE FETUSES:
The scattered occurrence of external, soft tissue and skeletal malformations throughout all test groups including the controls without a consistent pattern, without a clear dose-response relationship and/or at incidences, which were generally similar to historical control rates does not suggest any substance-induced origin of these findings. If all different types of malformations are summarized, the mean percentages of affected fetuses/litter with total malformations amounted to 7.0, 2.9, 4.1 and 2.9% at 0; 100; 300 or 1,000 mg/kg body weight/day, respectively. These overall incidences do not show any dose-response relationship and do not suggest any substance induced background.

Remarks on results including tables and figures

Summary of all classified fetal external, soft tissue, and skeletal observations:

		Test Group 0 control	Test Group 1 100 mg/kg bw/d	Test Gr 300 mg/k
Litters Evalueated	N	24	19	21
Fetuses Evaluated	N	155	142	125
Live	N	155	142	125
Dead	N	0	0	0
Total Malformations				
Fetal Incidence	N	5	5	4
	%	3.2	3.5	3.2
Litter Incidence	N	5 Fi	3	4
	%	21	16	19
Affected Fetuses/Litter	Mean %	7.0 Wi	2.9	4.1
	S.D.	20.84	8.09	9.3
Total Variations				
Fetal Incidence	N	129	122	106
	%	83	86	85
Litter Incidence	N	24Fi	19	20
	%	100	100	95

Affected Fetuses/Litter	Mean %	83.0Wi	85.6	82.
	S.D.	20.54	14.78	27.3

Statistics: Fi = fisher's exact test (one-sided), Wi = Wilcoxon-test (one-sided)

Summary:

There were no substance-related effects on the does concerning food consumption, change, uterine weights, corrected body weight change, clinical and necropsy obser including the limit dose of 1000 mg/kg body weight/day.

There were no differences of toxicological relevance between the control and the sul (100; 300 and 1000 mg/kg body weight/day) on the gestational parameters, i.e. in co number of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ra calculated for the pre- and the postimplantation losses.

No substance-related differences were recorded for placental and fetal body weights and skeletal examinations of the fetuses revealed no toxicological relevant difference and the substance-treated groups. Thus, under the conditions of this full-scale study administration of 1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester to pregnant fe during organo- and fetogenesis elicited no signs of maternal toxicity, had no influenc parameters and induced no signs of developmental toxicity up to and including a dos weight/day; especially, there were no indications of teratogenic effects which could b test substance administration.

Overall remarks, attachments

Overall remarks

Based on these results, the no observed adverse effect level (NOAEL) for maternal toxicity and prenatal developmental toxicity is 1000 mg/kg body weight/day. Testing at higher dose levels is not considered necessary as a dose of 1000 mg/kg body weight/day is in full accordance with the requirements for the LIMIT TEST (e.g. OECD Guidelines for Testing of Chemicals No. 414).

7.8.3 Toxicity to reproduction: other studies

Endpoint study record: Key.BASFAG60R0223/99095.Toxicity to reproduction: other studies

UUID	IUC5-a92de019-efc6-420e-bd9d-9ba11df3c833
Dossier UUID	0
Author	gerstma
Date	2009-10-23 22:56:36 CEST
Remarks	Added EU: REACH data protection flag

Administrative Data

EU: REACH

Purpose flag	key study (X) robust study summary (X) used for classification (X) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study (OECD)

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2002
Title	1,2-Cyclohexanedicarboxylic acid, diisononyl ester Pre-/Postnatal Developmental Toxicity Study in Wistar Rats Oral Administration (gavage)		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	60R0223/99095
Owner company	BASF SE		
Company study no.		**Report date**	2002-10-18

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Principles of method if other than guideline

The study was roughly based on the following test guidelines:

- EC Commission Directive 87/302/EEC of November 18, 1987 ; Part B : Methods for the determination of toxicity: Teratogenicity Study (rodent and non-rodent); Official Journal of the European Communities, No. L 133, pp. 24 - 26 (1988)
- EC Commission Directive 87/302/EEC of November 18, 1987 ; Part B : Methods for the determination of toxicity: One-Generation Reproduction Toxicity Test; Official Journal of the European Communities, No. L 133, pp. 43 - 46 (1988)
- OECD Guidelines for Testing of Chemicals; Proposal for updating Guideline 414: Prenatal Developmental Toxicity Study (January 22, 2001)
- OECD Guidelines for Testing of Chemicals; Guideline 415: One-Generation Reproduction Toxicity Study (May 1983)
- U.S. EPA, Health Effects Test Guidelines; OPPTS 870.3700: Prenatal Developmental Toxicity Study (August 1998)
- U.S. EPA, Health Effects Test Guidelines, OPPTS 870.3800: Reproduction and Fertility Effects (Aug. 1998)

Furthermore, this pre-/postnatal developmental toxicity study was based on the publication of Mylchreest et al. (1998).

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Test substance No.: 99/223-1
- Date of production: May 26, 1999
- Physical state: Liquid/colorless-clear
- Analytical purity: 99.7% (analytical report No. 99L00402)
- Lot/batch No.: R 5116/1 (#23725)
- Stability under test conditions: The stability under storage conditions was confirmed by reanalysis
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: Wistar, CrlGlxBrlHan:WI
- Source: Charles River Laboratories, Germany
- Age at study initiation: (P) about 10 weeks

- Weight at study initiation: (P) Females: mean 171.5 g
- Housing: individually in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, Germany (floor area of about 800 cm2), with the following exceptions: from day 18 of gestation until day 14 after birth, the pregnant animals and their litters were housed in Makrolon type M III cages (BECKER & CO)
- Diet (e.g. ad libitum): ground Kliba maintenance diet rat/mouse/hamster meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: gavage

Vehicle

olive oil

Details on exposure

PREPARATION OF DOSING SOLUTIONS:
For the preparation of the solutions the test substance was weighed in a graduated measuring flask depending on the dose group, topped up with olive oil and subsequently thoroughly mixed. In respect to the proven stability the test substance preparations were prepared at the beginning of the administration period and thereafter at 4 day intervals.

VEHICLE
- Justification for use and choice of vehicle (if other than water): due to solubility in vehicle
- Concentration in vehicle: 15, 20%
- Amount of vehicle (if gavage): 5 ml/kg bw
- Purity: Olive oil Ph.Eur./DAB

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The test substance solutions were analyzed by GC. Analytical verifications of the stability of the test substance in olive oil for a period of at least 4 days at room temperature were carried out before the study was initiated. Samples of the test substance solutions were sent to the analytical laboratory twice during the study period (at the beginning and towards the end) for verification of the concentrations

Duration of treatment / exposure

day 3 post coitum (p.c.) through day 20 post partum (p.p.)

Frequency of treatment

daily

Duration of test

The females were allowed to litter and rear their pups until day 21 after parturition. At this time all male pups and up to 3 female pups per litter were selected and raised until days 100 to 105 post partum and particularly examined for their sexual maturation. The study

was terminated with the terminal sacrifice of the selected offspring.

Doses / concentrations

750, 1000 mg/kg

Basis nominal conc.

No. of animals per sex per dose

10

Control animals

yes, concurrent vehicle

Details on study design

Details of mating procedure:
- M/F ratio per cage: 1/2
- Length of cohabitation: about 4.00 p.m. to about 7.30 a.m. on the following day
- Proof of pregnancy: sperm in vaginal smear referred to as day 0 of pregnancy

Study design:
- F0 generation females and their progeny:
The females were allowed to litter and rear their pups (Fl generation pups) for a predetermined time. After weaning of the surviving F1 pups on day 21 p.p., the dams were sacrificed. Those females, which failed to deliver, were sacrificed a few days after the expected date of parturition.
Anogenital distance (AG) measurements were performed on all live F1 pups on day 1 after birth. Furthermore, all surviving male pups were checked for the presence of signs of areolas/nipples from day 12 until day 15 p.p..
Furthermore, the non-selected female Fl pups were sacrificed on day 21 p.p. and discarded.

- Selected offspring (reared F1 weanlings):
After weaning, all live male pups and up to 3 female pups of each litter (the first 3 surviving females as a rule) were raised into adulthood until about day 100-105 after birth for the determination of their sexual maturation (testes descending, day of vaginal opening/balanopreputial separation) and further examinations of their reproductive organs. During this rearing phase the selected F1 offspring were not treated with the test substance.

Statistics

- DUNNETT-test (two-sided): Food consumption (F0 females), body weight and body weight change (F0 females and pups; for the pup weights, the litter means were used), duration of gestation, number of pups delivered per litter, anogenital distance, anogenital index, duration of sexual maturation (days to testes descending, preputial separation, days to vaginal opening)
- FISHER'S EXACT test: Mating index, fertility index, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, sexual maturation data (testes descending, preputial separation, vaginal opening)
- WILCOXON-test (one-sided): Proportions of pups reaching special criteria in each litter concerning sexual maturation data
- WILCOXON-test (one-sided) with Bonferoni- Holm-Adjustment: % sperm motility
- KRUSKALWALLIS test (two-sided) followed by WILCOXON test: Weight parameters

Any other information on materials and methods incl. tables

PARENTAL ANIMALS: OBSERVATIONS AND EXAMINATIONS

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: At least once daily a check was made for dead or moribund animals. All F0 females were checked daily for clinically evident signs of toxicity.
- Cage side observations checked in table were included.

BODY WEIGHT: Yes
- Time schedule for examinations: F0 females was determined on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c., if possible, at the same time of the day (in the morning). Females with litter were weighed on the day of parturition (day 0 p.p.) and on days 4, 7, 14 and 21 post partum.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

LITTER: OBSERVATIONS AND EXAMINATIONS
PARAMETERS EXAMINED
The following parameters were examined in F1 offspring:
number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies

CLINICAL OBSERVATIONS
All live pups were examined each day for clinical symptoms (including gross-morphological findings).

BODY WEIGHT
The pups were weighed on the day after birth (day 1 p.p.) and on days 4, 7, 14 and 21 after birth

SEXUAL MATURATION DATA
- All selected male offspring was evaluated daily for testes descending with examinations initiating on day 25 p.p..
- Selected female offspring (generally 3 females/litter) was evaluated daily for vaginal opening with examinations initiating on day 27 p.p..
- All selected male offspring were evaluated daily for balanopreputial separation with examinations initiating on day 40 p.p..
- After successful balanopreputial separation, the penis of all male offspring in test groups 0 and 2 was further inspected for any abnormalities using a magnifying glass.

POSTMOTREM EXAMINATION
ORGAN WEIGHTS
- female parental animals: anesthetized animals, liver, kidneys, uterus with oviducts and cervix uteri, ovaries

- male and female offspring: anesthetized animals, liver, kidneys, adrenal, glands, testes, epididymides (whole and cauda), seminal vesicles (with coagulating glands and their fluids), prostate gland, uterus with oviducts and cervix uteri, ovaries

HISTOPATHOLOGY
- The organs of all male offspring and of up to three female offspring/litter that were weighed were examined. Histological examination were performed on the testes of the first two male offspring per litter and on all gross lesions of the inner and outer sex organs of male offspring.
- Immediately after necropsy and organ weight determination the right cauda epididymis were taken from the selected male offspring of all dose groups. Sperm motility was determined

Results and discussions

Effect levels

Endpoint	NOAEL productive performance/systemic toxicity
Effect level	1000 mg/kg bw/day
Sex	female
Basis for effect level / Remarks	no substance-related effects
Endpoint	NOAEL pre-/postnatal developmental toxicity
Effect level	1000 mg/kg bw/day
Sex	male/female
Basis for effect level / Remarks	no substance-related effects on development

Observed effects

F0 females:

MORTALITY AND CLINICAL SIGNS
There were no substance-related mortalities in any of the female F0 animals in any of the groups. No clinical findings for which a substance-induced origin could be assumed were recorded in the dams during the gestation and lactation period.

FOOD CONSUMPTION
The F0 parental females did not show any substance-related changes of food consumption during gestation or lactation

BODY WEIGHTS
Mean body weights and body weight gains of all F0 parental females were not influenced by the test substance administration during gestation or lactation periods.

FEMALE REPRODUCTION AND DELIVERY DATA
The administration of the test compound did not adversely affect reproduction and delivery data of the F0 generation parental females at doses up to 1000 mg/kg body weight/day.

ORGAN WEIGHTS, GROSS AND HITOPATHOLOGICAL CHANGES
No treatment-related weight changes, gross lesions or microscopic findings were noted.

Litter/Pups:

PUP NUMBER AND VIABILITY
The mean number of delivered F1 pups/dam as well as the rate of liveborn and stillborn pups were not affected by the administration of the test substance. There was no substance-related influence up to a dose level of 1,000 mg/kg body weight/day on viability and mortality of the F1 pups.

PUP BODY WEIGHT
The body weights/body weight gains of F1 pups of the 750 mg/kg and the 1000 mg/kg groups did not show any relevant differences in comparison to the concurrent control group indicative for a substance-induced effect

PUP ANOGENTITAL DISTANCE/INDEX
In the male Fl pups of the 1000 mg/kg group the anogenital distance (AGD) and the calculated anogenital index (AGI, anogenital distance related to body weight) were marginally, but statistically significantly reduced (AGD about 7% and AGI about 8% lower than the respective control value). Moreover, the AGI of the female Fl pups of the same dose group showed also a slight reduction of about 8% (with statistical significance). Since all other corresponding parameters like like testes descent etc. were not affected, they are not considered to have any biological relevance and are assessed as spurious findings.

PUP CLINICAL SIGNS
There was no treatment-related increase in the presence of areolas/nipple anlagen in the 750 or 1000 mg/kg group F1 male pups

Selected Offspring (reared Fl weanlings)

MORTALITY AND CLINICAL SIGNS
There were no substance-induced mortalities in any of the selected male and female Fl animals in any of the dose groups. Neither substance-related clinical signs nor any disturbance of the general behavior were detected in the selected male and female F1 animals in any group during the whole study period

BODY WEIGHTS
There were no statistically significant or biologically relevant differences between the control rats and the animals of test groups 1 and 2 concerning body weights or body weight gains.

SEX MATURATION DATA
The mean age for testes descent was 26.9, 27.0 and 26.8 days for test groups 0, 1 and 2, indicating that this parameter was not affected.
The mean age for vaginal opening was 32.4, 32.9 and 32.8 days for test groups 0, 1 and 2, indicating that this parameter remained unaffected.
The mean age for balanopreputial separation was 43.8, 43.4 and 43.7 days for test groups 0, 1 and 2, indicating that this parameter was not affected.
There were no indications of a substance-induced effect on sperm motility in the selected F1 male offspring.

ORGAN WEIGHTS, GROSS AND HITOPATHOLOGICAL CHANGES
No treatment-related weight changes, gross lesions or microscopic findings were noted.

Remarks on results including tables and figures

The findings of this pre-/postnatal developmental toxicity study of the notified chemical shows no indications that the test substance induced any adverse effects in the parental female rats. There were no indications of any developmental toxicity in the F1 pups in terms of data obtained during gestation and lactation. No substance-related clinical and pathological observations were made for the F1 progeny. The administration of the test substance to the parental female rats showed no influence on sexual organ morphology and sexual maturation of the selected F1 rats of both genders, or on sperm motility of the males.

Based on these data, under the conditions of this study the NOAEL (no observed adverse effect level) for reproductive performance and systemic toxicity of the FO females is 1,000 mg/kg body weight/day.

The NOAEL for developmental toxicity (growth and development of the offspring including sexual organ morphology and sexual maturation) could be also fixed at 1,000 mg/kg body weight/day for the Fl progeny.

Overall remarks, attachments

Overall remarks

7.9 Specific investigations
7.9.3 Specific investigations: other studies
Endpoint study record: BASFAG48C0107/01181.Specific investigations: liver enzyme induction

UUID	IUC5-3d4b7866-16e0-4d94-a157-3c2bbbeeb3fc
Dossier UUID	0
Author	henscho / BASF SE / Ludwigshafen am Rhein / Germany
Date	2009-09-24 11:39:47 CEST
Remarks	

Administrative Data

Purpose flag	(X) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Comparable to guideline study

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2005
Title	1,2-Cyclohexane dicarboxylic acid, diisononyl ester Liver enzyme induction study in Wistar rats Administration in the diet over 2 weeks		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	48C0107/01181
Owner company	BASF SE		
Company study no.		**Report date**	2005-10-04

Data access

data submitter is data owner

Materials and methods

Type of effects studied

other: Liver enzyme induction

Type of method

in vivo

Endpoint addressed

not applicable

Test guideline

Qualifier no guideline available

Guideline

Deviations

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Synonym: DINCH
- Test substance No.: 01/0107-1
- Production-/filling date: May 08, 2000
- Physical state: Liquid/clear-colourless
- Analytical purity: 99.7 area-% (report no. 04L00301)
- Stability under test conditions: The stability of the test substance in the diet was demonstrated over a period of 50 days at room temperature in a previous study
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain, Wistar, CrlGlxBrlHan:WI
- Source: Charles River, Sulzfeld, Germany
- Age at study initiation: 5 weeks
- Weight at study initiation: mean 165.6 g (males), 133.4 g (females)
- Housing: singly in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area about 800 cm2)
- Diet (e.g. ad libitum): basic maintenance diet for mice/rats “GLP”, meal from Provimi KLIBA SA, Kaiseraugst / Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: 8-9 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): once before the start of the study
- Mixing appropriate amounts with (Type of food): The test substance was weighed out and thoroughly mixed with a small amount of food. Then corresponding amounts of food were added to this premix in order to obtain the desired concentration, and mixing was carried out for about 3 minutes in a Hobart-mixing bowl and 10 minutes in a Ruberg EM 100 laboratory mixer
- Storage temperature of food: room temperature

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

No analyses of the test substance preparation were carried out during the study.

Duration of treatment / exposure

14 days

Frequency of treatment

daily

Post exposure period

no post exposure period

Doses / concentrations

15000 ppm

Basis nominal in diet

1418 mg/kg bw/d

Basis analytical conc. for males

1568 mg/kg bw/d

Basis analytical conc. for females

No. of animals per sex per dose

5

Control animals

yes, plain diet

Examinations

Examinations

MORTALITY AND CLINICAL SIGNS
The animals were examined for evident signs of toxicity or mortality twice a day (in the morning and i n the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays. Additionally, further general clinical examinations were carried out daily.

FOOD CONSUMPTION AND SUBSTANCE INTAKE
Food consumption was determined weekly during the administration period and calculated as mean food consumption in grams per animal and day.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

BODY WEIGHTS
Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, the body weights were determined at the start of the administration period (study day 0) and thereafter at weekly intervals.

BIOANALYTICS
For the detection of possible enzyme induction, the following parameters were examined: Cytochrome P450 (Cyt.P450), ethoxyresorufin-O-deethylase (EROD), pentoxyresorufin-O-depentylase (PROD), benzoxyresorufin-O-debenzylase (BROD), 4-Methylumbelliferone-glucuronyltransferase (MUF-GT), 4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT)

Results and discussions

Details on results

MORTALITY AND CLINICAL SIGNS
No animal died during the study. No abnormal clinical signs were detected.

FOOD CONSUMPTION AND SUBSTANCE INTAKE
No treatment-related findings were observed.

BODY WEIGHTS
No treatment-related finding were observed

BIOANALYTICS
- Cyt. P450: Total Cyt.P450 in liver was statistically significantly increased by a factor of 2.2 and 2.2 for males and females, respectively, in 1,2-Cyclohexanedicarboxylic acid, diisononyl ester treated animals when compared to controls.
- EROD: EROD activities were statistically significantly increased by a factor of 2.7 and 1.6 for males and females, respectively, in 1,2-Cyclohexanedicarboxylic acid, diisononyl ester treated animals when compared to controls.
- PROD: BROD activities were statistically significantly increased by a factor of 11 and 24 for males and females, respectively, in 1,2- cyclohexanedicarboxylic acid, diisononyl ester treated animals when compared to controls.
- MUF-GT: MUF-GT activities were statistically significantly increased by a factor of 3.3 and 2.4 for males and females, respectively, in 1,2-Cyclohexanedicarboxylic acid, diisononyl ester treated animals when compared to controls.
- HOBI-GT: HOBI-GT activities were statistically significantly increased by a factor of 7.2 and 2.7 for males and females, respectively, in 1,2-Cyclohexanedicarboxylic acid, diisononyl ester treated animals when compared to controls.

All bioanalytical findings were considered to be treatment-related.

Remarks on results including tables and figures

Bioanalytical results:

			Males		Females
			Control	15000 ppm	Control
Cyt. P450		nm/mg prot.	0.84 ± 0.15	0.183 ± 0.29**	0.51 ± 0.13
Phase I - enzymes	EROD	pmol/min/mg prot	47.62 ± 2.10	129.27 ± 23.96**	16.95 ± 0.61
	PROD	pmol/min/mg prot	6.41 ± 1.06	194.83 ± 61.52**	3.40 ± 1.02
	BROD	pmol/min/mg prot	63.15 ± 3.89	707.50 ± 172.89**	17.67 ± 1.20
Phase II - enzymes	MUF-GT	FU/min/mg prot	322.50 ± 65.59	1077.45 ± 107.39**	364.95 ± 161.79
	HOBI-GT	FU/min/mg prot	29.58 ± 20.88	211.93 ± 77.48**	60.78 ± 18.81

** p<= 0.01

Overall remarks, attachments

Overall remarks

These findings indicate that 1,2-Cyclohexanedicarboxylic acid, diisononyl ester is an enzyme inductor of phase I- and phase II-liver-enzymes in male and female Wistar rats.

Endpoint study record: BASFAG99S0107/01082.Specific investigations: S-Phase Response Study

UUID	IUC5-bec9598b-1dbd-408b-a77b-dc8c2ac76f60
Dossier UUID	0
Author	henscho / BASF SE / Ludwigshafen am Rhein / Germany
Date	2009-09-24 11:46:53 CEST
Remarks	

Administrative Data

Purpose flag	(X) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Comparable to guideline study

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2005
Title	1,2 Cyclohexanedicarboxylic acid, diisononyl ester S-Phase Response Study in the liver of Wistar rats Administration in the diet for 1, 4 and 13 weeks		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	99S0107/01082
Owner company	BASF SE		
Company study no.		**Report date**	2005-12-02

Data access

data submitter is data owner

Materials and methods

Type of effects studied

other: S-Phase Response Study in the Liver

Type of method

in vivo

Endpoint addressed

not applicable

Test guideline

Qualifier	no guideline available

Guideline

Deviations

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2 Cyclohexanedicarboxylic acid, diisononyl ester
- Test substance No.: 01/0107-1
- Bottling date: May 08, 2000)
- Physical state: liquid/ colorless-clear
- Analytical purity: 99.6 g/100g (Study No. 01L00125)
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Stability under test conditions: The stability of the test substance in the diet was demonstrated over a period of 50 days at room temperature (08B0107/016011)
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Strain, Wistar, CrlGlxBrlHan:WI
- Source: Charles River, Sulzfeld, Germany
- Age at study initiation: approximately 10 weeks
- Weight at study initiation: mean 282.9 (males), 186.2 g (females)
- Housing: singly in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area about 800 cm2).
- Diet (e.g. ad libitum): ground Kliba maintenance diet mouse/rat “GLP”, meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: at least 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): weekly
- Mixing appropriate amounts with (Type of food): The test substance was weighed out and thoroughly mixed with a small amount of food. Then corresponding amounts of food, depending on the dose group, were added to this premix in order to obtain the desired concentration, and mixing was carried out for about 3 minutes in a Hobart-mixing bowl and 10 minutes in a Lödige M20 R laboratory mixer.
- Storage temperature of food: room temperature

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Analytical investigations were done by GC. The stability of the test substance in the diet over 50 days at room temperature was proven before the start of the study (analytical report: 08B0107/016011).

Duration of treatment / exposure

1, 4, 13 weeks

Frequency of treatment

daily

Post exposure period

no post exposure period

Doses / concentrations

40, 200 and 1000 mg/kg bw/day

Basis nominal in diet

No. of animals per sex per dose

10

Control animals

yes, plain diet

Further details on study design

Osmotic minipumps with BrdU were implanted one week prior to necropsy (dosing period of the pumps: 7 days)

Examinations

Examinations

MORTALITY AND CLINICAL SIGNS
The animals were examined for evident signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the

morning) on Saturdays, Sundays and public holidays. Additionally, further general clinical examinations were carried out weekly

FOOD CONSUMPTION AND SUBSTANCE INTAKE
Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. Food efficiency (group means) was calculated based upon individual values for body weight and food consumption

WATER CONSUMPTION
Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

BODY WEIGHTS
Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on day 0 (start of administration period) and thereafter at weekly intervals.
The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

NECROPSY
- Following weights were determined: anesthetised animals, liver, kidneys, thyroid glands (with parathyroid glands)
- Histopathology: all gross lesions, brain, pituitary gland, thyroid glands/ parathyroid glands, thymus, trachea, lungs, heart, liver, spleen, kidneys, adrenal glands, testes, epididymides, prostate, seminal vesicle, stomach (fore- and glandular stomach), duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes (mesenteric and mandibular lymph node), sciatic nerve, bone marrow (femur), eyes,spinal cord (cervical, thoracic and lumbar cord), skin
- Immunohistology: BrdU – stain (liver and kidneys), BrdU – calcitonin double stain (thyroid glands), TUNEL stain (liver and kidneys)

Any other information on materials and methods incl. tables

Statistics:

- DUNNET’s-test (two sided): Food consumption, body weight, body weight change, food efficiency
- KRUSKALWALLIS-test (two-sided) followed by WILCOXON test: Weight Parameters
- WILCOXON-test: Data of S-Phase Response and Apoptosis

Results and discussions

Details on results

MORTALITY AND CLINICAL SIGNS
Except single occurrences which were regarded as incidental, no substance-related effects were obtained in all groups. No substance-related mortalities were obtained in all groups.

FOOD CONSUMPTION AND SUBSTANCE INTAKE
All significant differences regarding food consumption observed were assessed as incidental and not related to the test substance, due to the isolated occurrences and alternating effects in the sense of increased as well as decreased food consumption. No substance-related effects on food efficiency were obtained.

WATER CONSUMPTION
Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

BODY WEIGHTS
No substance-related effects were obtained.

ABSOLUTE ORGAN WEIGHTS
The only significant weight changes were found as a weight increase in the liver of top dose females in the 1-week treatment group, in the liver of top dose males in the 4 –week treatment group and in the liver of females of all dose groups in the 4-week treatment group.
No significant weight changes were found for the kidneys and the thyroid glands as well as in the liver, kidneys and thyroid glands of the 13-week treatment groups.

RELATIVE ORGAN WEIGHTS
The only significant weight changes were found as a weight increase in the liver of top dose females in the 1-week treatment group, in the liver of the medium and top dose males in the 4-week treatment group and in the liver of females of all dose groups in the 4-week treatment group.
No significant weight changes were found for the kidneys and the thyroid glands as well as in the liver, kidneys and thyroid glands of the 13-week treatment groups.

GROSS LESIONS
The few single lesions recorded are regarded as spontaneous in nature and not related to treatment.

HISTOPATHOLOGICAL FINDINGS
All findings recorded for the liver and the kidneys were seen to be incidental or spontaneous in nature and not related to treatment.
In the thyroid glands, the finding of a “follicular cell hypertrophy” was noted in control as well as treated animals (1-week treatment: 2/2/2/7 (males)] and 0/1/3/5 (females); 4-week treatment: 2/2/2/8 (males) and 0/0/2/3 (females); 13-week treatment: 1/3/10/9 (males) and 0/0/0/10 (females)). There was an increase of males and females with a follicular cell hypertrophy in the top dose and - less pronounced - in the medium dose groups in either the males or females or both. After 13 weeks of treatment, an increase of altered colloid was as well recognized in some of the animals which showed a follicular cell hypertrophy (0/1/4/6 in males, 0/0/0/2 in females).

IMMUNOHISTOLOGY: S-PHASE RESPONSE
- Liver: There was a significant increase of cell proliferation noted after 1- and 4-week treatment periods in the male top dose groups. The induction of cell proliferation was slightly more pronounced after 1 week of treatment. In the male medium dose group, a significant induction of cell proliferation was only found in the one 1 hepatocytes after 1 week of treatment. Although not significant, a tendency for an increase was also seen in the male low dose group after 1 week of treatment and as a significant increase of cell proliferation also after the 4-week treatment period. After 1 week of treatment, a dose-dependent increase of cell proliferation was found in the female low, medium, and top dose groups. An increase of cell proliferation was also observed after the 4 -week treatment period, again in all dose groups, but not longer in a dose-dependent manner; the overall highest (relative) values were noted in the low dose group.
No induction of cell proliferation was found after the 13-week treatment period in males and females.
- Kidney: After 1 week of treatment, significant increased labeling indices were found in both evaluated compartments (cortex and outer stripe of medulla) in the male top dose group and in the cortex of the male medium dose group. No significant change was present in the male low dose group. An induction of cell proliferation was indicated by the increase labeling indices in the cortex after the 4-week treatment period, again in the male medium and top dose groups, but to a slightly lower extent. The significant increase of cell proliferation was following a dose-dependent pattern. After the 13-week treatment period, a

significant increase of the labeling indices was only found in the low dose group and only in the OSOM not in the cortex compartment. No indications for an increase of cell proliferation were noted for the medium and top dose groups. There were no significant changes regarding the labeling indices after a 1-, 4-, or 13-week treatment period in females.
- Thyroid glands: As well as after the 1-week and the 4-week treatment periods a significant and dosedependent increase of the labeling indices were noted, but after 4 weeks of treatment slightly less pronounced in both sexes.
No significant changes were recorded for the labeling indices of the treated animals after the 13-week treatment period in males and females.

IMMUNOHISTOLOGY: APOPTOSIS
- Liver: There was an overall very low number of positive labeled cells in the TUNEL–stain. In the treated male animals, there are no significant deviations recorded regarding the number of apoptotic cells. However, after the 1 - and the 4-week treatment periods the overall numbers of apoptotic cells seem to be slightly more in the treated animals when compared with the controls. This observation was not made after the 13-week treatment period. As for the males, there was an overall very low number of positive labeled cells in the TUNEL–stain in the females too. Significant deviations (increase) were observed for the low dose group (zone 2) after 1 week of treatment and for the medium dose group after 13 weeks of treatment.
- Kidneys: An overall very low number of positively labeled apoptotic cells was counted in males. Except for the cortex in the medium dose group after 4 weeks of treatment (slight significant increase), no significant deviations could be recognized. In females, a few positive labeled cells were counted in control and treated groups in both kidney compartments. No significant differences were observed for the treated groups except for a weak significancy in the low dose what was regarded to be of no biological relevance.

Remarks on results including tables and figures

Mean labeling index - BrdU-staining:

Organ	Treatment lenght	BrdU - Labeling index				
			control	40 mg/kg bw	200 mg/kg bw	1000 mg/kg bw
Liver- all zones	1 week	males	1.00 ± 0.36	1.41 ± 0.84	0.38 ± 0.51	2.09 ± 1.19
		females	1.57 ± 0.94	3.30 ± 1.49**	4.46 ± 1.90**	9.55 ± 2.98**
	4 week	males	1.00 ± 0.36	1.56 ± 0.31**	1.17 ± 0.62	1.60 ± 0.51*
		females	1.57 ± 0.94	3.59 ± 1.81**	2.55 ± 1.27**	2.98 ± 2.16**
	13 week	males	0.87 ± 0.19	0.78 ± 0.27	0.79 ± 0.16	0.63 ± 0.31
		females	1.73 ± 0.60	1.92 ± 0.99	1.15 ± 0.31	1.34 ± 0.54

Kidney - Cortex	1 week	males	1.25 ± 0.53	1.38 ± 0.44	1.64 ± 0.39*	1.95 ± 0.40**
		females	0.69 ± 0.25	0.65 ± 0.17	0.44 ± 0.13	0.54 ± 0.29
	4 week	males	1.25 ± 0.53	1.34 ± 0.53	1.54 ± 0.26*	1.60 ± 0.51
		females	0.69 ± 0.25	0.53 ± 0.16	0.62 ± 0.33	0.61 ± 0.25
	13 week	males	0.58 ± 0.27	0.59 ± 0.30	0.47 ± 0.13	0.67 ± 0.15
		females	0.58 ± 0.24	0.42 ± 0.24	0.39 ± 0.13	0.49 ± 0.13
Kidney - outer stripe of the outer medulla	1 week	males	1.55 ± 0.25	1.39 ± 0.37	1.61 ± 0.45	1.87 ± 0.40*
		females	1.70 ± 0.54	1.53 ± 0.41	1.64 ± 0.51	1.91 ± 0.42
	4 week	males	1.55 ± 0.24	1.55 ± 0.57	1.73 ± 0.67	1.48 ± 0.38
		females	1.70 ± 0.54	2.07 ± 0.69	1.94 ± 0.46	2.23 ± 0.70
	13 week	males	0.64 ± 0.25	1.04 ± 0.27*	0.86 ± 0.39	0.72 ± 0.26
		females	1.51 ± 0.47	1.13 ± 0.20	1.04 ± 0.33	1.14 ± 0.43
Thyroid gland - Follocle	1 week	males	1.32 ± 0.42	3.97 ± 1.71**	4.54 ± 1.05**	5.33 ± 2.46**
		females	1.33 ± 0.69	2.45 ± 1.25*	3.19 ± 1.36**	6.83 ± 2.28**
	4 week	males	1.32 ± 0.42	2.58 ± 0.54**	2.52 ± 0.78**	3.23 ± 1.17**
		females	1.33 ± 0.69	1.86 ± 0.64*	2.59 ± 0.59**	4.68 ± 1.97**
		males	1.35 ± 0.79	1.32 ± 0.36	1.53 ± 0.52	1.07 ± 0.52

	13 week	females	1.84 ± 0.44	1.16 ± 0.66	1.44 ± 1.12	1.99 ± 0.72

*p <= 0.05; **p <= 0.01

Mean total counts - TUNEL staining:

Organ	Treatment lenght	TUNEL counts				
			control	40 mg/kg bw	200 mg/kg bw	1000 mg/kg bw
Liver	1 week	males	4	8	8	12
		females	8	16	6	6
	4 week	males	4	14	15	21
		females	8	2	4	13
	13 week	males	11	2	4	5
		females	1	2	21**	9
Kidney - Cortex	1 week	males	4	5	7	13
		females	2	7	1	2
	4 week	males	4	3	16*	8
		females	2	1	6	7
	13 week	males	15	9	15	19
		females	9	16	3	10
	1 week	males	0	0	0	4

Kidney - outer stripe of the outer medulla		females	1	0	0	2
	4 week	males	0	3	2	1
		females	1	4	5	0
	13 week	males	3	0	0	3
		females	0	4*	5	3

*p <= 0.05; **p <= 0.01

Overall remarks, attachments

Overall remarks

In summary, a treatment-related gain in cell proliferation was found in the liver and the thyroid glands in all dose groups and each sex after treatment periods of 1 and 4 weeks. In kidneys, a slight but significant induction of cell proliferation was noted in top and medium dose males, only. After 13 weeks of treatment, no indications for an increased cell proliferation (BrdU-stain) was present in the three organs examined. Light microscopy detected as treatment-related finding a “follicular cell hypertrophy” in the thyroid glands after all three treatment periods

Endpoint study record: BASFAG48C0107/01180.Specific investigations: perchlorate discharge assay

UUID	IUC5-d1ad7845-0871-4ee4-8713-796a30f06713
Dossier UUID	0
Author	henscho / BASF SE / Ludwigshafen am Rhein / Germany
Date	2009-09-24 11:51:22 CEST
Remarks	

Administrative Data

Purpose flag	(X) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Comparable to guideline study

Data source

Reference

Reference type	study report		
Author	BASF AG	**Year**	2005
Title	1,2-Cyclohexanedicarboxylic acid, diisononyl ester Thyroid function study in male Wistar rats using perchlorate discharge as a diagnostic test Administration in the diet over 4 weeks		
Bibliographic source	Unpublished report		
Testing laboratory	Experimental Toxicology and Ecology, BASF AG	**Report no.**	48C0107/01180
Owner company	BASF SE		
Company study no.		**Report date**	2005-11-30

Data access

data submitter is data owner

Materials and methods

Type of effects studied

endocrine system modulation Thyroid function study using perchlorate discharge as a diagnostic test

Type of method

in vivo

Endpoint addressed

basic toxicokinetics

Test guideline

Qualifier no guideline available

Guideline

Deviations

Principles of method if other than guideline

Modified version of the perchlorate discharge assay acc. to Atterwill et al., 1987:
The perchlorate discharge assay, which involves the administration of 125iodide, was developed to detect changes in the human thyroid iodine accumulation and organification. The anion perchlorate (ClO4-) competes with iodine (I-) transport in the thyroid due to the similarity of the molecular weight and size. In subjects with normal uptake of iodide, perchlorate blocks further uptake of iodide by the thyroid but does not discharge organified iodine. Conversely, if iodide organification is incomplete, perchlorate blocks further iodide transport, and diffusion of iodide out of the thyroid cells proceeds. This is seen as discharge of radioactivity from the gland (Greenspan, 1991).

GLP compliance

yes (incl. certificate) Experimental Toxicology and Ecology, BASF AG

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 166412-78-8

Details on test material

- Name of test material (as cited in study report): 1,2-Cyclohexanedicarboxylic acid, diisononyl ester
- Synonym: DINCH
- Test substance No.: 01/0107-1
- Production-/filling date: May 08, 2000
- Physical state: Liquid/clear-colourless
- Analytical purity: 99.7 %
- Lot/batch No.: 46-0959 (Partie 33A/0)
- Stability under test conditions: The stability of the test substance in the diet was demonstrated over a period of 50 days at room temperature in a previous study (Analytical report, Project No. 08B0107/016011).
- Storage condition of test material: Room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Strain: Wistar, Crl:WI(Han)

- Source: Charles River, Sulzfeld, Germany
- Age at study initiation: 33 (±1) days
- Weight at study initiation: mean 171.9 g (males)
- Housing: singly in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area about 800 cm2).
- Diet (e.g. ad libitum): basic maintenance diet for mouse/rat “GLP”, meal from Provimi KLIBA SA, Kaiseraugst / Switzerland; ad libitum
- Water (e.g. ad libitum): drinking water; ad libitum
- Acclimation period: 8 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 24
- Humidity (%): 30 - 70
- Photoperiod (hrs dark / hrs light): 12 / 12

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

DIET PREPARATION
- Rate of preparation of diet (frequency): once before the start of the study
- Mixing appropriate amounts with (Type of food): The test substances were thoroughly mixed with a small amount of diet. Then were added to the food in order to obtain the desired concentrations, and mixing for about 10 minutes in a laboratory mixer.

Analytical verification of doses or concentrations

no

Duration of treatment / exposure

28 days

Frequency of treatment

daily

Post exposure period

no post exposure period

Doses / concentrations

15000 ppm

Basis nominal in diet

1301 mg/kg bw/d

Basis analytical conc.

No. of animals per sex per dose

6

Control animals

yes, plain diet

Further details on study design

1,2-Cyclohexanedicarboxylic acid, diisononyl ester (DINCH), Propylthiouracil (PTU) and Phenobarbital (PB) were each administered to 2 groups of 6 male Wistar rats over a period of 4 weeks. Groups received in the diet DINCH (15,000 ppm, groups 2 and 3), PTU (2,000 ppm, groups 4 and 5) and PB (1,000 ppm, groups 6 and 7). Control animals (groups 0 and 1) received ground diet during the test period.
On study day 29 the rats received intrapedtoneously 0.5 ml (1 µCurie) of radio labeled NaI (125iodide). Six hours after 125iodide administration, groups 0, 2, 4 and 6 were injected with 0.9% saline solution and groups 1, 3, 5 and 7 with potassium perchlorate (KCl04). After 2.5 minutes, the animals were sacrificed by cervical dislocation and the blood was sampled after decapitation of each animal.

Examinations

Examinations

The thyroid glands were removed, trimmed and weighed. Radioactivity was counted in the blood and thyroid and the ratio between 125iodide in thyroid and in blood was determined.

Positive control

- Propylthiouracil (PTU) - for direct mechanism
- Phenobarbital (PB) - for indirect toxic mechanisms

Any other information on materials and methods incl. tables

CLINICAL OBSERVATIONS

The animals were examined for overt signs of toxicity or mortality twice a day (in themorning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays. Additionally, further general clinical examinations were carried out daily.

BODY WEIGHT
During the administration period the body weight was determined on study days 0, 7,14, 21, 28 and 29.

FOOD CONSUMPTION AND SUBSTANE INTAKE
Individual food consumption was determined on study days 7, 14, 21, 26 and 29 and calculated as mean food consumption (g/animal/day).
The mean daily intake of each test substance was calculated based upon individual values for body weight and food consumption

CLINICAL CHEMISTRY
- Time schedule for collection of blood: day 27
- Anaesthetic used for blood collection: Yes (Isoflurane)
- Animals fasted: Yes
- How many animals: all
- Parameters examined: total triiodothyronine (T3), total thyroxine (T4), thyroid stimulating hormone (TSH)

BIOANALYTIC
- Time schedule for collection of blood: after cervical dislocation
- How many animals: all
- Parameters examined:125iodide in blood and thyroid samples via gamma counter

Results and discussions

Details on results

CLINICAL OBSERVATIONS
No abnormal clinical signs were observed and no animal died during the study.

BODY WEIGHT
The body weights of animals from dose groups 4 and 5 treated with 2,000 ppm of Propylthiouracil were found reduced from study day 7 to study day 29 (dose group 4, max. -35%; dose group 5, max. -36%). All other groups showed no significant deviations in comparison to the controls animals. However, as these results are
not relevant for the outcome of the study.

FOOD CONSUMPTION AND SUBSTANE INTAKE
A significant impairment in food consumption of animals from groups 4 and 5 treated with 2,000 ppm of Propylthiouracil from study day 7 to study day 29 (dose group 4, max. -52%; dose group 5 max. -54%) was observed.
All other groups showed no significant deviations in comparison to the controls animals.

CLINICAL CHEMISTRY
Thyroid hormones were found significantly altered after PTU treatment. Significant decreases of thyroxine (T4) as well as triiodothyronine (T3) levels were observed whereas thyroid stimulating hormone (TSH) was found increased when compared to the concurrent controls.
A slight, but not statistically significant, increase in TSH levels in rats treated with DINCH and PB was observed

BIOANALYTIC
- Day 29, after 0,9% saline solution administration (6 hours after 125iodine injection): Thyroid weights were observed significantly increased in rats treated with PTU (+449%), PB (+60%) and DINCH (+49%) as compared to the concurrent controls. 125Iodide levels in the blood and per gram of blood of animals treated with PTU were found significantly increased (+112% and +117%, respectively). The 125Iodide uptake in the thyroid of animals treated with DINCH, PTU and PB was found significantly increased (+96%, 64% and +193%, respectively). The unexpected increase of 125iodide uptake in the thyroid of PTU treated rats occurred due to the marked enlargement of the gland. 125Iidide per gram of thyroid was significantly reduced in animals treated with PTU (-68%). An increase in the 125iodide per gram of thyroid was observed in animals treated with DINCH (+38%) and PB (+87%) as compared to the control group. Thyroid to blood ratio was found 28% and 77% higher in groups of animals treated with DINCH and PB, respectively, while a significant reduction of 85% in the ratio was observed after PTU treatment

- Day 29, after potassium perchlorate administration (6 hours after 125iodine injection): Thyroid weights were significantly increased in rats treated with PTU (+312%) as compared to the concurrent controls. 125Iodide levels in the blood and per gram of blood of animals treated with PTU were found markedly increased (+234% and +238%, respectively). The 125iodide uptake in the thyroid and per gram of thyroid of animals treated with DINCH and PB was found significantly increased (+55% and +54%, respectively for DINCH and +187% and +117%, respectively for PB) while for PTU treated rats the incorporation of the labeled iodide into the gland as well as per gram of thyroid was observed significantly reduced (-33% and -84%, respectively). Thyroid to blood ratio was found 57% and 134% higher in groups of animals treated with DINCH and PB, respectively, while a significant reduction of 95% in the ratio was observed after PTU treatment.

Remarks on results including tables and figures

- 1,2 -Cyclohexanedicarboxylic acid, diisononyl ester (15000 ppm; 1301 mg/kg of body weight/day): marginal increase in TSH concentration, significant increase in thyroid weights, significant increase of 125iodide uptake in the thyroid, significant increase in the ratio of 125iodide measured in the thyroid versus blood.

- Propylthiouracil (2000 ppm; 133 mg/kg of body weight/day): significant decrease in T4 and T3 concentrations, significant increase in TSH concentration, marked increase in thyroid weights, significant reduction of 125iodide uptake in the thyroid after KClO4 administration, discharge of 125iodide after KClO4 administration, significant reduction in the ratio of 125iodide measured in the
thyroid versus blood.
- Phenobarbital (1000 ppm; 86 mg/kg of body weight/day): slight increase in TSH concentration, significant increase in thyroid weights, significant increase of 125iodide uptake in the thyroid, increase in the ratio of 125iodide measured in the thyroid versus blood.

Overall remarks, attachments

Overall remarks

Considering the significant increase of iodide organification as shown by an enhanced uptake of 125iodide in the thyroid gland after 4 weeks of 1,2 - Cyclohexanedicarboxylic acid, diisononyl ester administration and the absence of the labeled iodide discharged after co-administration with perchlorate, it can be concluded that the test substance has the potential, similar to Phenobarbital, to promote indirectly thyroid toxicity.

Reference substance: 1,2-Cyclohexandicarbonsäurediisononylester

UUID IUC4-41de2559-b66f-3b2c-ab8a-30139c2884a2
Dossier UUID 0
Author gerstma
Date 2009-10-23 11:36:06 CEST
Remarks Added EU: REACH data protection flag

General information

Reference substance name 1,2-Cyclohexandicarbonsäurediisononylester

Reference substance information

CAS information

CAS number 166412-78-8

Description

The given information is based on IUCLID4 chapter 1.1.0 General Substance Information and the IUCLID4 Substance Definition.

Synonyms

Name ELINCS listed

Molecular and structural information

Legal entity: BASF SE

UUID	IUC4-fa6b5320-73c6-367e-9073-49dcfd348866
Dossier UUID	0
Author	gerstma
Date	2009-10-22 10:48:00 CEST
Remarks	Added EU: REACH data protection flag

General information

Legal entity name	BASF SE
Legal entity type	company

Identifiers

Legal entity identifiers

EU: REACH

Identifier type	DUNS
ID	315000554

EU: REACH

Identifier type	VAT
ID	DE149145247

Contact information

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Sites

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