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Dossier

 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Notavailable,notasingleisomer

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1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Notavailable,notasingleisomer

Substance identification

Chemical name 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich

Reference substance

 / Notavailable,notasingleisomer

EC number

271-090-9

EC name

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich

CAS number

CAS name

IUPAC name

Not available, not a single isomer

Type of substance

Composition multi constituent substance

Origin organic

Trade names

Name

Jayflex DIN³

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1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich //
Not available, not a single isomer

Substance composition

1,2-Benzenedicarboxylic acid, di-C8-10 branched alkyl esters, C9-rich

Name 1,2-Benzenedicarboxylic acid, di-C8-10 branched alkyl esters, C9-rich
Brief description Di-isononyl phthalate is a di-ester of phthalic anhydride and isononanol. It is a high purity di-ester. It is identified by the following EINECS number 271-090-9, CAS number 68515-48-0 . See attached multi-constituent s

Constituents

/ 1,2-benzenedicarboxylic acid, di-isononyl ester

 [1,2-benzenedicarboxylic acid, di-isononyl ester](#)

Reference substance	EC number	EC name
	249-079-5	di-"isononyl" phthalate
	CAS number	CAS name
	IUPAC name	

1,2-benzenedicarboxylic acid, di-isononyl ester

/ 1,2-benzenedicarboxylic acid, isodecyl, isononyl ester

 [1,2-benzenedicarboxylic acid, isodecyl, isononyl ester](#)

Reference substance	EC number	EC name
	285-916-0	isodecyl isononyl phthalate
	CAS number	CAS name
	IUPAC name	

1,2-benzenedicarboxylic acid, isodecyl, isononyl ester

/ 1,2-benzenedicarboxylic acid, di-isodecyl ester

 [1,2-benzenedicarboxylic acid, di-isodecyl ester](#)

Reference substance	EC number	EC name
	247-977-1	di-"isodecyl" phthalate
	CAS number	CAS name
	IUPAC name	

1,2-benzenedicarboxylic acid, di-isodecyl ester

/ 1,2-benzenedicarboxylic acid, isooctyl isononyl ester

Reference substance  [1,2-benzenedicarboxylic acid, isooctyl isononyl ester](#)

EC number	EC name
306-190-4	isononyl isooctyl phthalate
CAS number	CAS name
IUPAC name	
1,2-benzenedicarboxylic acid, isooctyl isononyl ester	

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich / / Not available, not a single isomer

Identifiers

Regulatory programme identifiers

Other IT system identifiers

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Analytical information

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich / / Not available, not a single isomer

Joint submission

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich / / Not available, not a single isomer

Sponsors

- 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Suppliers

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Recipients

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Product and process oriented research and development

Classification and Labelling according to GHS

1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich

General information

Name 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich

Implementation EU

Remarks DNP was evaluated by the Classification and Labelling working group with a determination that classification was not required for any endpoint. Issues related to developmental and reproductive toxicity were considered by the Specialized Expert Group who also determined that data did not meet the standards for classification. The official results on Classification and Labeling are published on the website of the European Chemicals Bureau (Official journal of the European Union version C90/11 dated 13.4.2006).

Classification

Physical hazards

	Hazard statement	Reason for no classification
Explosives		conclusive but not sufficient for classification
Flammable gases		conclusive but not sufficient for classification
Flammable aerosols		conclusive but not sufficient for classification
Oxidizing gases		conclusive but not sufficient for classification
Gases under pressure		conclusive but not sufficient for classification
Flammable liquids		conclusive but not sufficient for classification
Flammable solids		conclusive but not sufficient for classification
Self-reactive substances and		conclusive but not sufficient for classification

mixtures

Pyrophoric liquids

conclusive but not sufficient for
classification

Pyrophoric solids

conclusive but not sufficient for
classification

Self heating
substances and
mixtures

conclusive but not sufficient for
classification

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

conclusive but not sufficient for
classification

Organic peroxides

conclusive but not sufficient for
classification

Substance and
mixtures corrosive
to metals

conclusive but not sufficient for
classification

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

conclusive but not sufficient for
classification

Skin
corrosion/irritation

conclusive but not sufficient for
classification

Serious damage/
eye irritation

conclusive but not sufficient for
classification

Respiratory
sensitization

conclusive but not sufficient for
classification

Skin sensitization

conclusive but not sufficient for
classification

Aspiration hazard

conclusive but not sufficient for
classification

Gen cell mutagenicity

Germ cell
mutagenicity

Hazard statement

Reason for no classification

conclusive but not sufficient for
classification

Carcinogenicity

Carcinogenicity

Hazard statement

Reason for no classification

conclusive but not sufficient for
classification

Reproductive
toxicity

Hazard statement

Reason for no classification

conclusive but not sufficient for
classification

Effects on or via
lactation

conclusive but not sufficient for
classification

Specific target organ toxicity - single

Specific target
organ toxicity -
single

Hazard statement

Reason for no classification

conclusive but not sufficient for
classification

Specific target organ toxicity - repeated

Specific target
organ toxicity -
repeated

Hazard statement

Reason for no classification

conclusive but not sufficient for
classification

Health hazards

Hazardous to the
aquatic
environment

Hazard statement

Reason for no classification

conclusive but not sufficient for
classification

Labelling

Signal word no signal word

Dossier > Document

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich / /
Not available, not a single isomer

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

1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich

General information

Name	1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich ✓ Not classified
Status	other: DINP was evaluated by the Classification and Labelling working group with a determination that classification was not required for any endpoint. DINP is not listed in Annex I of 67/548/EEC.
Remarks	DINP was evaluated by the Classification and Labelling working group with a determination that classification was not required for any endpoint. Issues related to developmental and reproductive toxicity were considered by the Specialized Expert Group who also determined that data did not meet the standards for classification. The official results on Classification and Labeling are published on the website of the European Chemicals Bureau (Official journal of the European Union version C90/11 dated 13.4.2006).

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 /		conclusive but not sufficient for classification

Corrosion	
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

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Technological process

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Estimated quantities

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Sites

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich / / Not available, not a single isomer

Form in the supply chain

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Uses and exposure

Overall use and exposure

Main use category

- ☒ Industrial use
- ☐ Professional use
- ☐ Consumer use

Specification for industrial and professional use

- ☒ Used in closed system
- ☒ Use resulting in inclusion into or onto matrix
- ☐ Non-dispersive use
- ☐ Dispersive use

Significant routes of exposure

Human exposure

- ☐ Oral
- ☐ Dermal
- ☐ By inhalation

Environmental exposure

- ☐ Water
- ☐ Air
- ☐ Solid waste
- ☐ Soil

Pattern of exposure

- ☐ Accidental / infrequent
- ☐ Occasional
- ☐ Continuous / frequent

Identified uses and exposure scenarios

Identified use

Application technique / Activity DINP is a plasticiser added to PVC (polyvinylchloride) to impart flexibility.

Use category other products: paints, coatings and inks

- ☒ Available as substance
- ☐ Substance in mixture

Industry category C20.3 - manufacturing: manufacture of paints, varnishes and similar coatings, printing ink and mastics

Type of article Other articles; specify: paints, coatings and inks

Use category plasticizer

- ☒ Available as substance
- ☒ Substance in mixture

Industry category C22.2 - manufacturing: manufacture of plastics products
category

Type of Other articles; specify: General purpose plasticiser added to PVC (polyvinylchloride), used in following industries: wire and cable (cable article)

Exposure scenario

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Uses advised against

Application DINP is restricted for use in toys and childcare articles in the European Union by Directive 2005/84/EC. The above restriction is based on a **technique** / review of the restrictions as required by Directive

Activity 2005/84/EC.

Use category other products: Toys and childcare articles that can be placed in the mouth.

☐ Available as substance

☐ Substance in mixture

Industry category C22.2 - manufacturing: manufacture of plastics products

Type of article Plastic products: Toys (no intended release)

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Waste from production and use

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich / / Not available, not a single isomer

Exposure estimates

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich / / Not available, not a single isomer

Biocidal information

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich // Not available, not a single isomer

Application for authorisation of uses

Dissemination Dossier
General inform Endpoint.001

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP.
Study period 2009-04-21 till 2009-05-15

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2009						

Materials and methods

Test guideline

Qualifier	Guideline
according to	other guideline: Ph. Eur. 2.2.1. Clarity and degree of opalescence of liquids.
according to	other guideline: Ph. Eur. 2.2.2. Degree of coloration of liquids.

Principles of method if other than guideline

Using identical tubes of colourless, transparent, neutral glass 2.0 ml of the liquid to be examined were compared with 2.0 ml of water. The colour and transparency were compared, viewing horizontally against a white background (translucent glass backlit by fluorescent lamp).

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Product name: Diisononyphthalat (Vestinol 9) 1284-19463
 - Lot no: 15.04.2009 13:00 Uhr

Results and discussion

Physical state at 20°C and 1013 hPa

liquid

Form

compact

Colour

The test item is a colourless and clear liquid.

Odour

slight

Substance type

organic

Applicant's summary and conclusion**Conclusions**

The test item is a colourless and clear liquid.

Executive summary

The test item is a colourless and clear liquid.

-  Dissemination Dossier
-  General inform Endpoint.002

Administrative Data

Purpose flag key study

Study result type experimental result

Study period

Reliability 2 (reliable with restrictions)

Rationale for reliability Measured according ASTM D 5386, but with no GLP conditions in place.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
company data		2008						

Materials and methods

Test guideline

Qualifier	Guideline	Deviation
according to	other guideline: ASTM D 5386	no

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Molecular weight = 418.3

Results and discussion

Physical state at 20°C and 1013 hPa

liquid

Form

viscous

Colour

Colour Pt-Co = < 30 (production specification), typically 10

Odour

odourless

Substance type

organic

Applicant's summary and conclusion

Conclusions

Di-Isononyl phthalate is a clear, colorless liquid with no odour at 20 degrees C and 1013 hPa.

Executive summary

Di-Isononyl phthalate is a clear, colorless liquid with no odour at 20 degrees C and 1013 hPa.

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Dissemination Dossier

General inform Endpoint.003

Administrative Data

Data source

Materials and methods

Results and discussion Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	other: experience
Reliability	2 (reliable with restrictions)
Rationale for reliability	Long term production demonstrates that the substance of the purity described in chapter 1.1 is a liquid at ambient temperature and is odourless.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
company data		2007							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: There is no standard test for appearance. Long term experience in production of the substance and comparison of the appearance with similar substances (esters of similar structure) demonstrate that the information is valid.	

Principles of method if other than guideline

There is no standard test for appearance. Long term experience in production of the substance and comparison of the appearance with similar substances (esters of similar structure) demonstrate that the information is valid.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Results and discussion

Physical state at 20°C and 1013 hPa

liquid

Colour

colourless

Odour

odourless

Substance type

organic

Applicant's summary and conclusion

Conclusions

There is no standard test for appearance. Long term experience in production of the substance and comparison of the appearance with similar substances (esters of similar structure) demonstrate that the information is valid.

Executive summary

There is no standard test for appearance. Long term experience in production of the substance and comparison of the appearance with similar substances (esters of similar structure) demonstrate that the information is valid.

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 Dissemination Dossier

 Melting Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	2009
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Study done according to ASTM method D9550, however GLP conditions not in place		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
company data		2009							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: ASTM D5950 modified	no data

Type of method

pour point

Principles of method if other than guideline

Method according ASTM D 5950, but without initial preheating.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Results and discussions

Melting / freezing point

Melt./Freez. pt.	Atm. pressure	Decomposition	Decomp. temp.	Sublimation	Subl. temp.	Remarks
< -50 °C	1 atm	no		no		No clear melting point exists. Measurement is difficult and poorly reproducible. In general, pour point gives a better indication of the change in physical form of this substancesical form of

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

Pour point of DINP is below minus 50 degrees Celsius.

Executive summary

Pour point of DINP is below minus 50 degrees Celsius.

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 [Melting Endpoint.002](#)

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[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	2009-04-21 till 2009-05-15
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Guideline study, but without GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2009							2009-05-29

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method A.1 (Melting / Freezing Temperature)	no
according to	OECD Guideline 102 (Melting point / Melting Range)	no

Type of method

thermal analysis

GLP compliance

no

Test materials

Test material equivalent to submission substance identity
no

Details on test material

- Product name: Diisononyphthalat (Vestinol 9) 1284-19463 - Lot no: 15.04.2009 13:00 Uhr

Results and discussions

Melting / freezing point

Melt./Freez. pt.	Atm. pressure	Decomposition	Decomp. temp.	Sublimation	Subl. temp.	Remarks
<= -80 °C	1013 hPa					No melting point was found. The substance had solidified amorphously and showed a glass transition at -88°C.

Applicant's summary and conclusion

Conclusions

No melting point was found. The substance had solidified amorphously and showed a glass transition at -88°C.

Executive summary

No melting point was found. The substance had solidified amorphously and showed a glass transition at -88°C.

Dissemination Dossier
Melting Endpoint.003

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP
Study period October 1-22, 2008

Data source

Reference	Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
	study report		2008						

Materials and methods

Test guideline	Qualifier	Guideline	Devi
	according to	other guideline: Method ASTM D 97-02	no

Type of method

pour point

GLP compliance

no

Test materials

Test material equivalent to submission substance identity
no

Details on test material

Identity: Di – "Isononyl" phthalate
POLYNT Trade Name: DIPLAST® NS
IUPAC chemical name: Bis (7 – methyloctyl) benzene – 1,2 – dicarboxylate
SYNONYMS: DINP
EINECS N°: 249-079-5
CAS N°: 28553-12-0
Physical state @ 25°C: Liquid
Storage conditions: Keep container tightly closed. Keep in a dry, cool and well ventilated place
Batch number: 2121208296
Expiry date: The product DIPLAST® NS due to its nature does not have expiry date.
Purity: 99.75%

Nature of impurities:
Alkyl benzoates 0.14%
<C9 Phthalates 0.09%
Bis (2 – ethylhexyl) phthalate 0.02%

Date received: 22 October 2008
Storage at testing facility: 5 years

Results and discussions

Melting / freezing point

Melt./Freez. pt.	Atm. pressure	Decomposition	Decomp. temp.	Sublimation	Subl. temp.
-54 °C					

Applicant's summary and conclusion**Conclusions**

The Pour – Point of DIPLAST® NS was determined to be -54 °C

Executive summary

The Pour – Point of DIPLAST® NS was determined to be -54 °C

Dissemination Dossier
Boiling Endpoint.001

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability guideline study, but without GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		1999						

Materials and methods

Test guideline

Qualifier	Guideline	Dev
according to	other guideline: dynamic under nitrogen	

Type of method

dynamic method dynamic under nitrogen

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): di-"isononyl" phthalate
- Analytical purity: 99.5%

Results and discussions

Boiling point

Boiling pt.	Atm. pressure	Decomposition	Decomp. temp.
252.4 °C	7 hPa		

Remarks on results including tables and figures

Substance decomposes before boiling at atmospheric pressure.

Dissemination Dossier
Boiling Endpoint.002

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP.

Study period

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2009						

Materials and methods

Test guideline

Qualifier	Guideline
according to	other guideline: ASTM E 537-07
according to	OECD Guideline 103 (Boiling point/boiling range)
according to	EPA OPPTS 830.7220 (Boiling Point / Boiling Range)
according to	EU Method A.2 (Boiling Temperature)

Type of method

differential scanning calorimetry method at 2°C/min

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Identity: Di – "Isononyl" phthalate
POLYNT Trade Name: DIPLAST® NS
IUPAC chemical name: Bis (7 – methyloctyl) benzene – 1,2 – dicarboxylate
SYNONYMS: DINP
EINECS N°: 249-079-5
CAS N°: 28553-12-0
Physical state @ 25°C: Liquid
Storage conditions: Keep container tightly closed. Keep in a dry, cool and well ventilated place.
Batch number: 2121208304
Expiry date: The product DIPLAST® NS due to its nature does not have expiry date.
Purity: 99,75%
Nature of impurities:
Alkyl benzoates 0,14%
<C9 Phthalates 0,09%
Bis (2 – ethylhexyl) phthalate 0,02%

Date received: 11 November 2008

Storage at testing facility: 5 years

Results and discussions

Boiling point**Boiling pt.**

331 °C

Atm. pressure

96.47 kPa

Decomposition

no

Decomp. temp.

Remarks on results including tables and figures

Applicant's summary and conclusion**Conclusions**

The boiling point of DIPLAST® NS was determined to be 331 °C at 96.47 kPa.

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 Dissemination Dossier

 Boiling Endpoint.003

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	estimated by calculation	Study period	2000
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Theoretical value based on GC analysis and Clausius Clapeyron equation		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Computer model	U.S. Environmental Protection Agency (USEPA)	2000	EPI Suite TM, Estimation Program Interface Suite, v3.12	USEPA, Washington, DC, USA					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: Computer model	no data

Type of method

other:

Principles of method if other than guideline

Boiling point calculation by MPBPWIN ver. 1.41, within EPISuite TM v3.12, using calculation method of Stein and Brown.

GLP compliance

no calculation, not applicable

Test materials

Test material equivalent to submission substance identity

yes

Results and discussions

Boiling point

Boiling pt.	Atm. pressure	Decomposition	Decomp. temp.	Remarks
> 400 °C	1 atm			Calculated value

Applicant's summary and conclusion

Conclusions

Boiling point for di-isononyl phthalate is above 400 degrees celsius.

Executive summary

Boiling point for di-isononyl phthalate is above 400 degrees celsius.

Dissemination Dossier
Boiling Endpoint.004

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP.
Study period 2009-04-21 till 209-05-15

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2009						

Materials and methods

Test guideline

Qualifier	Guideline	
according to	EU Method A.2 (Boiling Temperature)	
according to	OECD Guideline 103 (Boiling point/boiling range)	

Type of method

differential scanning calorimetry

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Product name: Diisononyphthalat (Vestinol 9) 1284-19463
- Lot no: 15.04.2009 13:00 Uhr

Results and discussions

Boiling point

Boiling pt.	Atm. pressure	Decomposition	Decomp. temp.
341 °C	1013 hPa		

Applicant's summary and conclusion

Conclusions

A boiling point of 341°C was calculated for Diisononylphthalate.

Executive summary

A boiling point of 341°C was calculated for Diisononylphthalate.

Dissemination Dossier
Density Endpoint.001

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP.

Study period 2009-04-21 till 2009-05-15

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2009						

Materials and methods

Test guideline

Qualifier	Guideline
according to	EU Method A.3 (Relative Density)
according to	OECD Guideline 109 (Density of Liquids and Solids)

Type of method

oscillating densitometer

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Product name: Diisononylphthalat (Vestinol 9) 1284-19463
- Lot no: 15.04.2009 13:00 Uhr

Results and discussion

Density

Type	Density	Temp.
relative density	0.97	20 °C

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

The relative density of Diisononylphthalate was measured at 20°C: 0.9740.

Executive summary

The relative density of Diisononylphthalate was measured at 20°C: 0.9740.

Dissemination Dossier
Density Endpoint.002

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP.
Study period October 1-22, 2008

Data source

Reference	Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report			2008						

Materials and methods

Test guideline

Qualifier	Guideline
according to	other guideline: ASTM D 4052-96
according to	EPA OPPTS 830.7300 (Density / Relative Density / Bulk Density)
according to	OECD Guideline 109 (Density of Liquids and Solids)
according to	EU Method A.3 (Relative Density)

Type of method

oscillating densitometer

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Identity: Di – "Isononyl" phthalate
POLYNT Trade Name: DIPLAST® NS
IUPAC chemical name: Bis (7 – methyloctyl) benzene – 1,2 – dicarboxylate
SYNONYMS: DINP
EINECS N°: 249-079-5
CAS N°: 28553-12-0
Physical state @ 25°C: Liquid
Storage conditions: Keep container tightly closed. Keep in a dry, cool and well ventilated place.
Batch number: 2121208296
Expiry date: The product DIPLAST® NS due to its nature does not have expiry date.
Purity: 99.75%
Nature of impurities:
Alkyl benzoates 0.14%
- <C9 Phthalates 0.09%
- Bis (2 – ethylhexyl) phthalate 0.02%
Date received: 22 October 2008
Storage at testing facility: 5 years

Results and discussion

Density**Type**

relative density

Density

0.97

Temp.

20 °C

Remarks on results including tables and figures

Applicant's summary and conclusion**Conclusions**

The density of DIPLAST® NS was determined to be 0.9741 g/cm³, corresponding to 974.1 kg/m³.

Dissemination Dossier
Density Endpoint.003

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Average manufacturing data over 2008, measured according ASTM D 4052

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
company data		2008						

Materials and methods

Test guideline

Qualifier	Guideline	Deviation
according to	other guideline: ASTM D 4052	

Type of method

oscillating densitometer

Principles of method if other than guideline

Standard method for density and relative density of liquids by Digital Density Meter.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Commercial product

Results and discussion

Density

Type	Density	Temp.
density	0.97 g/cm ³	20 °C

Remarks on results including tables and figures

Density = 0.971 g/cm³

Applicant's summary and conclusion

Conclusions

Density at 20 degrees C for di-isononyl phthalate is 0.971 g/cm³

Executive summary

Density at 20 degrees C for di-isononyl phthalate is 0.971 g/cm³

-  Dissemination Dossier
-  Granulometry Endpoint.001

Administrative Data

Data waiving study scientifically unjustified

Dissemination Dossier
Vapour Endpoint.001

Administrative Data

Purpose flag key study

Study estimated by calculation

Study period

result type

Reliability 2 (reliable with restrictions)

Rationale Recommended value from the European Commission risk assessment for 1,2-benzenedicarboxylic acid, di-c8-10-branched alkyl esters, C9 for reliability

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
publication		2003						

Materials and methods

Type of method

other: calculation

Principles of method if other than guideline

Vapour pressure normally evaluated at elevated temperature due to low volatility of the substance.
With extrapolation by linear regression using the Clausius-Clapeyron equation, estimates of vapour pressure values in the temperature range of greatest interest for environmental modelling (20°C to 30° C) can be obtained.

Test materials

Test material equivalent to submission substance identity

yes

Results and discussions

Vapour pressure

ca. 0.00006 Pa at 20 °C

Remarks

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

Vapour pressure for di-isononyl phthalate is 0.00006 Pa at 20 degrees C.

Executive summary

Vapour pressure for di-isononyl phthalate is 0.00006 Pa at 20 degrees C.

Dossier > Document

 Dissemination Dossier

 Partition Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	1999
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Test procedure followed a technically appropriate procedure to determine the octanol/water partition coefficient of substances with very low water solubility, but did not follow GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ellington J	1999	Octanol/water partition coefficients and water solubilities of phthalate esters.	J. Chem. Eng. Data 44(6): 1414-1418					

Materials and methods

Partition coefficient type

octanol-water

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 123 (Partition Coefficient (1-Octanol / Water), Slow-Stirring Method)	no

Type of method

other: See principles below.

Principles of method if other than guideline

Log KOW 25 deg C, Ellington JJ (1999), determination of octanol/water partition coefficients and water solubilities of di-n-hexyl, di-n-octyl and di-n-decyl phthalates by the slow stirring and no stirring methods. Chem. Eng. Data 44, 1414-1418

GLP compliance

no no GLP lab

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Purity: 99.4% (determined by GC/FT-IR/MS)

Analytical method

GC

Results and discussions

Partition coefficient

Type	Partition coefficient	Temp.	pH
log Pow	8.8	25 °C	7

Details on results

Expressed as Log KOW in company data

Applicant's summary and conclusion

Conclusions

Log KOW (Pow) for di-isononyl phthalate is 8.8 at 25 degrees C.

Executive summary

Log KOW (Pow) for di-isononyl phthalate is 8.8 at 25 degrees C.

Dissemination Dossier
Partition Endpoint.002

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP.
Study period 2009-04-23 till 2009-05-04

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2009						

Materials and methods

Partition coefficient type

octanol-water

Test guideline

Qualifier	Guideline
according to	EU Method A.8 (Partition Coefficient)
according to	OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method)

Type of method

HPLC method

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Lot/batch No.: 15.04.2009
- Sample No.: 1284/19463

Analytical method

HPLC

Results and discussions

Partition coefficient

Type	Partition coefficient	Temp.
log Pow	8.8 — 9.7	25 °C

Applicant's summary and conclusion

Conclusions

The partition coefficient log POW of DINP was measured by the HPLC-method. log pOW is in the range of 8.8 - 9.7.

Executive summary

The partition coefficient log POW of DINP was measured by the HPLC-method. log POW is in the range of 8.8 - 9.7.

Dossier > *Document*

 Dissemination Dossier

 Partition Endpoint.003

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	March 19-21, 2009
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Guideline study, but without GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2009							2009-03-26

Materials and methods

Partition coefficient type

octanol-water

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OPPTS 830.7570 (Partition Coefficient, n-octanol / H2O, Estimation by Liquid Chromatography) 1996	no
according to	OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method) 2004	no
according to	EU Method A.8 (Partition Coefficient)	no

Type of method

HPLC method

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Identity: Di – “Isononyl” phthalate POLYNT Trade Name: DIPLAST® NS IUPAC chemical name: Bis (7 – methyloctyl) benzene – 1,2 – dicarboxylate SYNONYMS:DINP EINECS N°: 249-079-5 CAS N°: 28553-12-0 Physical state @ 25°C: Liquid Storage conditions: Keep container tightly closed. Keep in a dry, cool and well ventilated place. Batch number: 2121208304 Expiry date: The product DIPLAST® NS due to its nature does not have expiry date. Purity: 99.75% Nature of impurities: Alkyl benzoates 0.14% <C9 Phthalates 0.09% Bis (2 – ethylhexyl) phthalate 0.02% Date received: 11 November 2008 Storage at testing facility: 5 years

Analytical method

HPLC According to the guidelines no quantitative analytical validation was required, only the determination of elution times was necessary. Determination of retention times: According to the OECD guideline 117 the retention times were determined by injection.

Results and discussions

Partition coefficient

Type	Partition coefficient	Temp.	pH
log Pow	7.4	55 °C	7.2

Remarks on results including tables and figures

Calibration data

Injection N°	Average Retention time	Average Log k	Literature Log P _{ow}
Thiourea (Dead Time)	1.140		
Acetanilide	1.299	-0.855	1
2-Nitrophenol	1.465	-0.545	1.8

Toluene	1.962	-0.142	2.7
Naphthalene	2.194	-0.034	3.6
Phenanthrene	3.110	0.237	4.5
DDT	4.203	0.429	6.5
Decachlorobiphenyl	28.838	1.385	8.2

Linear calibration line: $y = 3,447x + 3,8089$

Retention data for DIPLAST[®]NS

Injection N°	Retention time 1 st peak (7.1% Area)	Retention time 2 nd peak (22.7 % Area)	Retention time 3 rd peak (38.6 % Area)	Retention time 4 th peak (24.5 % Area)	Retention time 5 th peak (7.1% Area)	Log k' from 1 st peak	Log k' from 2 nd peak	Log k' from 3 rd peak	Log k' from 4 th peak	Log k' from 5 th peak
1	12.388	13.142	14.065	15.253	16.653	0.994	1.022	1.055	1.093	1.134
2	12.403	13.139	14.060	15.207	16.640	0.995	1.022	1.054	1.091	1.133
3	12.348	13.100	14.003	15.193	16.544	0.993	1.021	1.052	1.091	1.131
4	12.380	13.113	14.038	15.201	16.647	0.994	1.021	1.054	1.091	1.134
Mean						0.994	1.022	1.054	1.091	1.133

The DIPLAST NS was eluted as series of poorly resolved peaks considered as five main peaks.

Log₁₀P_{ow} of DIPLAST[®]NS was extrapolated from the correlation line as follows:

Log P_{ow} = 3.447 log k + 3.8089

Obtaining:

Log P_{ow} of the 1st peak = 7.2

Log P_{ow} of the 2nd peak = 7.3

Log P_{ow} of the 3rd peak = 7.4

Log P_{ow} of the 4th peak = 7.6

Log P_{ow} of the 5th peak = 7.7

Weighted mean: Log P_{ow} = 7.4

Applicant's summary and conclusion

Conclusions

The partition coefficient of the test item was determined to be log Pow=7.4 at pH= 7.2 and T= 55°C

 Dissemination Dossier
 Water sol Endpoint.001

Administrative Data

Purpose flag supporting study	
Study result type other: EU Risk Assessment Report	Study period
Reliability 1 (reliable without restriction)	
Rationale for reliability European Union Risk Assessment Report	

Data source

Reference				
Reference type	Author	Year	Title	Bibliographic source
	2003		European Union Risk Assessment Report Di-"Isononyl"phthalate	Testing laboratory Report no. Owner company

Materials and methods

Test guideline

Qualifier	Guideline
	other guideline: Several measurements in the 80s and 90s cited in the EU Risk Assessment

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity
 no

Results and discussions

Water solubility

ca. 0.6 µg/L

Temp. 20 °C

pH 4.6

Applicant's summary and conclusion

Interpretation of results

insoluble (< 0.1 mg/L)

Dissemination Dossier
Water sol Endpoint.002

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study.

Data source

Reference								
Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		1988						

Materials and methods

Test guideline		Qualifier		Guideline	Deviation
according to				EU Method A.6 (Water Solubility)	

Type of method

column elution method

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity
no

Details on test material

- Name of test material (as cited in study report): di-"isononyl" phthalate
- Analytical purity: pure

Results and discussions

Water solubility

< 0.1 mg/L

Temp. 25 °C

pH 7

Applicant's summary and conclusion

Interpretation of results

insoluble (< 0.1 mg/L)

Dissemination Dossier
Water sol Endpoint.003

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Guideline study, but without GLP.
Study period 2009-04-23 till 2009-05-04

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2009						

Materials and methods

Test guideline

Qualifier	Guideline	Devi
according to	EU Method A.6 (Water Solubility)	no
according to	OECD Guideline 105 (Water Solubility)	no

Type of method

column elution method

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Lot/batch No.: 15.04.2009
- Sample No.: 1284/19463

Results and discussions

Water solubility

<= 1.4 mg/L
Temp. 20 °C
pH 4.6

Applicant's summary and conclusion

Interpretation of results

slightly soluble (0.1-100 mg/L)

Conclusions

The water solubility of DINP was measured. DINP is slightly soluble in water, <1.4 mg/L.

Dissemination Dossier
Water sol Endpoint.004

Administrative Data

Purpose flag key study

Study result type experimental result

Study period

Reliability 2 (reliable with restrictions)

Rationale for reliability Test procedure followed a technically appropriate procedure to determine water solubility of substances with very low water solubility

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company studied
study report		1999						

Materials and methods

Type of method
analysis

Principles of method if other than guideline

equivalent or similar to OECD Guideline 105 (Water Solubility)

GLP compliance

no

Test materials

Test material equivalent to submission substance identity
yes

Details on test material

Substance type : Commercial product

Results and discussions

Water solubility

0.6 µg/L

Temp. 21 °C

pH 7

Remarks on results including tables and figures

Applicant's summary and conclusion

Interpretation of results

insoluble (< 0.1 mg/L)

Conclusions

Water solubility for di-isononyl phthalate is 0.6 microg/L at 21 degrees C.

Executive summary

Water solubility for di-isononyl phthalate is 0.6 microg/L at 21 degrees C.

Dossier > Document

 Dissemination Dossier

 Surface tension Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	2006
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Experimental result according Wilhelmy plate method, however GLP conditions not in place.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2006							

Materials and methods

Type of method

plate method

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: Wilmhelmy plate EC-M-F02	no

Principles of method if other than guideline

A universal method especially suited to check surface tension over long time intervals. A vertical plate of known perimeter is attached to a balance, and the force due to wetting is measured. The Wilhelmy plate consists of a thin plate usually on the order of a few centimeters square. The plate is often made from glass or platinum which may be roughened to ensure complete wetting. The plate is cleaned thoroughly and attached to a scale or balance via a thin metal wire. The force on the plate due to wetting is measured via a tensiometer or microbalance and used to

calculate the surface tension (σ) using the Wilhelmy equation: where l is the wetted length of the Wilhelmy plate and θ is the contact angle between the liquid phase and the plate. In practice the contact angle is rarely measured, instead either literature values are used, or complete wetting ($\theta = 0$) is assumed.

GLP compliance

no No GLP lab

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Commercial product

Results and discussions

Surface tension

30.7 mN/m

Temp. 20 °C

Concentration 100 vol%

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

Surface tension for di-isononyl phthalate is 30.7 mN/m at 20 degrees C.

Executive summary

Surface tension for di-isononyl phthalate is 30.7 mN/m at 20 degrees C.

Dissemination Dossier
Flash Endpoint.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Experimental result according ASTM D 93, however GLP conditions not in place.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2008						

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	ISO No., other: ASTM D 92	no

Type of method

open cup

Principles of method if other than guideline

Test done according ASTM D 92, Cleveland Open Cup

GLP compliance

no No GLP conditions in place

Test materials

Test material equivalent to submission substance identity

yes

Results and discussions

Flash point

236 °C
at 1013 hPa

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

Flash point for di-isononylphthalate is 236 degrees C at 1013 hPa.

Executive summary

Flash point for di-isononylphthalate is 236 degrees C at 1013 hPa.

Dissemination Dossier

Flash Endpoint.002

Administrative Data

Purpose flag supporting study

Study result type experimental result

Study period October 1-22, 2008

Reliability 2 (reliable with restrictions)

Rationale for reliability Guideline study, but without GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2008						

Materials and methods

Test guideline

Qualifier	Guideline	Devia
according to	ISO No., other: ASTM D 93 – 02	no
according to	EPA OPPTS 630.6315 (Flammability)	no
according to	EU Method A.9 (Flash-Point)	no

Type of method

closed cup

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Identity: Di – "Isononyl" phthalate

POLYNT Trade Name: DIPLAST® NS

IUPAC chemical name: Bis (7 – methyloctyl) benzene – 1,2 – dicarboxylate

SYNONYMS: DINP

EINECS N°: 249-079-5

CAS N°: 28553-12-0

Physical state @ 25°C: Liquid

Storage conditions: Keep container tightly closed. Keep in a dry, cool and well ventilated place.

Batch number: 2121208296

Expiry date: The product DIPLAST® NS due to its nature does not have expiry date.

Purity: 99.75%

Nature of impurities:

Alkyl benzoates 0.14%

<C9 Phthalates 0.09%

Bis (2 – ethylhexyl) phthalate 0.02%

Date received: 22 October 2008

Storage at testing facility 5 years

Results and discussions

Flash point

236 °C

at 101.7 kPa

Applicant's summary and conclusion**Conclusions**

The Flash point of DIPLAST® NS was determined to be 236°C.

 Dissemination Dossier

 Auto flamm Endpoint.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability Outside laboratory, GLP qualified.

Study period

Data source

Reference		Author		Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
Reference type		company data		2008						

Materials and methods

Test guideline		Qualifier		Guideline		Deviations	
according to				other guideline: ASTM E 659			

GLP compliance

yes Certified laboratory

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Commercial product

Results and discussions

Autoflammability / Self-ignition temperature

400 °C

at 1013 hPa

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

The auto ignition temperature for di-isononyl phthalate is 400 degrees C at 1013 hPa.

Executive summary

The auto ignition temperature for di-isononyl phthalate is 400 degrees C at 1013 hPa.

Dissemination Dossier
Flamm Endpoint.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability Tested by outside laboratory, GLP certified
Study period

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		2008						

Materials and methods

Test guideline

Qualifier	Guideline	Deviation
according to	other guideline: Intertek method	no

Principles of method if other than guideline

Test performed at atmospheric pressure using vertical flame propagation in a closed 2 litre capacity aluminium alloy vertical tube (100 mm diameter, 260 mm long) held within a temperature-controlled enclosure. Ignition by high tension spark gap mounted 10 mm above the base of the explosion tube.

GLP compliance

yes Certified laboratory

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Commercial substance

Results and discussions

Solid/liquid: Ignition on contact with air

no FFL: 0.3V% - UFL: 1.7V%

Remarks on results including tables and figures

LFL = Lower Flammability Limit

UFL = Upper Flammability limit

Applicant's summary and conclusion

Conclusions

Very low degree of flammability

Executive summary

Very low degree of flammability

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 Dissemination Dossier

 Expl Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	other: EU risk assessment	Study period	2003
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Recommended value from European Commision risk assesment		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	European Commission	2003	EU Risk Assessment Report - DINP Final Repoert, 2003	Risk Assessment report Vol. 36					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method A.14 (Explosive properties)	no

GLP compliance

no literature

Test materials

Test material equivalent to submission substance identity
yes

Details on test material

Commercial product

Results and discussions

Remarks on results including tables and figures

No explosion limits under standard conditions

Applicant's summary and conclusion

Conclusions

Di-isononyl phthalate does not have explosion limits under standard conditions.

Executive summary

Di-isononyl phthalate does not have explosion limits under standard conditions.

 Dissemination Dossier Oxid Endpoint.001

Administrative Data

Purpose flag supporting study

Study result type other: Company data

Study period

Reliability 2 (reliable with restrictions)

Rationale for reliability Recommended value from the European Commission risk assesment for 1,2-benzenedicarboxylic acid, di-C8-10-bra

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
publication		2003						

Materials and methods

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Commercial product

Results and discussions

Test result

other:

Not applicable

Remarks No oxidising properties

Applicant's summary and conclusion

Interpretation of results

no oxidising properties

Conclusions

Di-isononyl phthalate has no oxidizing properties

Executive summary

Di-isononyl phthalate has no oxidizing properties

-  Dissemination Dossier
-  Oxid Endpoint.002

Administrative Data

Data waiving study scientifically unjustified

 Dissemination Dossier

 Stability organic Endpoint.001

Administrative Data

Purpose flag key study

Study result type read-across based on grouping of substances (category approach)

Reliability 2 (reliable with restrictions)

Rationale for reliability No standard method, no GLP lab

Stuc

Data source

Reference								
Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
company data		2009						

Materials and methods

Test guideline				Deviatio				
Qualifier	Guideline							
according to	other guideline: Inhouse method			no				

Principles of method if other than guideline

The substance is placed in contact with organic solvents (THF, Acetone, White Spirit) in a ratio 50/50 in a glass bottle under the light. A GC light ends will be performed at regular times to compare it with the GC of a fresh sample.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Commercial product of Di-isodecyl phthalate.

Results and discussions

Test substance stable

yes

Degradation products

no

Remarks on results including tables and figures

Cross reading from DIDP results

-  Dissemination Dossier
-  Stability organic Endpoint.002

Administrative Data

Data waiving other justification

-  Dissemination Dossier
-  Dissociation Endpoint.001

Administrative Data

Data waiving study scientifically unjustified

Dissemination Dossier

Viscosity Endpoint.001

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability Guideline study, but without GLP.

Study period

2009-04-21 till 2009-05-15

Data source

Reference	Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
	study report		2009						

Materials and methods

Test guideline	Qualifier	Guideline	
	according to	OECD Test Guideline 114 (Viscosity of Liquids)	[r

Type of method

capillary method

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Product name: Diisononyphthalat (Vestinol 9) 1284-19463
- Lot no: 15.04.2009 13:00 Uhr

Results and discussions

Viscosity

77.6 mm²/s (static)

Temp. 20 °C

Remarks

27.7 mm²/s (static)

Temp. 40 °C

Remarks

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

The viscosity of Diisononylphthalate was measured at 20°C and at 40°C:

20°C: 77.6 mm²/s.

40°C: 27.7 mm²/s.

Executive summary

The viscosity of Diisononylphthalate was measured at 20°C and at 40°C:

20°C: 77.6 mm²/s.

40°C: 27.7 mm²/s.

Dissemination Dossier
Viscosity Endpoint.002

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability Measured according test method ASTM D 445, but GLP conditions not in place
Study period

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
company data		2008						

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: ASTM D 445	no

Type of method

capillary method

GLP compliance

no NO GLP certified laboratory

Test materials

Test material equivalent to submission substance identity
yes

Details on test material

commercial product

Results and discussions

Viscosity

93 mPa s (dynamic)

Temp. 20 °C

Remarks

Applicant's summary and conclusion

Conclusions

Viscosity of di-isononyl phthalate at 20 degrees c = 93 mPa.s

Executive summary

Viscosity of di-isononyl phthalate at 20 degrees c = 93 mPa.s

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 [Dissemination Dossier](#)

 [Viscosity Endpoint.003](#)

[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	October 01, 2008-March 26, 2009
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Guideline study, but without GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2009							2009-03-26

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: ASTM D 445-06	no
according to	EPA OPPTS 830.7100 (Viscosity)	no
according to	OECD Test Guideline 114 (Viscosity of Liquids)	no

Type of method

capillary method

GLP compliance

no

Test materials

Test material equivalent to submission substance identity
no

Details on test material

Identity: Di – “Isononyl” phthalate POLYNT Trade Name:DIPLAST® NS IUPAC chemical name: Bis (7 – methyloctyl) benzene – 1,2 – dicarboxylate SYNONYMS:DINP EINECS N°: 249-079-5 CAS N°: 28553-12-0 Physical state @ 25°C: Liquid Storage conditions: Keep container tightly closed. Keep in a dry, cool and well ventilated place. Batch number: 2121208296 Expiry date: The product DIPLAST® NS due to its nature does not have expiry date. Purity: 99.75% Nature of impurities: Alkyl benzoates 0.14% <C9 Phthalates 0.09% Bis (2 – ethylhexyl) phthalate 0.02% Date received: 22 October 2008 Storage at testing facility: 5 years

Results and discussions

Viscosity

77.1 mPa s (dynamic)

Temp. 20 °C

Remarks

27.2 mPa s (dynamic)

Temp. 40 °C

Remarks

Remarks on results including tables and figures

CALCULATION

Kinematic Viscosity $\nu(\text{mm}^2/\text{s}) = C \times t$

where C is the calibration constant of the viscometer and t the measured time

Dynamic Viscosity(mPa s) = $\nu \times \rho$

Where ν is the Kinematic Viscosity (mm^2/s) and ρ is the density (g/cm^3) at the same temperature used for the determination of the Kinematic Viscosity.

Applicant's summary and conclusion

Conclusions

The viscosity of DIPLAST® NS was determined to be 77.1 mPa s at 20.0°C and 27.2 mPa s at 40.0°C.

- Dissemination Dossier
- Other Endpoint.001

Administrative Data

Purpose flag supporting study

Study result type experimental result

Study period

Reliability 2 (reliable with restrictions)

Rationale for reliability Calculation based on measured density values at different temperatures measured according a standard method..

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
company data		2008						

Materials and methods

Endpoint investigated

other: Thermal expansion coefficient

Test guideline

Qualifier	Guideline	Deviation
according to	other guideline: ASTM D 4052	no

Principles of method if other than guideline

Thermal expansion coefficient is calculated from several density measurements at different temperatures between 20 and 100 degrees C.

GLP compliance

no No certified lab

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

commercial product

Results and discussions

Results

Thermal expansion coefficient for di-isononyl phthalate = 0.00077 within the temperature range from 20 to 100 deg C

Remarks on results including tables and figures

Thermal expansion coefficient for di-isononyl phthalate = 0.00077 within the temperature range from 20 to 100 deg C

-  Dissemination Dossier
-  Other Endpoint.002

Administrative Data

Purpose flag supporting study

Study result type experimental result

Study period

Reliability 2 (reliable with restrictions)

Rationale for reliability Measured according ASTM D 1045, but with no GLP conditions in place

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
company data		2008						

Materials and methods

Endpoint investigated

refractive index

Test guideline

Qualifier	Guideline	Deviation
according to	other guideline: ASTM D 1045	no

GLP compliance

no Lab is not certified

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

commercial product

Results and discussions

Results

Refractive index at 20 deg C is between 1.484 and 1.486

Remarks on results including tables and figures

1.484 and 1.486 are the product specification limits

Applicant's summary and conclusion

Conclusions

Refractive index for di-isononyl phthalate at 20 degrees C is between 1.484 and 1.486.

Executive summary

Refractive index for di-isononyl phthalate at 20 degrees C is between 1.484 and 1.486.

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 Dissemination Dossier

 Phototrans air Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	estimated by calculation	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is 2 because the data are calculated.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Computer model		2008							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed	other guideline: Computer model	

Principles of method if other than guideline

Calculated values using AOPWIN version 1.92, a subroutine of the computer program EPI SuiteTM version 4.0. Indirect photodegradation, or atmospheric oxidation potential, is based on the structure-activity relationship methods developed by R. Atkinson under the following conditions: Temperature: 25°C Sensitizer: OH- radical Concentration of Sensitizer: 1.5E6 OH-radicals/cm³

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

SMILES notation used: CC(CH)CC(CH)CCCOC(=O)c1ccccc1C(=O)OCCCC(CH)CC(CH)C

Study design

Light source

sunlight

Relative light intensity

1

Details on test conditions

Sensitiser (for indirect photolysis): OH Sensitiser concentration: 1500000 molecule/cm³

Results and discussions

Dissipation half-life of parent compound

DT50 5.4 h

Test condition Estimated value

Transformation products

no data

Remarks on results including tables and figures

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Rate constant (for indirect photolysis): $0.0000000000238 \text{ cm}^3/(\text{molecule} \cdot \text{sec})$ Degradation in %
(for indirect photolysis): 50 after 5.36 hour(s) or 0.45 days based on a 12 -hour day

Executive summary

Indirect photochemical degradation of DINP as mediated by OH-attack is estimated to have a half-life of 0.45 days or 5.4 hours based on a 12-hour sunlight day, a rate of $2.38 \times 10^{-11} \text{ cm}^3/\text{molecule} \cdot \text{sec}$, and an average OH- concentration of $1.5 \times 10^6 \text{ OH-}/\text{cm}^3$. A 12-hour day half-life value normalizes degradation to a standard day light period during which hydroxyl radicals needed for photolysis are generated in the atmosphere. Although DINP has the potential to degrade rapidly by OH-attack, multimedia distribution modeling indicates DINP is predicted to partition negligibly (0.1%) to the air compartment because it has a low vapor pressure (0.00006 Pa). Although DINP has a relatively short atmospheric oxidation half-life (5.5 hours), this process is unlikely to contribute significantly to the loss of DINP from the environment.

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 Dissemination Dossier

 Phototrans air Endpoint.002

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	estimated by calculation	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is 2 because the data are calculated.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Computer model		2008							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed	other guideline: Computer model	

Principles of method if other than guideline

Calculated values using AOPWIN version 1.92, a subroutine of the computer program EPI SuiteTM version 4.0. Indirect photodegradation, or atmospheric oxidation potential, is based on the structure-activity relationship methods developed by R. Atkinson under the following conditions: Temperature: 25°C Sensitizer: OH- radical Concentration of Sensitizer: 1.5E6 OH-radicals/cm³

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

SMILES notation used: CC(CH)CCCCCOC(=O)c1ccccc1C(=O)OCCCCCCC(CH)C

Study design

Light source

sunlight

Relative light intensity

1

Details on test conditions

Sensitiser (for indirect photolysis): OH Sensitiser concentration: 1500000 molecule/cm³

Results and discussions

Dissipation half-life of parent compound

DT50 5.5 h

Test condition Estimated value

Transformation products

no data

Remarks on results including tables and figures

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Rate constant (for indirect photolysis): $0.000000000234 \text{ cm}^3/(\text{molecule} \cdot \text{sec})$ Degradation in %
(for indirect photolysis): 50 after 5.49 hour(s) or 0.46 days based on a 12 -hour day

Executive summary

Indirect photochemical degradation of DINP as mediated by OH-attack is estimated to have a half-life of 0.46 days or 5.5 hours based on a 12-hour sunlight day, a rate of $2.38 \times 10^{-11} \text{ cm}^3/\text{molecule} \cdot \text{sec}$, and an average OH- concentration of $1.5 \times 10^6 \text{ OH-}/\text{cm}^3$. A 12-hour day half-life value normalizes degradation to a standard day light period during which hydroxyl radicals needed for photolysis are generated in the atmosphere. Although DINP has the potential to degrade rapidly by OH-attack, multimedia distribution modeling indicates DINP is predicted to partition negligibly (0.1%) to the air compartment because it has a low vapor pressure (0.00006 Pa). Although DINP has a relatively short atmospheric oxidation half-life (5.5 hours), this process is unlikely to contribute significantly to the loss of DINP from the environment.

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 Dissemination Dossier

 Hydrolysis Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussion Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	estimated by calculation	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is 2 because the data are calculated.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other:									
Computer model		2008							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed	other guideline: Computer Model	

Principles of method if other than guideline

Hydrolysis rate calculated by HYDROWIN version 2.00 based on work for EPA by T. Mill et al., a subroutine of the computer program EPI SuiteTM version 4.0.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

no

Details on test material

SMILES Notation used: CC(CH)CCCCCOC(=O)c1ccccc1C(=O)OCCCCCCC(CH)C

Results and discussion

Transformation products

not measured

Dissipation half-life of parent compound

pH	Temp.	Hydrolysis rate constant	Half-life	St. dev.	Type	Remarks (e.g. regression equation, r ² , DT90)
7	25 °C		3.43 yr			
8	25 °C		125.19 d			

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Hydrolysis will not significantly contribute to the removal of DINP from the environment. The DINP hydrolysis half-life (25 degrees C) is calculated as: pH 7 = 3.4 years pH 8 = 125.2 days

Executive summary

Results from the EQC (Equilibrium Criterion) Levels I and III distribution models (Mackay, 2001) show that because of its low water solubility, DINP is not expected to partition to the water compartment. However, abiotic degradation of any trace amounts of DINP which may be present in aquatic environments is unlikely to occur at a significant rate based on modeled data. The HYDROWIN model, a subroutine within the USEPA (2008) computer program, estimates a hydrolysis half-life for DINP of 3.4 years at pH 7 (25°C) and 125.2 days at pH 8 (25°C).

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 Dissemination Dossier

 Hydrolysis Endpoint.002

Administrative Data Data source

Materials and methods

Results and discussion Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	estimated by calculation	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is 2 because the data are calculated.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Computer model		2008							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed	other guideline: Computer Model	

Principles of method if other than guideline

Hydrolysis rate calculated by HYDROWIN version 2.00 based on work for EPA by T. Mill et al., a subroutine of the computer program EPI Suite™ version 4.0.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Radiolabelling

no

Details on test material

SMILES Notation used: CC(CH)CC(CH)CCCOC(=O)c1ccccc1C(=O)OCCCC(CH)CC(CH)C

Results and discussion

Transformation products

not measured

Dissipation half-life of parent compound

pH	Temp.	Hydrolysis rate constant	Half-life	St. dev.	Type	Remarks (e.g. regression equation, r ² , DT90)
7	25 °C		3.43 yr			
8	25 °C		125.19 d			

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Hydrolysis will not significantly contribute to the removal of DINP from the environment. The DINP hydrolysis half-life (25 degrees C) is calculated as: pH 7 = 3.4 years pH 8 = 125.2 days

Executive summary

Results from the EQC (Equilibrium Criterion) Levels I and III distribution models (Mackay, 2001) show that because of its low water solubility, DINP is not expected to partition to the water compartment. However, abiotic degradation of any trace amounts of DINP which may be present in aquatic environments is unlikely to occur at a significant rate based on modeled data. The HYDROWIN model, a subroutine within the USEPA (2008) computer program, estimates a hydrolysis half-life for DINP of 3.4 years at pH 7 (25°C) and 125.2 days at pH 8 (25°C).

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 Phototrans water Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	other: Technical discussion	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the information presents a technical summary rather than the results of a specific study.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Zepp R and Cline D	1977	Rates of direct photolysis in the aqueous environment	Environ. Sci. Technol. 11: 359-366					

Materials and methods

Principles of method if other than guideline

No applicable method: Direct photolysis will not contribute to the degradation of DINP in the aquatic environment because it does not absorb light at wavelengths >290 nm, in the range that contribute to this process.

Test materials

Test material equivalent to submission substance identity
yes

Radiolabelling

no

Results and discussions

Transformation products

no

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

DINP does not absorb light within a range of 290 to 750 nm. Therefore, direct photolysis will not contribute to the degradation of DINP in the aquatic environment because it does not absorb light at wavelengths >290 nm, i.e., in the range that contribute to this process.

Executive summary

Direct photochemical degradation in water occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then, in the resultant excited state, the chemical may undergo a transformation. A prerequisite for direct photodegradation is the ability of one or more bonds within a molecule to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. An approach to assessing the potential for DINP to undergo direct photochemical degradation is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by DINP molecules. DINP does not absorb light within a range of 290 to 750 nm. Therefore, direct photolysis will not contribute to the degradation of DINP in the aquatic environment because it does not absorb light at wavelengths >290 nm, i.e., in the range that contribute to this process.

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 [Phototrans soil Endpoint.001](#)

[Administrative Data](#) [Materials and methods](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	key study		
Study result type	other: Technical discussion	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the information presents a technical summary rather than the results of a specific study.		

Materials and methods

Test materials

Test material equivalent to submission substance identity

yes

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP; CAS #68515-48-0) does not absorb light within a range of 290 to 750 nm. Therefore, direct photolysis will not contribute to the degradation of DINP in the terrestrial environment because it does not absorb light at wavelengths >290 nm, i.e., in the range that contribute to this process.

Executive summary

Direct photochemical degradation in soil occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then, in the resultant excited state, the chemical may undergo a transformation. A prerequisite for direct photodegradation is the ability of one or more bonds within a molecule to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. An approach to assessing the potential for di-isononyl phthalate ester (DINP; CAS #68515-48 -0)to undergo direct photochemical degradation is

to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by DINP molecules. DINP does not absorb light within a range of 290 to 750 nm. Therefore, direct photolysis will not contribute to the degradation of DINP in the terrestrial environment because it does not absorb light at wavelengths >290 nm, i.e., in the range that contribute to this process.

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 Biodeg water screen Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability rating is a 1 because the study followed a standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study followed GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1983							
publication	Sugatt R, O'Grady D, Banerjee S, Howard P and Gledhill W	1984	Shake flask biodegradation of 14 commercial phthalate esters	Applied Environ Micro 47(4), 601-606					

Materials and methods

Test type

other: Simulation

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OPPTS 835.3170 (Shake Flask Die-away Test)	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: technical product

Study design

Oxygen conditions

aerobic

Inoculum or test system

activated sludge, adapted

Initial test substance concentration

Initial conc.
20 mg/L

Based on
test mat.

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
5		CO2 evolution	7 d	
12		CO2 evolution	9 d	
41		CO2 evolution	14 d	
57		CO2 evolution	21 d	
62		CO2 evolution	28 d	

Details on results

Day % Biodegradation 7 5 9 12 14 41 21 57 28 62

BOD5 / COD results

Overall remarks

Primary degradation was also determined based on measured disappearance of test substance. The result was > 99% after 28 days.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Interpretation of results

other: Rapidly biodegradable under acclimated conditions.

Conclusions

DINP is rapidly biodegradable under acclimated conditions.

Executive summary

DINP is rapidly biodegradable under acclimated conditions.

Dissemination Dossier

Biodeg water screen Endpoint.002

Administrative Data**Purpose flag** supporting study**Study result type** read-across from supporting substance (structural analogue or surrogate)**Study period** 1995**Reliability** 1 (reliable without restriction)**Rationale for reliability** Well documented GLP study performed by an accredited laboratory.**Data source****Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company stud
study report		1995						

Materials and methods**Test type**

ready biodegradability

Test guideline**Qualifier****Guideline**

according to

EU Method C.4-C (Determination of the "Ready" Biodegradability - Carbon Dioxide Evolution Test)

GLP compliance

yes (incl. certificate)

Test materials**Test material equivalent to submission substance identity**

no

Details on test material

- Name of test material (as cited in study report): Diisononylphthalat
- Physical state: liquid
- Analytical purity: 99,7 weight%
- Charge-No.: 1286-31619
- Sample-No: 1495/941006
- Stability under test conditions: stable

Study design**Oxygen conditions**

aerobic

Inoculum or test system

activated sludge, domestic, non-adapted

Details on inoculum

- Source of inoculum/activated sludge: municipal sewage plant (Marl-Ost), 17.01.1995
- Preparation of inoculum for exposure: yes, in accordance to instruction
- Concentration of sludge(Inoculum): 20ml Inoculum per sample
- Initial cell/biomass concentration (Inoculum): Bacterial count= 169 * 10000 CFU/ml (Colony Forming Units/ml) //Method: Koch'schen Plattengußverf
- Dry mass content (Inoculum): 4,276 g/L

Duration of test (contact time)

28 d

Initial test substance concentration**Initial conc.**

20.1 mg/L

Based on

test mat.

Parameter followed for biodegradation estimation**Parameter followed for biodegradation estimation**CO₂ evolution**Details on study design****TEST CONDITIONS**

The degradation was reviewed overall (28days) Test-Period on day 0, 2, 6, 9, 14, 20, 23, 28 and 29

- Test temperature: 21,2°C - 22,1°C, Mean 21,8°C
- pH (after 28days): 7,5 - 7,7
- Suspended solids concentration (samples): 28,5mg/L

Biological activity was stopped after the 28th day of incubation. Remaining CO₂ was trapped, too.

Reference substance

benzoic acid, sodium salt

Results and discussions**Test performance**

test fulfills validity criteria

% Degradation of test substance

% Degr.	St. dev.	Parameter	Sampling time	Remarks
81		CO ₂ evolution	28 d	average of two tests

Details on results

Diisononylphthalate displayed a > 60% degradation rate within the 10 day window, which qualifies DINP to be assumed as "readily biodegradable".

BOD₅ / COD results**Overall remarks****Applicant's summary and conclusion****Validity criteria fulfilled**

yes Guideline study under GLP conditions

Interpretation of results

readily biodegradable

Conclusions

DINP must be considered as readily biodegradable, fulfilling the 10 day test criterion as well as exceeding the minimum test requirement of 60 % degr;

Executive summary

DINP must be considered as readily biodegradable, fulfilling the 10 day test criterion , with 81% biodegradation exceeding the minimum test require

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Dissemination Dossier

Biodeg water screen Endpoint.003

Administrative Data

Data source

Materials and methods

Results and discussions

Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability rating is a 1 because the study followed an OECD standard guideline, which describes a procedure designed to evaluate this endpoint. The study was conducted under GLP and the results were reviewed for reliability and assessed as valid.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		2003							
publication	Staples C, Peterson D, Parkerton T and Adams W	1997	The environmental fate of phthalate esters: A literature review	Chemosphere 35(4):667-749					

Materials and methods

Test type

ready biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

The test substance in the Staples paper was an alkyl mono ester of phthalic acid. The mono ester alkyl group distribution of the test substance was approximately a 1:1 ratio of C8 and C10. The test substance was clear and purity ranged between 92 and 94% with the remaining impurities composed of the diester, and the phthalic acid and C8 and C10 alkyl alcohols used in the esterification process. The test substance in the Scholz paper was mono - isononyl phthalate ester

Study design

Oxygen conditions

aerobic

Inoculum or test system

activated sludge, domestic, non-adapted

Duration of test (contact time)

28 d

Parameter followed for biodegradation estimation

Parameter followed for biodegradation estimation
CO2 evolution

Reference substance

other: no data

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
ca. 90		CO2 evolution	28 d	

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Interpretation of results

readily biodegradable

Conclusions

The test substance was readily biodegradable.

Executive summary

Biodegradation results for a mono ester that is a mixture containing approximately equal amounts of monoesters with normal octyl (n-C8) and normal decyl (n-C10) side chains show that this mixture is readily biodegradable. The monoester of DINP, mono-isononyl phthalate (MINP), has been demonstrated to exhibit an equally high extent and rapid rate of biodegradation as was demonstrated by MC8/10PE. The n-C8/n-C10 mono ester as well as the corresponding mono - isononyl phthalate biodegraded to approximately 90% after 28 days and the results met the ready biodegradable criteria.

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 Dissemination Dossier

 Biodeg water screen Endpoint.004

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is a 2 because the study followed a test procedure that was similar to a current standard guideline, which describes a procedure designed to evaluate this endpoint, and the results were reviewed for reliability and assessed as valid.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	O'Grady D, Howard P and Werner A	1985	Activated sludge biodegradation of 12 commercial phthalate esters	Appl. Environ. Microbiol. 49(2): 443-445					
secondary source		1984							1984-12-15

Materials and methods

Test type

other: Simulation study

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	EPA OPPTS 835.5045 (Modified SCAS Test for Insoluble and Volatile Chemicals)	

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Diisononyl phthalate ester with unknown CAS number. Substance type: commercial product
Analytical purity: unknown Equal proportion blend of DINP supplied by different US manufacturers

Study design

Oxygen conditions

aerobic

Inoculum or test system

activated sludge, adapted

Details on inoculum

The acclimation period was 3 weeks. (These results were achieved after a 21-day draw and fill procedure.)

Initial test substance concentration

Initial conc.
1 mg/L

Based on
test mat.

Parameter followed for biodegradation estimation

Parameter followed for biodegradation estimation
Test mat. analysis

Details on study design

Daily operation of the semi-continuous activated sludge (SCAS) unit was conducted according to the Soap and Detergent Association procedure except the total volume was 2 liters instead of 1.5 liters. After the sludge was acclimated to the synthetic sewage feed and the dissolved organic carbon removal efficiency remained at >70%, the test substance feeding began. Once feeding began, a draw-and-fill procedure was started and maintained for 3 weeks. The test substance was added to the test system in an acetone stock solution to provide a concentration of 1 mg/L during week 1 and 3 mg/L during weeks 2 and 3. 24-hour test substance biodegradation was determined once a week during the 3-week draw-and-fill period, in the middle of each week by analyzing a 50 ml aliquot for each unit after feeding of test substance, synthetic sludge, and followed by a 23-hour aeration period.

Reference substance

ethylene glycol

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
68		Test mat. analysis	24 h	

BOD5 / COD results

Results with reference substance

No information was provided on the performance of the reference substance.

Overall remarks

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Interpretation of results

other: Rapidly biodegradable under acclimated conditions.

Conclusions

Di-isononyl phthalate ester can biodegrade rapidly under wastewater treatment simulation conditions, based on a 68% loss of test substance after 24 hours in a semi-continuous activated sludge test system.

Executive summary

Di-isononyl phthalate ester can biodegrade rapidly under wastewater treatment simulation conditions, based on a 68% loss of test substance after 24 hours in a semi-continuous activated sludge test system.

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Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	January 1995
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is a 2 because the study followed an OECD standard guideline, which describes a procedure designed to evaluate this endpoint, and the results were reviewed for reliability and assessed as valid. However, the study was not conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1995							1995-05-19

Materials and methods

Test type

ready biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test)	no

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: technical product

Study design

Oxygen conditions

aerobic

Inoculum or test system

activated sludge, domestic, non-adapted

Details on inoculum

Fresh activated sludge was used as the inoculum. The activated sludge was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, New Jersey. This treatment facility was selected because it deals predominantly with domestic sewage as specified in the guideline. There were no known contaminants in the fresh activated sludge believed to be present at levels high enough to interfere with this study. Fresh activated sludge was obtained on day -1 of the test. Duplicate 10 mL aliquots of the activated sludge were filtered through pre weighed Whatman 934-AH filter pads in a Buchner funnel and vacuum flask set up. The filter pads were placed in an aluminum pan and dried in an oven for ninety minutes at 103 degree C. After cooling the filters were reweighed and the mean total suspended solids concentration was determined to be 3.61 g/L. The activated sludge was then homogenized in a blender for two minutes at low speed. The homogenated sample was allowed to settle for sixty minutes, after which the supernatant was decanted (avoiding carry-over of sludge solids). An aliquot of the supernatant was used to determine microbial activity. The microbial activity was determined using an Easicult®-TTC dip slide. The agar stick was removed from the culturing tube and the agar dipped into the supernatant aliquot. Excess supernatant was blotted off with a clean paper towel, and the agar stick was then placed back into the culture tube. The whole unit was placed into a dark environmental chamber for 48 hours at 20 +/- 1 degree C monitored by Aquatic Toxicology Data Systems (ATDS). Based on comparison of the density of colonies growing on the agar with the model density chart provided by the supplier, the microbial activity was determined to be 10E5 CFU/mL. The remaining decanted sludge supernatant was used for final preparation of the test medium on day -1.

Duration of test (contact time)

28 d

Initial test substance concentration

Initial conc.
ca. 50 mg/L

Based on
test mat.

Parameter followed for biodegradation estimation

Parameter followed for biodegradation estimation
O₂ consumption

Details on study design

Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance. The empirical formula and the theoretical oxygen demand (ThOD) of the test substance were calculated from elemental analysis data (assuming 100 gram test substance). Sodium benzoate ThOD was calculated using the empirical formula and was determined to be 1.67 mg O₂/mg sodium benzoate. The ThOD calculation of the test and positive control substance was based on Annex IV of OECD 301F1 guideline. Activated sludge and test medium were combined prior to test substance addition. Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride). Test vessels were 1L glass flasks placed in a water bath and electronically monitored for oxygen consumption. Test substance was tested in triplicate, controls and blanks were tested in duplicate. Test substance (1,2-benzenedicarboxylic acid, diiso-C10 alkyl esters) concentration was approximately 50 mg/L. The positive control (sodium benzoate) concentration was approximately 50 mg/L. Test temperature was 22 +/- 1 Deg C. All test vessels were stirred constantly for 28 days using magnetic stir bars.

Reference substance

benzoic acid, sodium salt

Results and discussions

Test performance

By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. Oxygen uptake in the blanks was within guideline limits. No excursions from the protocol were noted. Positive Reference Substance (Na Benzoate): Day % Degradation* 1 32, 36 2 56, 66 5 76, 89, 82 * replicate data

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
7.77		O2 consumption	11 d	
14.8		O2 consumption	12 d	
50.5		O2 consumption	18 d	
61.2		O2 consumption	22 d	
70.5		O2 consumption	28 d	

Details on results

The biodegradation half-life <3 weeks. By day 28, 70.5% biodegradation of the test substance was observed. 10% biodegradation was achieved on approximately day 12 and 60% biodegradation on approximately day 22.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Interpretation of results

readily biodegradable

Conclusions

The test substance is readily biodegradable, and biodegrades to a high extent, 70.5% after 28 days.

Executive summary

The test substance is readily biodegradable, and biodegrades to a high extent, 70.5% after 28 days.

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 Biodeg water sim Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is a 2 because, although the data were developed using a non standard test procedure, the information is well documented and the testing followed accepted scientific procedures		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		2008							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Sphingobium chungbukense was grown in a mineral salts medium (pH 7.0, 30 degrees C, and stirring at 200 rpm). The maximum specific rate of DINP degradation was determined by using GS/MS.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Radiolabelling

no

Details on test material

>99% Sigma-Aldrich, USA

Study design

Oxygen conditions

aerobic

Inoculum or test system

other: Sediment microorganism: *S. chungbukense*

Details on source and properties of surface water

mineral salt medium (MSM) containing the following: (g L⁻¹) K₂HPO₄, 1.6; KH₂PO₄, 0.2; (NH₄)₂SO₄, 1.0; Mg SO₄ 7H₂O, 0.2; NaCl, 0.1; CaCl₂ 2H₂O, 0.02; FeSO₄ H₂O, 0.01; Na₂MoO₄ 2H₂O, 0.05; MnSO₄ H₂O, 0.05; and Na₂WO₄ 2H₂O

Details on inoculum

Sphingobium chungbukense KCTC 2955, originally isolated from sediment

Duration of test (contact time)

> 3 — < 10 d

Initial test substance concentration

Initial conc.

> 50 — < 500 mg/L

Based on

test mat.

Parameter followed for biodegradation estimation

Test mat. analysis

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
ca. 100	2.1	Test mat. analysis	3 d	

Transformation products

yes

Identity of degradation products

No.	Identifier	Identity
#1	Common name	monoisononyl phthalate (MINP)
#2	Common name	phthalic acid (PA)
#3	Common name	isononyl alcohol (INA)
#4	Common name	1,3-isobenzofurandione (IBF)

Details on transformation products

MINP and PA were formed rapidly as a major product in the initial 24–30 h but their concentration decreased progressively. In this study, small amounts of isononyl alcohol (INA) and 1,3-isobenzofurandione (IBF) were also produced during degradation; they were completely degraded after 60 h. These results suggest that *S. chungbukense* has the potential to remove DINP from the environmental without producing toxic by-products.

Evaporation of parent compound

not measured

Volatile metabolites

not measured

Residues

not measured

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Microorganisms originally isolated from sediment are capable of degrading di-isononyl phthalate (DINP) to a great extent in isolated culture in the laboratory.

Executive summary

Microorganisms originally isolated from sediment are capable of degrading di-isononyl phthalate (DINP) to a great extent in isolated culture in the laboratory. These bacteria may have the same capability to degrade DINP in their natural sediment environment.

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 Biodeg water sim Endpoint.002

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Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Value cited in the European Commission risk assessment for 1,2-benzenedicarboxylic acid, di-C8-10- branched alkyl esters, C9-rich, CAS#: 68515-48 -0, EINECS#: 271-090 -0, and:di-"isononyl" phthalate (DINP), CAS#: 28553 -12 -0, EINECS#: 249-079-5.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: PhD dissertation	Furtman K	1993	Phthalates in the aquatic environment	Regional Water and Wastewater Authority, Nordrhein-Westfalen, Germany					
publication		2003							

Materials and methods

Test guideline

Qualifier

Guideline

Deviations

no guideline available

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

no

Study design

Oxygen conditions

aerobic

Inoculum or test system

other: Treated wastewater

Duration of test (contact time)

7 d

Initial test substance concentration

Initial conc.

3 µg/L

Based on

test mat.

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
91		Test mat. analysis	7 d	

Half-life of parent compound / 50% disappearance time (DT50)

Compartment	Half-life	St. dev.	Type	Remarks (e.g. regr. equ., r^2 , DT90)
other: Wastewater	ca. 1 d		(pseudo-)first order (= DT50)	

Evaporation of parent compound

not measured

Volatile metabolites

not measured

Residues

not measured

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-n-nonyl phthalate degraded 91% after 7 days incubation with wastewater at 25 degrees C.

Executive summary

Di-n-nonyl phthalate degraded 91% after 7 days incubation with wastewater at 25 degrees C.

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 Biodeg water sim Endpoint.003

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is a 2 because, although the data were developed using a non standard test procedure, the information is well documented and the testing followed accepted scientific procedures.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Otton V, Sura S, Blair J, Ikononou M and Gobas F	2008	Biodegradation of mono-alkyl phthalate esters in natural sediments	Chemosphere					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed	other guideline: Followed sound scientific testing principles for determining biodegradability in sediment.	

Principles of method if other than guideline

Aerobic biodegradation in natural sediment as determined in closed glass test systems and based

on analytical measurements of the parent test substance over time.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

no

Details on test material

The test substance was synthesized by Chemsyn Science Laboratories (Lenexa, KS) and had 97–99% purity as esters as determined by HPLC. The test substance was produced from an isomeric mixture of isononyl alcohols.

Study design

Oxygen conditions

aerobic

Inoculum or test system

natural sediment

Details on source and properties of sediment

Surface sediment samples were collected using a petit ponar grab sampler from two locations in False Creek (called ‘North Central’ and ‘Marina South’ in Mackintosh et al., 2004), an urbanized marine inlet in Vancouver. The top layer (0.5–1.0 cm) of each grab sample was transferred to cleaned 250 ml glass jars with foil-lined lids. The filled jars were immediately placed on ice for transport to the laboratory. Sediments were either used immediately or were stored at –20 °C in the dark until use. The pH of the sediments was measured in the field using pHDrion pH-indicator strips. Frozen sediments were thawed at room temperature and any pebbles or vegetative material were removed. Autoclaved sediments were used as controls to measure any degradation or loss of chemical not due to microbial degradation. They were prepared by autoclaving three consecutive times at 120 °C for 20 min, followed by 24 h cooling periods at 22 ± 1 °C. The total organic carbon content and moisture content of the sediments were measured at

the Institute of Ocean Sciences (Sidney, BC) using a Control Equipment Corporation 440 Elemental Analyzer according to Van Iperen and Helder, 1985 J. Estimates of the number of bacteria culturable on agar under aerobic conditions were obtained using EasiCult® dip-slides (Orion Diagnostica, Espoo, Finland). The agar dipslides were inoculated by dipping into a 1:2000 dilution of sediment with sterilized milli-Q water. The organic carbon content of the sediment was $2.90 \pm 0.17\%$ (mean $\pm$ SD, $n = 4$) for the marine sediments from False Creek. The pH of the sediment was 6 ± 0.2 . The number of culturable bacteria was high in marine sediment samples (i.e. $>10^8$ g⁻¹ sediment, wet weight). The agar slides inoculated with autoclaved marine or freshwater sediment were blank, verifying the effectiveness of the sterilization procedure.

Details on inoculum

The inoculum consisted of the natural microorganisms found in the sediment samples. No outside source of bacteria were added.

Duration of test (contact time)

ca. 72 h

Initial test substance concentration

Initial conc.
ca. 2 μ g/g sediment (wet weight)

Based on
test mat.

Details on study design

Sediment samples (4.0 g wet weight) were transferred to solvent-rinsed 20 ml glass scintillation vials. Autoclaved sediment samples were used as a control to determine loss of the mono phthalate ester (MPE) by processes other than biotic. A small volume (8 μ l) of a 1 g l⁻¹ MPE solution in acetonitrile was added to the sediment slurry and gently mixed on a vortex mixer. Sediments were exposed to the MPE in the dark. The final concentration of the MPE was approximately 2 μ g g⁻¹ sediment (wet weight). The incubation vials were capped with foil-lined caps and wrapped in foil to eliminate the potential of photolysis. The proportion of headspace air-to-sediment at the beginning of the incubation was 4.5:1, based on the volume of sediment in the scintillation vial. The spiked sediments were incubated in triplicate at 22 ± 1 °C and at 5 ± 1 °C. At various time points, 0.5 g subsamples were removed and transferred to clean glass scintillation vials for analysis. A 10 ml volume of acetonitrile was added to 0.5 g subsamples to stop biodegradation. The sediments were not agitated or actively oxygenated during the incubations, except when removing subsamples. Incubation experiments were repeated up to nine times.

Results and discussions

Test performance

After an initial lag phase, MiNP concentration followed an exponential decay pattern during incubation, while no significant change in concentration was observed in treatments with autoclaved sediments. This indicates that microbial activity was responsible for the observed degradation of the MINP. The lag phase varied from 20 to 70 h among individual incubations. After the initial lag phase, MiNP concentration in sediments dropped quickly over time. First-order biodegradation half-life for MiNP was 23 ± 5 h based on 4 replicates.

Half-life of parent compound / 50% disappearance time (DT50)

Compartment	Half-life	St. dev.	Type	Remarks (e.g. regr. equ., r^2 , DT90)
sediment	23 h	5	(pseudo-)first order (= DT50)	

Other kinetic parameters

first order rate constant The kinetics of biodegradation were determined from linear regression of the slope (after the lag phase) on a plot of the logarithm of concentration in subsamples of the sediment incubation versus time.

Transformation products

yes

Identity of degradation products

No.	Identifier	Identity
#1	Common name	Phthalic Acid
#2	Common name	Isononyl Alcohol

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The mono ester of di-isononyl phthalate (mono isononyl phthalate) exhibited a relatively rapid rate of biodegradation in a natural marine sediment, with a measured half-life of 23 ± 5 hours, based on the results of four replicates.

Executive summary

The mono ester of di-isononyl phthalate (mono isononyl phthalate) was found to biodegrade at a relatively rapid rate in a natural marine sediment, with a measured half-life of 23 ± 5 hours, based on the results of four replicates. The investigators also evaluated the biodegradability of other mono phthalate esters (MPEs) in marine sediment and some MPEs in freshwater sediments and found that, in general, all the MPEs exhibited relatively similar half-lives in marine and freshwater sediment, ranging from 16 to 39 hours. Although the biodegradability of mono isononyl phthalate was not evaluated in freshwater sediment, based on biodegradation data for other MPEs in both sediments, mono isononyl phthalate would also be expected to exhibit a half-life in freshwater sediment equivalent to its half-life in marine sediment.

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Administrative Data

Data waiving study scientifically unjustified

Applicant's summary and conclusion

Executive summary

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 Bioaccumulation Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is a 2 because, although the data were developed using a test procedure that does not have an established formal test guideline, the information is well documented and the testing followed accepted scientific procedures.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Mackintosh C, Maldonado J, Hongwu J, Hoover N, Chong A, Ikonomou M and Gobas F	2004	Distribution of phthalate esters in a marine aquatic food-web: Comparison to polychlorinated biphenyls	Environ Sci Technol 38, 2011-2020					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Various marine species were field collected and their tissues analyzed for phthalate diesters including diisononyl phthalate ester congeners. The sum of diisononyl phthalate ester congeners analyzed in tissue from each species was used to define the food-web and calculate food-web magnification factors.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

no

Details on test material

The field study analyzed sample tissues for phthalate diesters including diisononyl phthalate ester congeners.

Test organisms

Details on test organisms

18 Species were field collected including: Green Algae - *Enteromorpha intestinalis* Brown Algae - *Nereocystis leutkeana*; *Fucus gardneri* Plankton - various species Blue Mussel - *Mytilus edulis* Pacific Oyster - *Crassostrea gigas* Geoduck Clam - *Panope abrupta* Manila Clam - *Tapes philippinarum* Dungeness Crab - *Cancer magister* Purple Seastar - *Pisaster ochraceus* Juv. Shiner Perch - *Cymatogaster aggregata* Pacific Herring - *Clupea harengus* Pile Perch - *Rhacochilus vacca* Striped Sea Perch - *Embiotoca lateralis* Pacific Staghorn Sculpin - *Leptocottus armatus* English Sole - *Pleuronectes ventulus* Whitespotted Greenling - *Hexagrammos stelleri* Spiny Dogfish - *Squalus acanthias* Surf Scoter - *Melanitta perspicillata*

Study design

Route of exposure

feed

Test type

field study

Water media type

saltwater

Test conditions

Reference substance (positive control)

yes polychlorinated biphenyl congeners

Results and discussions

Bioaccumulation factor

Conc. in environment / dose	Type	Value	Time Basis of plateau	Calculation basis	Remarks
	other: Food Web Magnification Factor	0.46			A FWMF that is greater than 1.0 is an indication of chemical biomagnification within a food web, whereas a value of less than 1.0 indicates biodilution or dilution from lower to higher trophic levels.

Details on results

Isotope analysis revealed a general pattern of enrichment of analyzed isotopes beginning with the sediment and moving through the food web to plankton, macroalgae and bivalves, small forage fish, large fish, marine birds, dungeness crabs, and dogfish. There was a 3-4% increase on average for each succeeding trophic level. Which illustrates that the food web investigated is sufficiently long to test for the occurrence of biomagnification. Concentrations of twenty target PCB (polychlorinated biphenyl) congeners and thirteen phthalate diesters were detected at levels above the MDL (minimum detection limit) in the majority of biota species. The lipid equivalent concentrations of the high molecular phthalate esters (DEHP [diethylhexyl phthalate], DnOP [di-n-octyl phthalate], C8 [di-iso-octyl phthalate], DnNP [di-n-nonyl phthalate], C9 [di-iso-nonyl phthalate], and C10 [di-iso-decyl phthalate]) significantly declined with increasing trophic position and isotope levels in the food-web. FWMF (food web magnification factor) values for the PCBs ranged from 1.7 to 8.8, indicating biomagnification. FWMF values for the high molecular weight phthalate esters (C8 to C10) ranged from 0.28 to 0.50, indicating that these phthalate diesters did not biomagnify in the aquatic food web studied.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The data from this food-web study show that diisononyl phthalate ester did not biomagnify through a food-web that included 18 species, but rather decreased in tissue concentration with increasing trophic position.

Executive summary

The authors concluded that high molecular weight phthalate diesters, including DINP, do not biomagnify through the food-web, but rather decrease in tissue concentration with increasing trophic position. Decreasing concentrations, also referred to as biodilution, can be quantified by food-web magnification factors (FWMFs). A FWMF that is greater than 1.0 is an indication of chemical biomagnification within a food web, whereas a value of less than 1.0 indicates biodilution or dilution from lower to higher trophic levels. The authors showed that lipid equivalent concentrations of the high molecular weight phthalate diesters significantly declined with increasing trophic level and that FWMFs ranged from 0.29 for DEHP, a C8 PE, to 0.50 for a di-iso-decyl phthalate ester. The FWMF for DINP was 0.46.

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Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is a 2 because although the study followed a standard guideline, which describes a procedure designed to evaluate this endpoint, the study did not follow GLP, there were limited data on water quality parameters, and the reported exposure concentration was at a level above the test substance water solubility, which prevents calculation of an accurate BCF value.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: publication; handbook	Chemicals Inspection & Testing Institute	1992	Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan	Japan Chemical Industry Ecology-Toxicology & Information Center, Japan; pp. 3-91					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according	OECD Guideline 305 C (Bioaccumulation: Test for the Degree of	

to Bioconcentration in Fish)

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

no

Test organisms

Test organisms (species)

Cyprinus carpio

Details on test organisms

Fish weight approximately 30 grams; fish length approximately 10 cm.

Study design

Route of exposure

aqueous

Water media type

freshwater

Total exposure / uptake duration

56 d

Test conditions

Nominal and measured concentrations

0.1 mg/L nominal concentration

Results and discussions

Lipid content

≥ 2 — ≤ 6 %

Time point

Remarks

Bioaccumulation factor

Conc. in environment / dose	Type	Value	Basis	Time of plateau	Calculation basis	Remarks
		BCF	< 14.4	no data		

Depuration

Elimination	Endpoint	Depuration time (DT)
no		

Details on results

BCF = <14.4; log BCF = <1.16

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

1,2-Benzenedicarboxylic acid, diisodecyl ester, has a low potential to bioconcentrate based on a measured BCF of <14.4 from an exposure concentration of 0.1 mg/L.

Executive summary

1,2-Benzenedicarboxylic acid, diisodecyl ester, has a low potential to bioconcentrate based on a measured BCF of <14.4. As an analog to DINP, these data suggest that DINP will also exhibit an equally low potential to bioconcentrate.

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 Bioaccumulation Endpoint.003

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	3 (not reliable)		
Rationale for reliability	Test procedure analyses do not accurately measure parent test substance concentrations and the exposure solutions allow for excess test material to interfere with quantifying the amount of test material in the organisms.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Brown D and Thompson R	1982	Phthalates and the aquatic environment: Part II. The bioconcentration and depuration of di-2-ethylhexyl phthalate (DEHP) and di-isodecyl phthalate (DIDP) in mussels (Mytilus edulis)	Chemosphere 11(4): 427-435					

Materials and methods

GLP compliance

no

Test materials**Test material equivalent to submission substance identity**

no

Radiolabelling

yes

Details on test material

The ¹⁴C samples of di-isodecyl phthalate ester (DIDP) were synthesised from phthalic anhydride, labelled in the benzene ring, and an isodecyl alcohol. The chemical and radiochemical purity was found to be >97.5% as checked by GLC using two different columns. The sample was supplied in acetone solution at specific activity of 4.7 mCi/g. An accurate CAS and EC number cannot be identified because the synthesis is not equivalent to standard manufacturing processes for DIDP.

Details on sampling

Five mussels from each test vessel were sacrificed for analysis at intervals throughout the 28 day bioconcentration phase on days 1, 3, 7, 14, 21, 24, and 28 and on days 29, 31, 35, and 42 during the 14 day depuration phase.

Vehicle

yes

Test organisms**Test organisms (species)**

other: *Mytilus edulis*

Details on test organisms

Mytilus edulis were collected locally, to Devon, England, and 30 randomly selected mussels from those collected had a mean shell length (30 mussels) of 22.6 mm (range 20-28 mm) and 15 randomly selected mussels had a mean wet tissue weight of 472 mg (range 274-772 mg).

Study design

Route of exposure

aqueous

Test type

flow-through

Water media type

saltwater

Total exposure / uptake duration

28 d

Total depuration duration

14 d

Test conditions

Hardness

Not provided

Test temperature

15 +/- 1 degrees C

pH

Not provided

Dissolved oxygen

Not provided

TOC

Not provided

Salinity

Not provided

Nominal and measured concentrations

Nominal concentrations were 5 and 50 ug/L, which measured 4.4 and 41.7 ug/L as determined by total radioactivity.

Details on test conditions

Groups of 80 mussels were exposed for 28 days to each of the following nominal concentrations: DIDP at 5 ug/l and 50 ug/l, and a solvent control (0.5 ml acetone/litre). Following this bioconcentration phase, the mussels were maintained in clean seawater for a depuration period of 14 days. The test vessels were glass reinforced plastic tanks with a 35 L capacity, each receiving a continuous supply of filtered (5 µm) seawater at a flow rate of 500 ml/min, and at a temperature controlled to 15 +/- 1°C. The mussels were fed by continuous addition of a cultured unicellular alga (*Platlnnonas suecica*) to the dilution water, to provide approximately 2000 algae/ml in the supply to each vessel. The phthalates were prepared as stock solutions in acetone at 2000 times the required exposure level using the appropriate 14C labelled sample for the 5 g/l levels, and a mixture of 1 part 14C labelled phthalate and 9 parts non-labelled material for the 50 ug/l levels. The stock solutions were delivered by peristaltic pump to a stirred mixing chamber to mix with the seawater immediately prior to delivery to the test vessel. Acetone alone was similarly added to the supply to the solvent control, all vessels having a nominal acetone concentration of 0.5 ml/L.

Reference substance (positive control)

no

Details on estimation of bioconcentration

Calculations were made using total radioactivity.

Results and discussions

Lipid content

Time point

Remarks Not measured

Metabolites

Metabolites were not measured.

Results with reference substance (positive control)

A reference substance was not tested.

Details on results

Estimated plateau levels and bioconcentration factors for DIDP during 28-day uptake phase:
Estimated Mean Plateau Concentration Level Bioconcentration Mean Bioconcentration (ug/L)
(ug/mg) at Plateau at Plateau 4.4 17.5 3977 3488 41.7 125 2998

Remarks on results including tables and figures

Non-quantitative observations were made at intervals during the study of the deposition of faecal/pseudofaecal material in the test vessels, of byssal thread attachment, and of the general appearance and activity of the mussels. There was no evidence of any adverse effects on the mussels, and all groups appeared to be actively feeding throughout the study. The mussels were also examined daily for mortalities, defined as mussels displaying an abnormal degree of shell opening and subsequent inability to close the shell within 1 minute of removal from the test vessels. Throughout the test period there was 1 death in the solvent control.

Applicant's summary and conclusion

Validity criteria fulfilled

no The study is not valid due to an inaccurate calculation of BCF.

Conclusions

The exposure of the mussel, *Mytilus edulis*, to C14 radiolabelled DIDP led to a plateau level of radioactive residues corresponding to a mean bioconcentration factor (BCF) of approximately 3,500. Additionally, these residues were rapidly lost from the mussels (T1/2 was approximately 31/2 days) and the 28 day exposure period to nominal levels of 5 ug/l and 50 ug/l of DIDP produced no apparent adverse effects. However, the BCF was calculated based on total radioactivity and consequently the reported BCF value is not valid because total activity does not differentiate between the parent test substance and metabolites.

Executive summary

The exposure of the mussel, *Mytilus edulis*, to C14 radiolabelled DIDP led to a plateau level of radioactive residues corresponding to a mean bioconcentration factor (BCF) of approximately 3,500. Additionally, these residues were rapidly lost from the mussels (T1/2 was approximately 31/2 days) and the 28 day exposure period to nominal levels of 5 ug/l and 50 ug/l of DIDP produced no apparent adverse effects. However, the BCF was calculated based on total radioactivity and consequently the reported BCF value is not valid because total activity does not differentiate between the parent test substance and

metabolites.

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[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	4 (not assignable)		
Rationale for reliability	The reliability rating is a 4 because although the study followed a standard guideline, which describes a procedure designed to evaluate this endpoint, the study did not follow GLP, there were limited data on water quality parameters, and the reported exposure concentration was at a level above the test substance water solubility, which prevents calculation of an accurate BCF value.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: publication; handbook	Chemical Inspection & Testing Institute	1992	Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan	Japan Chemical Industry Ecology-Toxicology & Information Center, Japan; pp. 3-91					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according	OECD Guideline 305 C (Bioaccumulation: Test for the Degree of	

to Bioconcentration in Fish)

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

no

Test organisms

Test organisms (species)

Cyprinus carpio

Details on test organisms

Fish weight approximately 30 grams; fish length approximately 10 cm.

Study design

Route of exposure

aqueous

Water media type

freshwater

Total exposure / uptake duration

56 d

Test conditions

Nominal and measured concentrations

1 mg/L nominal concentration

Results and discussions

Lipid content

≥ 2 — ≤ 6 %

Time point

Remarks

Bioaccumulation factor

Conc. in environment / dose	Type	Value	Basis	Time of plateau	Calculation basis	Remarks
	BCF	< 3.6	no data			

Depuration

Elimination

Endpoint

Depuration time (DT)

no

Details on results

BCF = <3.6; log BCF = <0.56

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

1,2-Benzenedicarboxylic acid, diisodecyl ester, has a low potential to bioconcentrate based on a measured BCF of <3.6 from an exposure concentration of 1 mg/L.

Executive summary

1,2-Benzenedicarboxylic acid, diisodecyl ester, has a low potential to bioconcentrate based on a measured BCF of <3.6. As an analog to DINP, these data suggest that DINP will also exhibit an equally low potential to bioconcentrate.

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 Dissemination Dossier

 Bioaccumulation Endpoint.005

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	other: summary	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the information presents the summary of an evaluation of data for a group of phthalate diesters rather than the results of a specific study.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Gobas F, Mackintosh C, Webster G, Ikonomou M, Parkerton T and Robillard K	2003	Bioaccumulation of Phthalate Esters in Aquatic Food-Webs	In: Phthalate Esters. Staples C (Ed.) Springer-Verlag, Berlin, Germany					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline required		

Principles of method if other than guideline

Organisms from various trophic levels in sediment and water were taken from sampling sites in False Creek Harbor, Vancouver, BC, Canada and examined for high molecular weight phthalate ester content.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Various phthalate diesters

Results and discussions

Bioaccumulation factor

Conc. in environment / dose	Type	Value	Basis	Time of plateau	Calculation basis	Remarks
						A compilation of laboratory and field studies support the conclusion that phthalate esters do not appear to be bioaccumulative.

Applicant's summary and conclusion

Validity criteria fulfilled

not applicable Summary from book chapter titled Bioaccumulation of Phthalate Esters in Aquatic Food-Webs.

Executive summary

Organisms from various trophic levels in sediment and water samples taken from various sampling sites in False Creek Harbor, Vancouver, BC, Canada, were examined for high molecular weight phthalate ester content. The conclusions on high molecular weight phthalate ester biomagnification from this study are summarized in the following abstract taken from a book chapter titled Bioaccumulation of Phthalate Esters in Aquatic Food-Webs.

This chapter explores the bioaccumulation behavior of several phthalate esters in aquatic food-webs. It includes (i) a compilation of bioconcentration data from reported laboratory studies in the literature, (ii) an overview and discussion of the results from a recently completed food-web bioaccumulation field study and (iii) an analysis of the results of a bioaccumulation modeling study. The study concludes that laboratory and field studies indicate that phthalate esters do not biomagnify in aquatic food-webs. Higher molecular weight phthalate esters (DEHP [diethylhexyl phthalate], DnOP [di-n-octyl phthalate] and DnNP [di-n-nonyl phthalate]) show evidence of trophic dilution in aquatic food-webs, which is consistent with findings from laboratory studies and modeling studies which indicate that metabolic transformation is a key mitigating factor. Bioaccumulation patterns of DBP [dibutyl phthalate], DiBP [diisobutyl phthalate] and BBP [butyl benzyl phthalate] indicate no significant relationship with trophic position consistent with a lipid-water partitioning model. The lowest molecular weight phthalate esters (DMP [dimethyl phthalate] and DEP [diethyl phthalate]) show bioaccumulation factors in laboratory and field studies that are greater than predicted from a lipid-water partitioning model. The considerable variability in the field-derived bioaccumulation factors (BAFs) for lower molecular weight phthalate esters across aquatic species suggests that species-specific differences in metabolic transformation can have a significant effect on observed bioaccumulation. With some exceptions discussed below, the bioconcentration and bioaccumulation factors of the phthalate esters, discussed in this paper, are below the UNEP [United Nations Environment Programme] bioaccumulation criterion of 5,000. The low bioavailability of the high molecular weight phthalate esters in natural waters is the main reason why the BAFs of the higher molecular weight phthalate esters are below the UNEP bioaccumulation criterion. Since the intention of the bioaccumulation criteria is to identify substances as being "bioaccumulative" if they (like PCBs [polychlorinated biphenyls]) biomagnify in the food-web, current evidence supports the conclusion that phthalate esters do not appear to be "bioaccumulative".

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 Dissemination Dossier

 Bioaccumulation Endpoint.006

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	August - September 2000
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is a 2 because, although the data were developed using a non standard test procedure, the information is well documented and the testing followed accepted scientific procedures.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		2002							2002-07-22

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		no

Principles of method if other than guideline

This study was performed to determine the elimination rate constant for the test substance from rainbow trout (*Oncorhynchus mykiss*) tissue. The test substance was administered to the test system via the diet. The low water solubility and high Kow of DINP prevent conducting an aqueous exposure BCF study.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Radiolabelling

no

Details on test material

Substance type: commercial product

Details on sampling

Fish samples were collected from each treatment and the control on days 7 and 14 of the uptake phase and on days 0, 1, 3, and 8 of the depuration phase. Day 0 depuration samples were removed approximately 5 hours after the beginning of depuration. Ten fish were removed from each tank at each sampling interval with the exception of day 7 of the uptake phase. Only four fish were removed at this sample interval.

Vehicle

yes

Details on preparation of test solutions or sediment

As this was a dietary study, the vehicle was Salmon Starter obtained from Zeigler Bros., Inc., Gardners, PA, USA. A semi-static exposure system was constructed to provide a sufficient volume of test water to the test tanks. The test water was delivered to the test chambers at a rate of approximately 56 ml/min using a peristaltic pump. This allowed for at least five volume replacements through each test chamber per day. The appropriate amount of the test substance was added to the test feed to achieve a nominal concentrations of 1200 ppm. Untreated food was fed to the control fish. The treated and untreated diets were measured daily and fed to the fish as a single feeding. After 14 days of exposure to the treated feed, the fish were transferred to clean tanks and fed untreated food for 8 days. Transfer of the fish, treated and untreated, marked the beginning of the depuration phase.

Test organisms

Test organisms (species)

Oncorhynchus mykiss

Details on test organisms

The test organisms were supplied by Pierce Associates, Inc., West Buxton, ME, USA. The test organisms were quarantined and observed for parasites and disease for 14 days prior to use in the test, and were held in test water at approximately 15 degrees C, that was continuously aerated to provide a dissolved oxygen concentration of at least 80% of the air saturation value. Fish were not treated for disease or parasites before use in this study. Fish were held under static conditions using biological and mechanical filtration, and were fed Salmon Starter and Artemia nauplii (<24 hours old). Test organism loading at test initiation was 0.32 grams of fish per liter; 55 organisms were used in the control system and 55 organisms in the treatment system. Length and weight measurements of a subsample of the stock population were recorded prior to the start of the study to estimate a mean organism weight. Length and weight measurements were also recorded on fish removed at each sampling period. Total length was measured in all instances.

Study design

Route of exposure

feed

Test type

semi-static

Water media type

freshwater

Total exposure / uptake duration

14 d

Total depuration duration

8 d

Test conditions

Hardness

Hardness as CaCO₃ (mg/L) in water batches used during the test ranged from 100 to 122 with one value as low as 96. Water quality results for one of the water batches had one hardness value outside of the recommended range. Because there was no mortality in the study, those waters are

not believed to have adversely affected the study.

Test temperature

Parameter Test Day Control Treated Diet (°C) 0 14.9 14.9 2 14.0 14.0 7 14.3 14.2 9 13.8 13.7 14
14.7 14.6 2 dep 14.1 14.0 8 dep 14.2 14.1

pH

Parameter Test Day Control Treated Diet pH 0 7.5 7.5 2 6.8 6.8 7 6.9 7.0 9 6.9 6.9 14 6.7 6.7 2
dep 7.1 7.1 8 dep 7.6 7.6

Dissolved oxygen

Dissolved oxygen concentrations did not fall below 60% saturation. Parameter Test Day Control
Treated Diet (mg/L) 0 9.4 9.4 2 7.2 7.2 7 8.1 7.7 9 7.0 6.4 14 6.7 6.2 2 dep 8.1 7.9 8 dep 8.6 8.1

TOC

TOC in water batches used during the test were all <0.1 ppm.

Nominal and measured concentrations

The nominal diet loading was 200 mg/kg feed. Analyses of feed for the uptake phase of the test
was as follows: Pre-Study Day 14 Control ND ND Treated 187 177 Feed 185 178 186 Mean 186
178

Details on test conditions

The test water is prepared from UV-sterilized, deionized well water that is treated and distributed throughout the testing facility via PVC and stainless-steel pipes. Batches of 500 to 1000 L of this deionized water are then reconstituted in the laboratory to meet aquatic toxicity testing needs, following Method 8010E of Standard Methods for the Examination of Water and Wastewater, 18th edition. Diurnal light was approximately 16 hours light and 8 hours dark with a gradual intensity conversion between periods. Daylight intensity ranged from 504 to 733 Lux during full daylight periods of the study. Observations for mortality, abnormal behavior and appearance of the fish were performed on all test chambers daily. Water quality measurements (pH, dissolved oxygen, specific conductance and temperature) were performed twice per week on the test treatment and the control. No un-dissolved test substance was observed in the test chambers during the study. To maintain good hygiene, fecal material and uneaten food were siphoned from the bottom of each test chamber approximately 30 minutes to 1 hour after feeding. The test chambers were 60 L glass aquaria containing approximately 16 L of test water. The test chambers were covered with glass tank lids to minimize contamination or evaporation. A stainless steel screen was placed over the standpipe to prevent fish from becoming trapped in the

drain.

Reference substance (positive control)

no

Details on estimation of bioconcentration

Test results were used to derive the elimination rate constant (K_{elim}) as a function of the concentration of the test substance in fish versus time. Calculations were performed using MicroSoft® Excel 97 software and are consistent with methods outlined in the OECD test guidelines [Bioconcentration: Flow-Through Fish Test. OECD Guidelines for Testing of Chemicals. Section 3: Degradation and Accumulation, Guideline 305, adopted 14-Jun-96].

Results and discussions

Lipid content

2.47 %

Time other: The percentage lipid in the fish did not vary significantly between the point beginning and the end of the uptake phase or between treatments.

Remarks The mean percent lipid was 2.47% and was determined using three measurements.

Bioaccumulation factor

Conc. in environment / dose	Type	Value	Basis	Time of plateau	Calculation basis	Remarks
178	BCF	< 3	other: L/kg wet wt., adjusted to a 5% lipid content	14 d	kinetic	The half-life value of <1 day was used to calculate a BCF in fish of <3 L/kg

Depuration

Elimination	Endpoint	Depuration time (DT)
yes	other: Elimination rate constant = 1.16 ug/g day-1	

Kinetic parameters

Elimination rate constant = 1.16 ug/g day-1

Metabolites

Metabolites were not measured.

Results with reference substance (positive control)

A reference substance was not tested.

Details on results

There was no difference in mortality, growth, or lipid content between the treatment and the control after 14 days of exposure or at the end of the study (22 days). The following presents the mean weights, percent survival, and final lipid content. Initial Weight (g) Final Weight (g) Survival (Day 14 Uptake) (Day 8 Depuration) After 14 days Final Lipid Content Treatment Mean SD N Mean SD N % Control 1.035 0.212 10 1.504 0.298 10 100 2.42 Treated Diet 1.046 0.168 10 1.499 0.181 10 100 2.37 The calculated elimination rate constants for the test substance is 1.16 ug/g day⁻¹.

Reported statistics

Test results were used to derive the elimination rate constant (K_{Elim}) as a function of the concentration of the test substance in fish versus time. Calculations were performed using MicroSoft® Excel 97 software and are consistent with methods outlined in the OECD test guidelines [Bioconcentration: Flow-Through Fish Test. OECD Guidelines for Testing of Chemicals. Section 3: Degradation and Accumulation, Guideline 305, adopted 14-Jun-96].

Remarks on results including tables and figures

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) has a low potential to bioaccumulate in the environment based on results from a fish dietary lab study. The elimination rate constant was calculated as 1.16 ug/g day⁻¹. The low water solubility and high K_{ow} of DINP prevent conducting an aqueous exposure BCF study. At the end of the exposure period, fish were sampled after different depuration times (0, 0.6, 1, 3 days). Results demonstrated limited bioaccumulation with a lipid normalized biomagnification factor (BMF, concentration ratio in tissue to that in diet) of <0.1 and rapid subsequent depuration with a tissue elimination half-life of <1 day. The half-life value of <1 day was used to calculate a BCF in fish of approximately 1 L/kg for DINP.

Executive summary

Di-isooctyl phthalate ester (DINP) has a low potential to bioaccumulate in the environment based on results from a fish dietary lab study. The elimination rate constant was calculated as $1.16 \text{ ug/g day}^{-1}$. The low water solubility and high K_{ow} of DINP prevent conducting an aqueous exposure bioconcentration factor (BCF) study. At the end of the exposure period, fish were sampled after different depuration times (0, 0.6, 1, 3 days). Results demonstrated limited bioaccumulation with a lipid normalized biomagnification factor (BMF, concentration ratio in tissue to that in diet) of 0.1 and rapid subsequent depuration with a tissue elimination half-life of less than 1 day. The half-life value of less than 1 day was used to calculate a BCF in fish of $<3 \text{ L/kg}$ for DINP.

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 Dissemination Dossier

 Bioaccu terr Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	April 1996
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study procedure followed an accepted test guideline and was conducted under GLP. The data are consistent with known toxicological properties of similar high molecular weight phthalate ester substances.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1996							1996-07-08

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to other guideline:	OECD Guideline 207 (Earthworm, Acute Toxicity Test)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Radiolabelling

no

Details on test material

Substance type: technical product

Study design

Details on sampling

Test soils dosed with diisononyl phthalate ester (DINP) were analyzed at test initiation and termination, days -1 and 14, respectively.

Vehicle

no

Details on preparation and application of test substrate

Test soil was homogenized prior to use by placing soil into 4L size plastic (HDPE) containers and mixing on a jar mill for approximately 15 minutes. After homogenizing, a sample was removed to determine the moisture fraction. The moisture fraction was measured by placing soil into a crystallizing dish and weighing it (initial wt.). The sample and crystallizing dish were then placed in an oven at 100 degrees C for 23 hours. After drying and cooling in a desiccator, the final weight of the sample and crystallizing dish was measured. Treatments were prepared by adding the appropriate amount of test material to a soil sample. The treatment soils were stirred copiously in stainless steel bowls with stainless steel spoons for 15 to 30 minutes and held overnight at room temperature. On day 0, each treatment was divided into 5 replicates. The test chambers were one pint glass canning jars containing approximately 200g of a soil control or treatment and the appropriate amount of hydration water to bring the soils up to 75% of their water holding capacity. The worms were then transferred to randomized test chambers, which were capped with a lid containing a 1/8 inch hole in the center.

Test organisms

Test organisms (species)

Eisenia fetida

Details on test organisms

Worms, *Eisenia fetida*, were obtained from Carolina Biological, Burlington, NC, USA. Species were verified based on: Earthworms of Ontario, J.W. Reynolds, Life Sciences Miscellaneous Publishing, The Royal Ontario Museum, 15 June 1977 Average weight of worms was 0.393g (sd = 0.090), based on a subsample (n=20) of worms. The organism loading rate during the study was 10 worms/~200 g soil.

Total exposure / uptake duration

14 d

Total depuration duration

0 d

Nominal and measured concentrations

Soil samples were dosed with 10,000 mg test substance/kg soil. Soil were analyzed for DINP concentration at test initiation and termination. The soil analytical results are based on the mean of duplicate samples for each soil sampled: DIDP Conc. Day -1: 7270 mg/kg soil, dry wt. DIDP Conc. Day 14: 7872 mg/kg soil, dry wt. Average Conc.: 7591 mg/kg soil, dry wt.

Test conditions

Test temperature

Test temperature ranged from 18.2 to 20.6 degrees C as measured continuously and recorded by computer for the first 6 days, then measured daily thereafter.

pH

Soil pH was measured at test initiation (day 0) of the study. Ten grams of each treatment were mixed with 20mL of distilled water. The pH value of the slurry was measured using a soil pH probe. Soil pH ranged from 6.9 to 7.2.

Moisture

The water holding capacity of each soil was determined as follows: 25g of the dried sample was placed into a 100mL glass beaker with 25mL of reverse osmosis water and mixed thoroughly. This slurry was added to a pre-weighed, wetted filter paper and funnel (initial weight = wt. of funnel, wetted paper, + 25 g soil). The filter paper (#113 Whatman 150mm) was wetted with 9mL of reverse osmosis water. The glass funnel measured 10cm top diameter with an approximately 30mm stem. The slurry, filter and funnel were covered with aluminum foil and allowed to stand for 3 hours at which time a final weight was measured. The water holding capacity, expressed in mL water/100g of soil, is the difference between the final and initial weights multiplied by 4 to achieve 100g equivalence.

Details on test conditions

Natural soil was obtained from Snyder Research Farm located in Pittstown, NJ, USA, which was managed by Rutgers University. The soil was sieved through a 10 mesh sieve prior to use to remove large particles. Artificial soil was prepared using 70% sand, 20% clay, 10% peat, and CaCO₃ to adjust pH.

Results and discussions

Bioconcentration factor

Type	Value	Basis	Time of plateau	Calculation basis	Remarks
BSAF	0.018	whole body w.w.			

Details on results

The biota-soil accumulation factor (BSAF) as measured in a natural soil was 0.018 based on a DINP concentration in the earthworm of 134 mg/kg (wet weight) and in soil of 7591 mg/kg (dry weight). A BSAF value of <1 indicates a lack of bioaccumulation.

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

The biota-soil accumulation factor (BSAF) as measured in a natural soil was 0.018 based on a DINP concentration in the earthworm of 134 mg/kg (wet weight) and in soil of 7591 mg/kg (dry weight). A BSAF value of <1 indicates a lack of bioaccumulation.

Executive summary

Data to assess the potential for terrestrial bioaccumulation of DINP were reported in a 14-day earthworm (*Eisenia fetida*) toxicity study. The biota-soil accumulation factor (BSAF) as measured in a natural soil was 0.018 based on a DINP concentration in the earthworm of 134 mg/kg (wet weight) and in soil of 7591 mg/kg (dry weight). A BSAF value of <1 indicates a lack of bioaccumulation.

Administrative Data

Purpose flag key study

Study result type estimated by calculation Study period Not applicable

Reliability 2 (reliable with restrictions)

Rationale for The reliability rating is 2 because the data are calculated.

reliability

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Computer model		2008							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: Computer model	

Principles of method if other than guideline

The calculated value was determined using PCKOCWIN version 2.00, a subroutine within the computer program EPI SuiteTM version 4.0.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Radiolabelling

no

Details on test material

SMILES Notation used: CC(CH)CC(CH)CCCOC(=O)c1ccccc1C(=O)OCCCC(CH)CC(CH)C

Study design

Test temperature

N/A

Batch equilibrium or other method

Analytical monitoring

no

Computational methods

The calculated value was determined using Soil Adsorption Coefficient Program (PCKOCWIN) version 2.00, a subroutine within the computer program EPI Suite™ version 4.0. PCKOCWIN estimates the soil adsorption coefficient (Koc) of organic compounds. Koc can be defined as "the ratio of the amount of chemical adsorbed per unit weight of organic carbon (oc) in the soil or sediment to the concentration of the chemical in solution at equilibrium" (Lyman, 1990); it is represented by the following equation (Lyman, 1990): $Koc = (\text{ug adsorbed/g organic carbon}) / (\text{ug/mL solution})$

Results and discussions

Adsorption coefficient Koc

793100

log Koc

5.9

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Estimated di-isononyl phthalate log K_{oc} is 5.9.

Executive summary

Estimated di-isononyl phthalate log K_{oc} is 5.9.

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 [Adsorption Endpoint.002](#)

[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale reliability	for	The reliability rating is 2 because the data are calculated.	

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Computer model		2008							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: Computer model	

Principles of method if other than guideline

The calculated value was determined using PCKOCWIN version 2.00, a subroutine within the computer program EPI SuiteTM version 4.0.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

no

Details on test material

SMILES Notation used: CC(CH)CC(CH)CCCOC(=O)c1ccccc1C(=O)OCCCC(CH)CC(CH)C

Study design

Test temperature

N/A

~~Batch equilibrium or other method~~

Analytical monitoring

no

Computational methods

The calculated value was determined using Soil Adsorption Coefficient Program (PCKOCWIN) version 2.00, a subroutine within the computer program EPI Suite™ version 4.0. PCKOCWIN estimates the soil adsorption coefficient (Koc) of organic compounds. Koc can be defined as "the ratio of the amount of chemical adsorbed per unit weight of organic carbon (oc) in the soil or sediment to the concentration of the chemical in solution at equilibrium" (Lyman, 1990); it is represented by the following equation (Lyman, 1990): $Koc = (\text{ug adsorbed/g organic carbon}) / (\text{ug/mL solution})$

Results and discussions

Adsorption coefficient Koc

947900

log Koc

6

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Estimated di-isononyl phthalate log Koc is 6.0.

Executive summary

Estimated di-isononyl phthalate log Koc is 6.0.

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate) Study period Not applicable
Reliability	2 (reliable with restrictions)
Rationale for reliability	The reliability rating is a 2 because the study followed a U.S. EPA standard for guideline, which describes a procedure designed to evaluate this endpoint, and the results were reviewed for reliability and assessed as valid. However, the study was not conducted under GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory no.	Report Owner company	Report study no.	Report date
publication	Williams M, Adams W, Parkerton T, Biddinger G and Robillard K	1995	Sediment sorption coefficients for four phthalate esters: Experimental results and model theory.	Environ. Toxicol. Chem. 14: 1477-1486				

Materials and methods

Study type

adsorption

Media

sediment

Type of method

other: radiolabeled method

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OTS 796.2750 (Sediment and Soil Adsorption Isotherm)	

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Radiolabelling

yes

Details on test material

DIDP was ¹⁴C-uniformly ring-labeled with a radiopurity of 92.5%. Analyses showed that the radiolabeled DIDP was equivalent to the commercial substance.

Study design

Test temperature

25 degrees C

Batch equilibrium or other method

Analytical monitoring

yes

Details on sampling

Solid-to-solution ratios were 1:50 and 1:500 for each soil type. The test was conducted in triplicate at six concentrations. Triplicate 1.0 ml aliquots of each test solution at time 0 were analyzed by LSC (liquid scintillation counting) to establish actual concentrations. Test systems were incubated for 7 days after which samples were analyzed by LSC.

Details on matrix

Three sediments were used: U.S. EPA 8 (0.15% organic carbon, pH = 8.32), U.S. EPA 18 (0.66% organic carbon, pH = 7.76), and U.S. EPA 21 (1.88% organic carbon, pH = 7.60).

Details on test conditions

Test systems were incubated on a rotary shaker, in the dark at 25 degrees C.

Results and discussions

Adsorption coefficient K_{oc}

286000

log K_{oc}

5.46

Results: Batch equilibrium or other method

Recovery of test material

Mass balance determinations for DIDP were: EPA Soil 8 - 103% at initiation EPA Soil 18 - 105% at initiation EPA Soil 21 - 106% at initiation

Concentration of test substance at end of adsorption equilibration period

Mass balance determinations for DIDP were: EPA Soil 8 - 103% at termination EPA Soil 18 - 102% at termination EPA Soil 21 - 107% at termination

Transformation products

not measured

Remarks on results including tables and figures

The following table summarizes the sediment type and the dependency of log K_{oc} from organic content of sediment.

Sediment characteristics and K_{oc} values from Williamset al.(1995)

Sediment Type	OC (a) (%)	Sand (%)	Silt (%)	Clay (%)	CEC (b) (meq/100 g)	pH	K _{oc} (l/kg)
EPA 8 (c)	0.15	82.4	6.8	10.7	3.72	8.32	111,000

EPA 18	0.66	34.6	39.5	25.8	15.43	7.76	601,000
EPA 21	1.88	50.2	7.1	42.7	8.33	7.60	145,000

- a Organic carbon
- b Cation exchange capacity
- c U.S. Environmental Protection Agency soil type

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Sediment partition coefficients were measured for the commercial phthalate ester, disodecyl phthalate (DIDP). The Freundlich equation was used to calculate the organic carbon-normalized sediment/water partition coefficient (Koc), which averaged 2.86 E5 for DIDP.

Executive summary

Sediment partition coefficients were measured for the commercial phthalate ester, disodecyl phthalate (DIDP). The experimental procedure was based on the U.S. Environmental Protection Agency (EPA) Test Guideline 796.2750, "Sediment and Soil Adsorption Isotherm." Three sediments were used: EPA 8 (0.15% organic carbon), EPA 18 (0.66% organic carbon), and EPA 21 (1.88% organic carbon). The Freundlich equation was used to calculate the organic carbon-normalized sediment/water partition coefficient (Koc), which averaged 2.86 E5 for DIDP.

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 Dissemination Dossier

 Henry law Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	estimated by calculation	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Recommended value from European Commission risk assessment for 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich, CAS#:68515-48-0, EINECS#: 271-091-9.and:di-"isononyl" phthalate (DINP), CAS#: 28553-12-0, EINECS#: 249-079-5.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		2003							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline required		

Principles of method if other than guideline

Henry's Law constant is calculated based on vapor pressure and water solubility.

GLP compliance

no not applicable

Test materials

Test material equivalent to submission substance identity

yes

Results and discussions

Henry's Law constant H

H 41.4 Pa m³/mol

Temp. (°C) 25

Atm. press.

Applicant's summary and conclusion

Conclusions

Henry's Law constant for DINP, 41.4 Pa.m³/mole at 25 degrees C, indicates that volatilization from water is not expected to occur at a rapid rate, but may occur at a significant rate.

Executive summary

Henry's Law constant for DINP, 41.4 Pa.m³/mole at 25 degrees C, indicates that volatilization from water is not expected to occur at a rapid rate, but may occur at a significant rate.

Dossier > Document

 Dissemination Dossier

 Distribution Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag key study

Study result type estimated by calculation Study period Not applicable

Reliability 2 (reliable with restrictions)

Rationale for reliability The reliability rating is 2 because the data are calculated.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Mackay D, DiGuardo A, Paterson S and Cowan C	1996	Evaluating the environmental fate of a variety of types of chemicals using the EQC model	Environ. Toxicol. Chem. 15: 1627-1637					
other: Mathematical model	Mackay D	1997	Level III Fugacity-Based Environmental Equilibrium Partitioning Model, Version 1.01	Environmental Modelling Centre, Trent University, Ontario, Canada					
publication	Mackay D	2001	Multimedia environmental models: The fugacity	Second edition, pp. 1-261, Lewis Publisher,					

approach Boca Raton,
Fl, USA

Materials and methods

Model

Calculation according to Mackay, Level III

Calculation programme

Level III simulation using the Mackay Multimedia Environmental Model, version 2.80, released 2004, available at the Canadian Environmental Modelling Centre, Trent University, Canada.

Release year

2004

Media

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

Test materials

Test material equivalent to submission substance identity

yes

Test substance input data

Parameter Value w/ Units Molecular Weight 418.62 Temperature 25° C Log Kow 8.6 Water Solubility 0.000308 g/m3 Vapor Pressure 0.00000681 Pa Melting Point -48°C Emissions rates used in the calculation: Compartment Rate (kg/hr) Air 1000 Water 1000 Soil 1000

Results and discussions

Percent distribution in media

Air (%)

0.9

Water (%)

8.2

Soil (%)

68.5

Sediment (%)

22.4

Susp. sediment (%)

0

Biota (%)

0

Aerosol (%)

0

Remarks on results including tables and figures

The potential environmental distribution of di-isononyl phthalate ester in 4 environmental compartments as calculated using a fugacity model, the Mackay Level III, under the default emission scenario (1000 kg/h into each of air water and soil compartments):

Relative distribution when released (see half-life values above):

Air	0.9%
Soil	68.5%
Water	8.2%
Sediment	22.4%

Level III simulation using the Mackay Multimedia Environmental Model (Mackay, 2001). Mass balances are calculated for the four bulk media of air (gas + aerosol), water (solution + suspended sediment + biota), soil, (solids + air + water), and sediment (solids + pore water). Equilibrium exists within, but not between media. Physical-chemical properties are used to quantify a chemical's behavior in an evaluative environment. Three types of chemicals are treated in this model: chemicals that partition into all media (Type 1), non volatile chemicals (Type 2), and chemicals with zero, or near-zero, solubility (Type 3). The model cannot treat ionizing or speciating substances. The Level III model assumes a simple, evaluative environment with user-defined volumes and densities for the following homogeneous environmental media (or compartments): air, water, soil, sediment, suspended sediment, fish and aerosols.

This model provides a description of a chemical's fate including the important degradation and advection losses and the intermedia transport processes. The distribution of the chemical between media depends on how the chemical enters the system, e.g. to air, to water, or to both. This mode of entry also affects persistence or residence time. The rates of intermedia transport are controlled by a series of 12 transport velocities. Reaction half-lives are requested for all 7 media. The advective residence time selected for air also applies to aerosols and the residence time for water applies to suspended sediment and fish. The advective residence time of aerosols, suspended sediment and fish cannot be specified independently of the air and water residence times.

Applicant's summary and conclusion

Conclusions

The Mackay Level III equilibrium model estimates that DINP will partition largely to the soil compartment (approximately 69%), followed by the sediment (approximately 22%), water (approximately 8%), and air (less than 1%) compartments, based on all available measured data and the model's default emission rates.

Executive summary

The Mackay Level III equilibrium model estimates that DINP will partition largely to the soil compartment (approximately 69%), followed by the

sediment (approximately 22%), water (approximately 8%), and air (less than 1%) compartments, based on all available measured data and the

model's default emission rates.

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 Dissemination Dossier

 Distribution Endpoint.002

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	estimated by calculation	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability rating is 2 because the data are calculated.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Mackay D, DiGuardo A, Paterson S and Cowan C	1996	Evaluating the environmental fate of a variety of types of chemicals using the EQC model	Environ. Toxicol. Chem. 15: 1627-1637					
other: Mathematical model	Mackay D, DiGuardo A, Paterson S and Cowan C	1997	EQC Model ver. 1.01	Available from the Environmental Centre, Trent University, Canada.					
publication	Mackay D	2001	Multimedia environmental models: The fugacity approach	Second edition, pp. 1-261, Lewis Publisher, Boca Raton,					

FL, USA

Materials and methods

Model

Calculation according to Mackay, Level I

Calculation programme

Level I simulation using the Mackay Multimedia Environmental Model, version 3.0, released 2004, available at the Canadian Environmental Modelling Centre, Trent University, Canada.

Release year

1997

Media

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

Test materials

Test material equivalent to submission substance identity

yes

Test substance input data

Parameter Value w/ Units Molecular Weight 418.62 Temperature 25° C Log Kow 8.6 Water Solubility 0.000308 g/m3 Vapor Pressure 0.00000681 Pa Melting Point -48°C

Results and discussions

Percent distribution in media

Air (%)

0

Water (%)

0

Soil (%)

97.7

Sediment (%)

2.2

Susp. sediment (%)

0.1

Biota (%)

0

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

The Mackay Level I equilibrium model estimates that di-isononyl phthalate ester will partition largely to the soil compartment (approximately 98%), followed by the sediment (approximately 2%) compartment.

Executive summary

The Mackay Level I equilibrium model estimates that di-isononyl phthalate ester will partition largely to the soil compartment (approximately 98%), followed by the sediment (approximately 2%) compartment.

 Dissemination Dossier

 Data eco Summary

Administrative Data

Results and discussions

PNEC soil in mg/kg soil dw 30 Assessment factor Extrapolation method

PNEC oral in kg/kg food 150 Assessment factor

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 Dissemination Dossier

 Fishtox Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	read-across from supporting substance (structural analogue or surrogate)	Study period	1995-04-03 - 1995-04-07
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1995-06-08

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method C.1 (Acute Toxicity for Fish)	

Principles of method if other than guideline

Tested with emulsifier.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Physical state: clear liquid - Analytical purity: 99,7 Gew. % - Date of manufacture: 30.06.1992
- Lot/batch No.: 1286-31619 - Sample-No.: 1495/941006 - Stability: stable

Analytical monitoring

yes

Details on sampling

Direct sampling from test container

Vehicle

yes

Details on test solutions

Castor oil + 40 EO

Test organisms

Test organisms (species)

Brachydanio rerio (new name: Danio rerio)

Details on test organisms

TEST ORGANISM - Source: West Aquarium, Bad Lauterberg - Length at study initiation (length definition, mean, range and SD): 2.3cm. The deviation from the reference value (3cm +/- 0.5cm) is not result-related. - Feeding during test: no ACCLIMATION / QUARANTINE - Acclimation period: 14 days quarantine - Acclimation conditions (same as test or not): 20°C +/- 2°C, dechlorinate drinking water (flow through) - Food type: TetraMin - Amount: 1% of the body weight - Health during acclimation (any mortality observed): 7 days (before beginning of the test)= <=5%

Study design

Test type
semi-static

Water media type

freshwater

Limit test

yes

Total exposure duration

96 h Remarks semi static

Post exposure observation period

normal behavior

Test conditions

Hardness
approx. 13 °dH

Test temperature

Mean= 20°C, Min. 19°C, Max. 20°C

pH

7,7 - 8,3, during the study

Dissolved oxygen

O2-value during the study= 90 up to 99% - Saturation

Salinity

fresh water

Nominal and measured concentrations

100 mg/l nominal; 102 mg/l measured

Details on test conditions

TEST SYSTEM - Test vessel: - Material, size, headspace, fill volume: 10L (daily renewal) - Aeration: continued in the test-vessel - Renewal rate of test solution (frequency/flow rate): semi static, daily exchange of water - No. of organisms per vessel: 10 animals TEST MEDIUM / WATER PARAMETERS - Source/preparation of dilution water: Drinking Water from Gelsenwasser AG OTHER TEST CONDITIONS - Photoperiod (light/dark frequency): 16/8 h - tested with emulsifier (MARLOWET R 40, Ricinusöl + 40 EO, Castor oil + 40 EO)

Reference substance (positive control)

not required

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 102 mg/L	meas. (arithm. mean)	test mat.	mortality	

Details on results

- Mortality of control: no mortality - Concentration achieved through use of emulsifier

Reported statistics and error estimates

Limit test with no mortalities, therefore statistics were not necessary.

Any other information on results incl. tables

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The acute zebrafish (*Danio rerio*) toxicity data reported for di-isononyl phthalate ester (DINP) are consistent with the data for several fish species as summarized by Staples et al. (1997). These data clearly show that DINP does not produce acute toxicity to fish at or below its maximum attainable water solubility.

Executive summary

The acute toxicity of di-isononyl phthalate ester (DINP) as measured by mortality to zebrafish (*Danio rerio*) was evaluated in freshwater. DINP combined with an emulsifier did not produce acute lethal toxicity to *Danio rerio* at its maximum water solubility (under the conditions of this study).

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 Fishtox Endpoint.002

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1983							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ Toxicol Chem 16(5):875-891					

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publication 200
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Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Water quality samples were taken daily. Analytical samples were taken at time zero and on a composite of replicates at study termination.

Vehicle

no

Details on test solutions

Test treatments were prepared by using a proportional diluter modified to enhance mixing of

phthalates. The dilution water was Wareham, Mass., USA, town water (untreated and unchlorinated). A concentrated stock solution was prepared and combined with dilution water prior to pumping into the diluter.

Test organisms

Test organisms (species)

Pimephales promelas

Study design

Test type

static

Water media type

freshwater

Limit test

no

Total exposure duration

96 h Remarks

Test conditions

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 0.1 mg/L	meas. (not specified)	test mat.	mortality	
96 h	NOEC	0.1 mg/L	meas. (not specified)	test mat.	mortality	

Details on results

96 hr LC50 >0.10 mg/L. Diisononyl phthalate ester did not produce acute lethal toxicity at the highest achievable concentration under the conditions of this test.

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Bionomial Probability.

Any other information on results incl. tables

It was concluded in the European Risk Assessment Report (2003) for DINP that no chemical toxic effect could be attributed to the substances tested in any of the valid studies used to assess the aquatic toxicity of DINP. The report went on to state that "a two-generation test with *Oryzias latipes* showed that oral intake of 20 mg/kg had no adverse effect upon reproduction and growth. It can therefore be concluded that based on the available data, DINP has no adverse effects upon fish and that a NOEC cannot be determined."

European Commission (2003). Risk-Assessment Report Vol. 35, 2003 on: 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich, CAS#: 68515-48 -0, EINECS#: 271-090 -0, and:di-"isononyl" phthalate (DINP), CAS#: 268553 -12 -0, EINECS#: 249-079-5. Publication: EUR 20784 EN.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The acute fathead minnow (*Pimephales promelas*) toxicity data reported for di-isononyl phthalate ester (DINP) are consistent with the data for several fish species as summarized by Staples et al. (1997). These data clearly show that DINP does not produce acute toxicity to fish at or below its maximum attainable water solubility.

Executive summary

The acute toxicity of di-isononyl phthalate ester (DINP) as measured by mortality to fathead minnow (*Pimephales promelas*) was evaluated in freshwater. DINP did not produce acute lethal toxicity to fathead minnow at its maximum water solubility (0.10 mg/L) under the conditions of this study.

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 Fishtox Endpoint.003

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1983							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ. Toxicol. Chem. 16(5):875-891					

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Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Water quality samples were taken daily. Analytical samples were taken at time zero and on a composite of replicates at study termination.

Vehicle

no

Details on test solutions

Test treatments were prepared by using a proportional diluter modified to enhance mixing of

phthalates. The dilution water was Wareham, Mass., USA, town water (untreated and unchlorinated). A concentrated stock solution was prepared and combined with dilution water prior to pumping into the diluter.

Test organisms

Test organisms (species)

Lepomis macrochirus

Study design

Test type

static

Water media type

freshwater

Limit test

no

Total exposure duration

96 h Remarks

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 0.14 mg/L	meas. (not specified)	test mat.	mortality	
96 h	NOEC	0.14 mg/L	meas. (not specified)	test mat.	mortality	

Details on results

96 hr LC50 >0.14 mg/L. Diisononyl phthalate ester did not produce acute lethal toxicity at the highest achievable concentration under the conditions of this test.

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Bionomial Probability.

Any other information on results incl. tables

It was concluded in the European Risk Assessment Report (2003) for DINP that no chemical toxic effect could be attributed to the substances tested in any of the valid studies used to assess the aquatic toxicity of DINP. The report went on to state that "a two-generation test with *Oryzias latipes* showed that oral intake of 20 mg/kg had no adverse effect upon reproduction and growth. It can therefore be concluded that based on the available data, DINP has no adverse effects upon fish and that a NOEC cannot be determined."

European Commission (2003). Risk-Assessment Report Vol. 35, 2003 on: 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich, CAS#: 68515-48 -0, EINECS#: 271-090 -0, and:di-"isononyl" phthalate (DINP), CAS#: 268553 -12 -0, EINECS#: 249-079-5. Publication: EUR 20784 EN.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The acute bluegill fish (*Lepomis macrochirus*) toxicity data reported for di-isononyl phthalate ester (DINP) are consistent with the data for several fish species as summarized by Staples et al. (1997). These data clearly show that DINP does not produce acute toxicity to fish at or below its maximum attainable water solubility.

Executive summary

The acute toxicity of di-isononyl phthalate ester (DINP) as measured by mortality to bluegill fish (*Lepomis macrochirus*) was evaluated in freshwater. DINP did not produce acute lethal toxicity to bluegill fish at its maximum water solubility (0.14 mg/L) under the conditions of this study.

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[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1983							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ. Toxicol. Chem. 16(5):875-891					

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Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Water quality samples were taken daily. Analytical samples were taken at time zero and on a composite of replicates at study termination.

Vehicle

no

Details on test solutions

Test treatments were prepared by using a proportional diluter modified to enhance mixing of

phthalates. The dilution water was Wareham, Mass., USA, town water (untreated and unchlorinated). A concentrated stock solution was prepared and combined with dilution water prior to pumping into the diluter.

Test organisms

Test organisms (species)

Pimephales promelas

Study design

Test type

flow-through

Water media type

freshwater

Limit test

no

Total exposure duration

96 h Remarks

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 0.19 mg/L	meas. (not specified)	test mat.	mortality	
96 h	NOEC	0.19 mg/L	meas. (not specified)	test mat.	mortality	

Details on results

96 hr LC50 >0.19 mg/L. Diisononyl phthalate ester did not produce acute lethal toxicity at the highest achievable concentration under the conditions of this test.

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Bionomial Probability.

Any other information on results incl. tables

It was concluded in the European Risk Assessment Report (2003) for DINP that no chemical toxic effect could be attributed to the substances tested in any of the valid studies used to assess the aquatic toxicity of DINP. The report went on to state that "a two-generation test with *Oryzias latipes* showed that oral intake of 20 mg/kg had no adverse effect upon reproduction and growth. It can therefore be concluded that based on the available data, DINP has no adverse effects upon fish and that a NOEC cannot be determined."

European Commission (2003). Risk-Assessment Report Vol. 35, 2003 on: 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich, CAS#: 68515-48 -0, EINECS#: 271-090 -0, and:di-"isononyl" phthalate (DINP), CAS#: 268553 -12 -0, EINECS#: 249-079-5. Publication: EUR 20784 EN.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The acute fathead minnow (*Pimephales promelas*) toxicity data reported for di-isononyl phthalate ester (DINP) are consistent with the data for several fish species as summarized by Staples et al. (1997). These data clearly show that DINP does not produce acute toxicity to fish at or below its maximum attainable water solubility.

Executive summary

The acute toxicity of di-isodecyl phthalate ester (DINP) as measured by mortality to fathead minnow (*Pimephales promelas*) was evaluated in freshwater. DINP did not produce acute lethal toxicity to fathead minnow at its maximum water solubility (0.19 mg/L) under the conditions of this study.

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[Dissemination Dossier](#)

[Fishtox Endpoint.005](#)

[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1983							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ. Toxicol. Chem. 16(5):875-891					

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Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial substance

Analytical monitoring

yes

Details on sampling

Water quality samples were taken daily. Analytical samples were taken at time zero and on a composite of replicates at study termination.

Vehicle

no

Details on test solutions

Test treatments were prepared by using a proportional diluter modified to enhance mixing of

phthalates. The dilution water was Wareham, Mass., USA, town water (untreated and unchlorinated). A concentrated stock solution was prepared and combined with dilution water prior to pumping into the diluter.

Test organisms

Test organisms (species)

Oncorhynchus mykiss

Details on test organisms

Mean length = 39 mm Mean wet weight = 0.5 g

Study design

Test type

flow-through

Water media type

freshwater

Limit test

no

Total exposure duration

96 h Remarks

Test conditions

Hardness

Range was 20 to 26 mg/L as CaCO₃

Test temperature

11 degrees C

pH

Range was 7.1 to 7.4

Dissolved oxygen

Range was 10.0 to 10.1 mg/L

Nominal and measured concentrations

Nominal test concentrations: control, control, 0.012, 0.025, 0.050, 0.10, and 0.20 µl/L Mean measured test concentrations: < 0.0068 (control), 0.0087, 0.019, 0.032, 0.062, and 0.16 mg/L

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 0.16 mg/L	meas. (not specified)	test mat.	mortality	
96 h	NOEC	0.16 mg/L	meas. (not specified)	test mat.	mortality	See other information on results below.

Details on results

96 hr LC50 >0.16 mg/L. Diisononyl phthalate ester did not produce acute lethal toxicity at the highest achievable concentration under the conditions of this test. Measured values dropped slightly during the exposure period. Droplets of undissolved test material were observed on the surface of all test solutions throughout the exposure period and a film of undissolved test material was observed on the surface of the two high concentrations throughout the test.

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Binomial Probability.

Any other information on results incl. tables

It was concluded in the European Risk Assessment Report (2003) for DINP that no chemical toxic effect could be attributed to the substances tested in any of the valid studies used to assess the aquatic toxicity of DINP. The report went on to state that "a two-generation test with *Oryzias latipes* showed that oral

intake of 20 mg/kg had no adverse effect upon reproduction and growth. It can therefore be concluded that based on the available data, DINP has no adverse effects upon fish and that a NOEC cannot be determined."

European Commission (2003). Risk-Assessment Report Vol. 35, 2003 on: 1,2-benzenedicarboxylic acid, di-C8-10-

branched alkyl esters, C9-rich, CAS#: 68515-48 -0, EINECS#: 271-090 -0, and:di-"isononyl" phthalate (DINP), CAS#: 268553 -12 -0, EINECS#: 249-079-5. Publication: EUR 20784 EN.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The acute rainbow trout (*Oncorhynchus mykiss*) toxicity data reported for di-isononyl phthalate ester (DINP) are consistent with the data for several fish species as summarized by Staples et al. (1997). These data clearly show that DINP does not produce acute toxicity to fish at or below its maximum attainable water solubility.

Executive summary

The acute toxicity of di-isononyl phthalate ester (DINP) as measured by mortality to rainbow trout (*Oncorhynchus mykiss*) was evaluated in freshwater. DINP did not produce acute lethal toxicity to *Oncorhynchus mykiss* at its maximum water solubility (0.16 mg/L) under the conditions of this study.

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[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1984							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ Toxicol Chem 16(5):875-891					

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Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Water quality samples were taken daily. Analytical samples were taken at time zero and on a composite of replicates at study termination.

Vehicle

no

Details on test solutions

Test treatments were prepared by using a proportional diluter modified to enhance mixing of

phthalates. The dilution water was Wareham, Mass., USA, town water (untreated and unchlorinated). A concentrated stock solution was prepared and combined with dilution water prior to pumping into the diluter.

Test organisms

Test organisms (species)

Cyprinodon variegatus

Study design

Test type

flow-through

Water media type

saltwater

Limit test

no

Total exposure duration

96 h Remarks

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 0.52 mg/L	meas. (not specified)	test mat.	mortality	
96 h	NOEC	0.52 mg/L	meas. (not specified)	test mat.	mortality	

Details on results

96 hr LC50 >0.52 mg/L. Diisononyl phthalate ester did not produce acute lethal toxicity at the highest achievable concentration under the conditions of this test.

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Binomial Probability.

Any other information on results incl. tables

It was concluded in the European Risk Assessment Report (2003) for DINP that no chemical toxic effect could be attributed to the substances tested in any of the valid studies used to assess the aquatic toxicity of DINP. The report went on to state that "a two-generation test with *Oryzias latipes* showed that oral intake of 20 mg/kg had no adverse effect upon reproduction and growth. It can therefore be concluded that based on the available data, DINP has no adverse effects upon fish and that a NOEC cannot be determined."

European Commission (2003). Risk-Assessment Report Vol. 35, 2003 on: 1,2-benzenedicarboxylic acid, di-C8-10-

branched alkyl esters, C9-rich, CAS#: 68515-48 -0, EINECS#: 271-090 -0, and: di-"isononyl" phthalate (DINP), CAS#: 268553 -12 -0, EINECS#: 249-079-5. Publication: EUR 20784 EN.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The acute sheepshead minnow (*Cyprinodon variegatus*) toxicity data reported for di-isononyl phthalate ester (DINP) are consistent with the data for several fish species as summarized by Staples et al. (1997). These data clearly show that DINP does not produce acute toxicity to fish at or below its maximum attainable water solubility.

Executive summary

The acute toxicity of di-isodecyl phthalate ester (DIDP) as measured by mortality to sheepshead minnow (*Cyprinodon variegatus*) was evaluated in saltwater. DIDP did not produce acute lethal toxicity to sheepshead minnow at its maximum water solubility (0.52 mg/L) under the conditions of this study.

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Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	2002
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the study generally followed an OECD standard guideline, applied valid scientific testing principles, and the results were reviewed for reliability and assessed as valid. However, the study was not conducted under GLP. study.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		2002							
publication		2006							2005-05-23

Materials and methods

Test type

life cycle: reproduction, (sub)lethal effects

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	other guideline: OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test) with evaluation of two generations	not applicable

Principles of method if other than guideline

Examination of the reproductive effects of DINP in Japanese medaka following long-term dietary exposure. Medaka were used because they are sensitive to estrogen and estrogenic compounds producing ovotestis and other reproductive effects. Medaka reach sexual maturity in 40 to 60 days post-hatch, which allows for a multigenerational study to be completed in one year. The test substance was administered via the diet since this is the major route of exposure to hydrophobic compounds.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Substance type: commercial product - Name of test material (as cited in study report): diisononyl phthalate (DINP) - Substance type: technical material; an orthophthalic diester containing isometric mixtures of branched aliphatic alcohols, primarily in the C8 - 10 range. - Physical state: liquid - Analytical purity: > 99.7 % identified by gas chromatography and found to be within commercial specification. - Impurities (identity and concentrations): no details given but stated that it did not contain any that would affect the outcome of the study. - Purity test date: not given - Lot/batch No.: not given - Expiration date of the lot/batch: not given - Stability under test conditions: the concentration and homogeneity of the diet containing the DINP were confirmed analytically prior to start of exposure and once at the end of each exposed generation. The mean phthalate concentration in treatment diet was $21.9 \pm 2.8 \mu\text{g/g}$. - Storage condition of test material: not given

Analytical monitoring

yes

Details on sampling

The test diet was analyzed three times during the study: pre-study, day 136, and day 284.

Vehicle

yes

Details on test solutions

The test substance was administered through the diet. PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances) - Method: incorporated into Tetramin flake food (5% lipid content) at a nominal concentration of 20 µg/g (1 µg/g fish/day). - Controls: x 2 groups; untreated and carrier treated diet - Chemical name of vehicle (organic solvent, emulsifier or dispersant): Acetone - Concentration of vehicle in test medium (stock solution and final test solution(s): including acetone carrier control, 20mL/1000g flake food to evenly distribute the test material (where relevant) in the diet. Following spiking, acetone was allowed to evaporate overnight under ambient laboratory conditions. - Evidence of undissolved material (e.g. precipitate, surface film, etc): N/A

Test organisms

Test organisms (species)

Oryzias latipes

Details on test organisms

TEST ORGANISM - Common name: Japanese medaka - Strain: golden - Source: Carolina Biological Supply Co. (Burlington, NC, USA) used to establish an in-house breeding colony
POST-HATCH FEEDING - Start date: Juvenile fish, older than 14 days posthatch were fed - Type/source of feed: Salmon Starter, Tetramin, nematode worms and/or *Artemia nauplii* during culturing - Frequency of feeding: twice daily

Study design

Test type

flow-through

Water media type

freshwater

Limit test

yes

Total exposure duration

284 d Remarks

Test conditions

Hardness

Test conducted but results not reported

Test temperature

Temperature was measured daily in each replicate chamber and was maintained at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the duration of the study.

pH

Range: 6.5 - 7.7 Untreated Acetone Test Control Control Substance pH 6.5-7.6 6.9-7.7 6.8-7.7

Dissolved oxygen

> 60% saturation Untreated Acetone Test Control Control Substance Dissolved Oxygen 7.0-8.8 7.1-8.7 6.8-8.8 (mg/l)

Nominal and measured concentrations

A single dietary concentration of the test substance was prepared at a nominal loading of 20 $\mu\text{g/g}$. The mean measured concentration of the test substance in the test diet was $21.9 \pm 2.8 \mu\text{g/g}$ and ranged from $18.5 \pm 0.40 \mu\text{g/g}$ to $24.5 \pm 1.44 \mu\text{g/g}$.

Details on test conditions

Test vessel: Open Glass aquaria (20L), with stainless-steel standpipes set at 10L volume. Diet Preparation: A single dietary concentration was prepared at a nominal loading of 20 $\mu\text{g/g}$. The exposure concentration represented a reasonable worst-case scenario based on US EPA field measurements and equilibrium partitioning theory predictions for concentrations in prey organisms. The experimental diet was prepared by adding the test substance to TetraMin flake fish food (5% lipid content) with the help of acetone as a solvent in order to evenly distribute the test substance in the fish food. The solvent was allowed to evaporate overnight under ambient laboratory conditions. An acetone solvent control was included with an untreated control group and prepared under similar conditions. The diets were stored at $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the duration of the study to prevent spoilage. The feed was analyzed by GC-MSD for concentration verification purposes prior to, during, and after the study. Methods: A flow-through exposure system was constructed to provide a sufficient volume of water to the exposure chambers. The flow rate (approximately 250 ml/min) was monitored daily and adjusted as necessary in order to maintain optimal water quality for the test organisms. Five replicate chambers were prepared for each of the three treatment groups (treated, solvent control, untreated control). Test chambers were 19 liter glass aquaria with stainless steel standpipes cut to allow a test volume of 10 liters of water. Embryos collected during the study were hatched in 250 ml glass beakers under continuous aeration. Test water in the hatching chambers was replaced as needed to preserve water quality and maintain embryo health. During overlap between generations, juvenile fish were housed in

glass cylinders suspended in the test chambers to prevent losses due to consumption by adult fish. Replicate integrity was maintained throughout the study. For the F0 generation, 50 Medaka larvae per replicate (a total of 250 fish per group) were fed the appropriate diet at a rate of 50% body weight per day. The food ration was periodically adjusted to compensate for growth of the fish during the study. Feeding was also supplemented three times weekly with freshly hatched brine shrimp (*Artemia* spp.). Observations for mortality were performed and recorded daily. Dead organisms were recorded and removed from the test chambers. Test chambers were also cleaned periodically to remove accumulated organic material. Once adults reached sexual maturity (about 40 to 60 days post-hatch (DPH)), eggs were collected on sponge filters. Egg production was observed for approximately 3 to 4 weeks and recorded before eggs were collected for hatching of the next generation. Eggs for hatching were collected once all replicates were observed producing eggs. During the embryo-rearing stages of the study the rearing chambers were observed daily and all embryos with fungus or an opaque appearance were removed. Eggs from each replicate were hatched to provide 50 larval fish for the next generation. The remaining collected eggs were counted and frozen for chemical analysis. At termination of each generation (140 DPH) there remained at least 25 fish per replicate (125 fish per group). Population, individual, and biochemical test parameters were evaluated at this time. Male and female fish were processed separately. Fish were evaluated for morphometric parameters such as total weight, standard length, gonad weight, and gonadal-somatic index. As with the original population (F0), the F1 fish were allowed to spawn (F2 generation) and fecundity, egg viability, and embryo development were evaluated. Adult F1 fish were also evaluated based on histopathology, morphological characteristics, and biochemical parameters. F1 larvae were evaluated for lesion occurrence, stage development, post-hatch survival and growth. The F2 larvae were allowed to grow out until 42 DPH, at which time they were weighed, measured, sacrificed, and processed for histopathology evaluation.

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
284 d	NOEC	>= 18.5 — <= 24.5 ug/g feed	meas. (not specified)	test mat.	other: Survival, development, reproduction, carcinogenicity	

Details on results

No significant difference in survival was observed between the treated group and the controls.

Mean Percent Mortality Generation F0 F1 F2 Control 11.2 24.6 14.4 Solvent Control 7.6 18.0 14.0 Test Substance 7.6 22.4 12.4 There were no treatment-related gross lesions or disease processes observed in the adult fish. No significant reduction growth or egg production was observed between the treated group and the controls. However, the untreated group produced fewer eggs than both the treated group and the solvent control group in both generations. Sex ratio (male to female) was similar among all groups (approximately 1:1) for the F0, F1 and F2 generations. Phenotypic and histological gender classifications were in agreement for both males and females. There was no statistically significant gonadal-somatic index (GSI) differences among the treated and control groups in either the F0 or the F1 generation. F2 generation juvenile fish were processed on Day 42 post-hatch. The primary organs examined for general developmental conditions and the occurrence of lesions included the brain, digestive system, liver, kidney, gonads, and skeleton. There were no treatment-related histological lesions observed in the fish. The results demonstrate that the test substance did not elicit endocrine-mediated effects such as testis-ova, intersex conditions, or sex reversal in Japanese Medaka. EROD activity of the F1 generation male and female Medaka were not significantly different among the control and treated fish. Activity in male fish ranged from 0.04 to 0.08 pmol/min/mg protein and for female fish ranged from 0.09 to 0.12 pmol/min/mg protein. Significant induction or reduction in EROD activity was considered to be 2-fold different than the solvent control for either male or female hepatic samples. There were also no major metabolic differences among the groups with respect to testosterone hydroxylase activity. Hepatic vitellogenin production was not detectable in Western Blot analysis of F0 and F1 generation male Medaka at 140 DPH. The F0 females showed faint banding at approximately 170 kDa and the F1 females showed a 2 to 3-fold increase in staining when compared to the untreated control group. Positive controls (17 β -estradiol) had a 5 to 6-fold increase in staining in both males and females when compared to the untreated control group. Histological and biochemical endpoints are addressed in a separate peer-reviewed article: Patyna P. et. al. (2006). Hazard evaluation of diisononyl phthalate and diisodecyl phthalate in a Japanese medaka multigenerational assay. *Ecotoxicol. Environ. Safety* 65(1): 36-47.

Results with reference substance (positive control)

A positive control was not included.

Reported statistics and error estimates

An Analysis of Variance (ANOVA) was used to determine differences in the groups based on survival, growth, and fecundity. The ANOVA was determined using the General Linear Models procedure of SAS.

Remarks on results including tables and figures

Diet analysis:

The concentration and homogeneity of the DINP diet was confirmed analytically prior to start of exposure and once at the end of each exposed generation. The mean phthalate concentrations in

the DINP treated diet was $21.9 \pm 2.8 \mu\text{g/g}$.

Survival:

The long term survival data for the Japanese medaka F_0 , F_1 and F_2 generations are summarized in Table 2 below. Survival at 140 days post hatch (DPH) was decreased only in the F_1 generation DINP fish ($63\% \pm 5.4$; range 56-68%). However, all other F_1 generation groups exhibited wide replicate variation in survival (e.g. untreated control 62 -84%). Comparable survival was observed for all treatment groups in the F_0 and F_2 generation fish. There were no treatment-related gross lesions or disease processes observed in the adult fish.

Table 2.

Survival of F_0 , F_1 and F_2 generation Japanese medaka adults
% Survival^a $\pm$ SD (range)

Generation	Day	Untreated control	Acetone control	DINP
F_0	140	89 ± 3.3 (84-92)	92 ± 6.2 (86-100)	80 ± 17.6 (52-96)
F_1	140	75 ± 8.1 (62-84)	82 ± 6.5 (72-90)	$63 \pm 5.4^*$ (56-68)
F_2	40	86 ± 8.9 (76-96)	8.6 ± 8.1 (72-92)	92 ± 3.7 86-96

* $P \leq 0.05$ compared to untreated control

^aResults from 250 fish from five replicate tanks ($N = 50$) in each treatment

Embryo development:

Eggs were collected from the F_0 and F_1 generation fish to assess embryo development, monitor the occurrence of gross lesions, and determine posthatch survival (Table 3 below). The only statistically significant observation found was a transient delay in red blood cell (RBC) pigmentation. This short-lasting anomaly was corrected prior to hatching. Embryos from the F_0 generation acetone control fish exhibited a greater occurrence of this delay (exhibited a greater occurrence of this delay ($P < 0.05$) than the untreated control group in one of the two assays conducted. F_0 generation DINP-treated embryos exhibited a greater occurrence of this delay ($P < 0.05$) than the control groups in one of the two assays conducted. The affected embryos appeared to continue to develop normally and there were no significant changes in embryo survival among treatment groups. Posthatch larval survival was significantly decreased in one assay of the DINP-treated F_0 generation though not in the second assay. Other observations during the 10 -day developmental period included under-inflation of the swim bladder and some craniofacial abnormalities; however, DINP treated embryos appeared to show lower overall rates of histopathological effects than the controls. There were no statistically significant differences in development of these nonlethal effects in control and treated F_0 and F_1 generation offspring.

Table 3.

Histopathology effects and posthatch survival observations

Generation	Group	RBC pigmentation ^a (%)		Posthatch survival (%)	
		Assay 1	Assay 2	Assay 1	Assay 2
F ₀ offspring N= 50/assay	Untreated	0	0	100	94 ± 9
	Acetone	48 ± 33*	31 ± 8*	92 ± 6	98 ± 4
	DINP	70 ± 35*	96 ± 6***	74 ± 15*	82 ± 18
F ₁ offspring N= 50/assay	Untreated	0	0	100	90 ± 2
	Acetone	20 ± 10	10 ± 7	100	90 ± 90
	DINP	30 ± 18	80 ± 35*, **	80 ± 10	100

* $P \leq 0.05$ compared to untreated control

** $P \leq 0.05$ compared to acetone control

^aLoss of red blood cell pigmentation. Results shown from two separate embryo assays in each generation

Gonadal-somatic index:

There were no statistically significant total body weight differences among treatment groups in either the F₀ or the F₁ generation. The standard deviation within treatment groups and replicates for all morphometric parameters was large (see Table 4 below). Based on all the morphometric indices, the F₀ generation fish were 30% smaller than the F₁ generation fish in the treated group. There were no statistically significant gonad weight differences in the treated groups in either the F₀ or the F₁ generation male and female fish. The gonad weights were approximately 3 -5 fold less in the F₀ generation than in the F₁ generation. There were no statistically significant differences in the GSI (gonadal-somatic index) among the treated groups in either the F₀ or the F₁ generation.

Table 4

F₀ and F₁ generation total bodyweight, gonad weight and gonadal –somatic index (mean±SD) after 140 days of dietary exposure

Endpoint ^a	Generation	Sex	Untreated	N	Acetone	N	DINP
Total body weight (mg)	F ₀	M	170±69	50	140±46	52	160±58
	F ₀	F	150±56	35	130±36	41	160±51
	F ₁	M	240±87	72	180±70	68	210±66
	F ₁	F	200±66	47	170±59	66	210±48
Gonad weight (mg)	F ₀	M	0.71±0.62	23	0.92±0.54	24	0.87±0.72
	F ₀	F	9.6±5.2	19	6.3±4.8	20	6.2±3.9
	F ₁	M	3.3±1.7	50	3.9±1.6	48	4.2±2
	F ₁	F	21±20	47	28±18	50	29±14
GSI ^b	F ₀	M	0.5±0.44	23	0.7±0.37	24	0.6±0.52
	F ₀	F	6.5±3.49	19	4.9±2.84	20	5.1±2.67
	F ₁	M	1.4±0.66	50	2.1±0.98	48	2.1±1.24
	F ₁	F	11.1±10.22	47	15.9±8.01	50	13.6±5.13

M: Male F: Female

^aThere were no statistically significant differences among the treatment groups

^bGSI, gonad weight /total body weight x 100

Egg production:

In both the F₀ and the F₁ generations, the total egg production (eggs per tank) was not significantly different in the DINP group compared to the control groups (Table 5, below). However, the untreated control groups from both generations produced fewer eggs than the acetone control and the DINP group. Dividing the total egg production by the number of females per tank reduces bias caused by an increased number of females in any replicate. Egg production per female for both generations followed a trend similar to that seen in the total egg production where the DINP-treated females produced more eggs than the other groups. In the F₁ DINP group, there was an increase in egg production (two-fold greater than untreated control) on a per female basis. The large standard deviation in DINP replicate egg production resulted in this increase not being statistically significant.

Table 5

Egg production per tank and per female (mean ± SD)^a

Endpoint	Generation	Untreated	Acetone	DINP
Eggs per tank	F ₀	598±222.1	711± 251.4	777±375.1
	F ₁	1263±466.5	1865±582.8	2388±1042.
Eggs per female	F ₀	98±41.6	106±59.2	145±70.0
	F ₁	133±17.9	142±35.0	311±141.9

^aNo statistically significant differences among the treatment groups

Histology:

In the F₀ and F₁ generations, fish were processed for histopathological evaluation at days 42, 81 and 140 posthatch. The F₂ generation juvenile fish were processed on day 42 posthatch. The primary organs examined for general developmental conditions and the occurrence of lesions included the brain, digestive system, liver, kidney, gonads and skeleton. There were no treatment-related histological lesions observed in the fish. The phenotypic and histological gender classification for both males and females were in agreement. There were no shifts in the male-to-female ratio in any of the treatment groups. None of the treatments elicited endocrine-mediated effects such as testis-ova, intersex conditions, or sex reversal in Japanese medaka.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) did not produce significant carcinogenic, teratological, or reproductive effects in the Medaka fish (*Oryzias latipes*) exposed to DINP through the diet at a nominal concentration of 20 ug/g feed (measured concentrations ranged from 18.5 to 24.5 ug/g feed).

Executive summary

Specific guidelines for the study were not available. Therefore, the study was performed following procedures outlined in existing chronic fish toxicity test guidelines. The results showed that di-isononyl phthalate ester (DINP) did not produce significant carcinogenic, teratological, or reproductive effects in *Oryzias latipes* in a 284 -day chronic toxicity study. Measured feed concentrations of DINP through the study ranged from 18.5 to 24.5 ug/g feed.

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[Administrative Data](#)

[Data source](#)

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Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1984							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ Toxicol Chem 16(5):875-891					

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Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Analytical samples taken at time zero and on a composite of replicates at termination. Measured values declined during study exposure. The concentration of test substance measured in the high treatment solution is considered the maximum solubility achievable under the conditions of this test.

Vehicle

no

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

Daphnia were <24 hours old and obtained from in-house stock.

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

48 h

Remarks

Test conditions

Hardness

The range of total hardness as CaCO₃ of the dilution water was 150 to 170 mg/L.

Test temperature

Test temperature = 22 +/- 2 degree C.

pH

The pH was 8.4.

Dissolved oxygen

Dissolved oxygen ranged from 8.6 to 9.7 at initiation and 8.3 on day 2.

Nominal and measured concentrations

Nominal test concentrations: control and 0.20 mg/L. Mean measured test concentrations of time 0 and 48 hr values: <0.014 and 0.06 mg/L.

Details on test conditions

The test treatment was prepared by mixing the test substance and dilution water (fortified well water) using a magnetic stir bar at a loading of 10 µl per liter. Exposure solution was removed from below the surface of the mixing vessel after settling for 1 hour and transferred to a bottle and allowed to settle again for 24 hours, after which solution from below the surface was removed for use in the test. Three replicates of five organisms were tested at a single treatment level. Test vessels were 250 ml beakers with 200 ml of test solution. Analytical method was Gas Liquid Chromatography (GLC).

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC50	> 0.06 mg/L	meas. (not specified)	test mat.	mobility	
48 h	NOEC	0.06 mg/L	meas. (not specified)	test mat.	mobility	

Details on results

48 hr EC50 >0.06 mg/L (no effects at test substance saturation). 48 hr EC50 >0.086 mg/L (based upon time zero analytical samples; no effects at test substance saturation). Value was recalculated as > 0.06 mg/L as per U.S. EPA current practices using mean of measured initiation and termination samples as reported in Staples et al. (1997). Mean measured values were used in the final EC50 calculation. This study was conducted as a limit test. % Immobility results at 48 hrs per replicate for control and treatment level: Conc. (mg/L) Rep1/Rep2/Rep3 Control 0 / 0 / 0 0.06 0 / 0 / 0

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Binomial Probability.

Remarks on results including tables and figures

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) does not produce acute toxicity (immobility) to *Daphnia magna* at its maximum water solubility (0.06 mg/L) under the conditions of this study.

Executive summary

The acute toxicity of di-isodecyl phthalate ester (DINP) as measured by immobility to the water flea (*Daphnia magna*) was evaluated in freshwater. DINP did not produce acute toxicity to *Daphnia* at its maximum water solubility (0.06 mg/L) under the conditions of this study.

Dossier > Document

 Dissemination Dossier

 Daphniatox Endpoint.002

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1984							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ Toxicol Chem 16(5):875-891					

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Materials and methods

Test guideline

Qualifier Guideline

according to other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians

Deviations

no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Vehicle

no

Test organisms

Test organisms (species)

Mysidopsis bahia (new name: Americamysis bahia)

Study design

Test type

static

Water media type

saltwater

Limit test

no

Total exposure duration

96 h

Remarks

Test conditions

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 0.39 mg/L	meas. (initial)	test mat.	mortality	
96 h	NOEC	0.39 mg/L	meas. (initial)	test mat.	mortality	

Details on results

96 hr LC50 >0.39 mg/L. Di-isononyl phthalate ester did not produce acute lethal toxicity at the highest achievable concentration under the conditions of this test. Mean measured values were used in the LC50 calculation.

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Bionomial Probability.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) does not produce acute lethal toxicity to *Mysidopsis bahia* at its maximum water solubility (0.39 mg/L) under the conditions of this study.

Executive summary

The acute toxicity of di-isononyl phthalate ester (DINP) as measured by lethality to *Mysidopsis bahia* was evaluated in saltwater. DINP did not produce acute toxicity to mysids at its maximum water solubility (0.39 mg/L) under the conditions of this study.

Dossier > Document

 Dissemination Dossier

 Daphniatox Endpoint.003

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	17.01.1995 to 19.01.1995
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a EU standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1995-04-03

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method C.2 (Acute Toxicity for Daphnia) Study undertaken as specified by 92/69 EEC, Dec 1992. The test method C.2 is not explicitly stated in the report, but can be inferred.	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): VESTINOL 9 - Substance type: Technical material - Physical state: Colorless liquid - Stability under test conditions: No data - Storage condition of test material: No data

Analytical monitoring

yes

Details on sampling

- Concentrations: Nominal values of 0 (control) , 100 mg/L and 0 (vehicle control) mg/L - Sampling method: No data - Sample storage conditions before analysis: No data

Vehicle

yes

Details on test solutions

Stock solution prepared by adding 1g VESTINOL 9 and 1g MARLOWET R40 in 1L of deionized water. MARLOWET R 40 (Ricinusoil 40 EO; castor oil ethoxylate) . The concentration of the solublizer in the test solution was 100mg/l. The solublizer is referred to as the vehicle in the rest of this summary. test medium was Elendt synthetic fresh water (composition reported in test report)

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

TEST ORGANISM - Common name: Daphnia - Strain: Daphnia magna Straus, clone 5 - Source: Bred in-house - Age at study initiation (mean and range, SD): The offspring produced overnight were transferred into the test water - Method of breeding: Culture conditions ensured parthenogenic reproduction - Feeding during test: None

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

48 h

Remarks

Test conditions**Hardness**

No data

Test temperature

20+/- 1°C

pH

8.0 (control), 7.8 (test) and 7.9 (vehicle control)

Dissolved oxygen

8.4 (control), 7.9 (test) and 7.9 (vehicle control) mg/L

Salinity

Not applicable

Nominal and measured concentrations

Nominal: 0 (control), 100 (test) and 0 (vehicle control) mg test material/L Measured: < 30 (control), 74 (test) and < 30 (vehicle control) mg test material/L The geometric mean of the analytical values after 0h (82 mg/L) and 48h (67mg/L) exposure deviated more than 20% from

the nominal values. The geometric value was taken for the exposure concentration.

Details on test conditions

TEST SYSTEM - Test vessel: - Type (delete if not applicable): open - Material, size, headspace, fill volume: Round-bottom test tubes calibrated to 10ml - Aeration: None - No. of organisms per vessel: 5 - No. of vessels per concentration (replicates): 4 - No. of vessels per control (replicates): 4 - No. of vessels per vehicle control (replicates): 4 - Biomass loading rate: 2 ml per test organism TEST MEDIUM / WATER PARAMETERS - Source/preparation of dilution water: Elendt artificial water OTHER TEST CONDITIONS - Adjustment of pH: None - Photoperiod: Continuous dark EFFECT PARAMETERS MEASURED (with observation intervals if applicable) : Immobilization TEST CONCENTRATIONS - Spacing factor for test concentrations: Not applicable

Reference substance (positive control)

no historic positive control data available in test report

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
24 h	EC50	> 74 mg/L	meas. (geom. mean)	test mat.	mobility	not applicable
48 h	EC50	> 74 mg/L	meas. (geom. mean)	test mat.	mobility	Not applicable

Reported statistics and error estimates

No statistics employed.

Remarks on results including tables and figures

Cumulative number of immobilized Daphnia during the course of the test, absolute and % values

Treatment	24h values			48h values		
	Number		%	Number		imm
	mobile	immobile		mobile	immobile	
Control	20	0	0	18	2	
VESTINOL 9 74 (mg/L)	19	1	5	18	2	
Vehicle	20	0	0	20	0	

control					
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Applicant's summary and conclusion

Validity criteria fulfilled

yes 1) not more the 10% of the control Daphnids immobilized 2) Dissolved oxygen concentration at end of test >3 mg/L

Conclusions

Di-isononyl phthalate ester (DINP) does not produce acute toxicity (immobility) to *Daphnia magna* at its maximum water solubility with a solubilizer (74 mg/L) under the conditions of this study.

Executive summary

In a 48 hour acute toxicity study, *Daphnia magna* were exposed to Di-isononyl phthalate ester (DINP) at a measured concentration of 74 mg/L (nominal concentration 100 mg/L) under static conditions. The 48 hour EC₅₀ was >74 mg/l. The study is considered suitable for classification and satisfies the guideline requirements for acute aquatic invertebrate toxicity studies.

Di-isononyl phthalate ester (DINP) does not produce acute toxicity (immobility) to *Daphnia magna* at its maximum water solubility with a solubilizer (74 mg/L) under the conditions of this study.

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 Dissemination Dossier

 Daphniatox Endpoint.004

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1984							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ Toxicol Chem 16(5):875-891					

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Materials and methods

Test guideline

Qualifier Guideline

according to other guideline: USEPA, (660/3-75-009) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians

Deviations

no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Vehicle

no

Test organisms

Test organisms (species)

other: Paratanytarsus parthenogenetica

Study design

Test type

static

Water media type

freshwater

Limit test

no

Total exposure duration

96 h

Remarks

Test conditions**Reference substance (positive control)**

no

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	LC50	> 0.08 mg/L	meas. (initial)	test mat.	mortality	
48 h	NOEC	0.08 mg/L	meas. (initial)	test mat.	mortality	

Reported statistics and error estimates

Statistical methods applied the following procedures: Moving average angle, Probit, and Bionomial Probability.

Applicant's summary and conclusion**Validity criteria fulfilled**

yes

Conclusions

Di-isononyl phthalate ester (DINP) does not produce acute toxicity to Paratanytarsus

parthenogenetica at its maximum water solubility (0.08 mg/L) under the conditions of this study.

Executive summary

The acute toxicity of di-isononyl phthalate ester (DINP) as measured by lethality to a midge (*Paratanytarsus parthenogenetica*) was evaluated in freshwater. DINP did not produce acute toxicity to a midge at its maximum water solubility (0.08 mg/L) under the conditions of this study.

Dossier > Document

 Dissemination Dossier

 Daphniatox Endpoint.005

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a U.S. EPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		1998							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Vehicle

yes

Details on test solutions

Test substance exposure solutions were prepared by adding 1 mg/L of the test substance and 10 mg/L of dispersant (Marlowet 40) to one liter of dilution medium. The dilution medium was Elendt's medium (Elendt and Bias, 1990), which was pH adjusted to 8 and aerated for >2 hours prior to use.

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

Organisms were supplied by in-house continuous cultures. Age = <24 hours old. Test organisms were not fed during the study.

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

48 h

Remarks

Test conditions

Test temperature

19 to 21 degrees C

pH

8

Dissolved oxygen

8 to 9 mg/L

Nominal and measured concentrations

A chronic and acute study were conducted following the same test solution preparation procedures. The nominal test substance loading was 1 mg/L for both tests. The mean measured test substance concentrations for the 21-day period were 1.0 mg/L in new exposure solutions and 1.0 mg/L in old exposure solutions, which represents 100 and 100%, respectively, of the nominally added test substance. However, the analytical results for the acute study were not included in the article. Because test conditions were similar for both the chronic and acute studies, the exposure concentrations in the acute study are likely to have been equivalent to the day 21 results.

Details on test conditions

Test substance exposure solutions were prepared by adding 1 mg/L of the test substance and 10 mg/L of dispersant (Marlowet 40) to one liter of dilution medium. The dilution medium was Elendt's medium (Elendt and Bias, 1990), which was pH adjusted to 8 and aerated for >2 hours prior to use. Two replicate test systems, containing five daphnids each (<24 hours old), were prepared for the single treatment, control and dispersant control. Test chambers were glass beakers with loose fitting lids. Each beaker contained 80 ml of exposure solution with a depth of approximately 5 cm. The study was conducted under static conditions in a temperature controlled room. Lighting = 16 hours light and 8 hours dark with a 15 minute transition period.

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	LL50	> 1 mg/L	nominal	test mat.	mortality	
48 h	NOELR	1 mg/L	nominal	test mat.	mortality	
48 h	EC50	> 1 mg/L	meas. (arithm. mean)	test mat.	mobility	Analytical data are from the chronic study conducted after the acute study. Since the solution preparation procedures were similar for the two studies, the analytical results from the chronic study are used to estimate the acute solution concentration.
48 h	NOEC	1 mg/L	meas. (arithm. mean)	test mat.	mobility	Analytical data are from the chronic study conducted after the acute study. Since the solution preparation procedures were similar for the two studies, the analytical results from the chronic study are used to estimate the acute solution concentration.

Details on results

LL50 >1.0 mg/L (with 10 mg/L dispersant), based on nominal loading level of the test substance. LC50 >1.0 mg/L (with 10 mg/L dispersant), based on analytical measurements for chronic study run subsequently with same test substance at same loading level. NOELR = 1.0 mg/L (with 10 mg/L dispersant), based on nominal loading level. NOELR = 1.0 mg/L (with 10 mg/L dispersant), based on analytical measurements for chronic study run subsequently with same test substance at same loading level. No immobilization or flotation effects were observed over the 48-hour test period in either the treatment or control solutions.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) does not produce acute toxicity (immobility) to *Daphnia magna*, based on the results from a study that evaluated DINP in the presence of a surfactant and at a loading level above its water solubility.

Executive summary

The acute toxicity of di-isononyl phthalate ester (DINP) as measured by immobility to the water flea (*Daphnia magna*) was evaluated in freshwater.

Di-isononyl phthalate ester (DINP) was not acutely toxic to *Daphnia* and results from this study are consistent with the extant aquatic toxicity database for DINP. Testing in this study was conducted at a loading rate with a surfactant that exceeds the water solubility of DINP (1 mg/L) after it was demonstrated that such a procedure was able to satisfactorily disperse the test substance and prevent flotation of the test organisms (a documented problem that can occur when evaluating the toxicity of similar substances).

Dossier > Document

 Dissemination Dossier

 Chondrophniatox Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	05.07.1995-26.07.1995
Reliability	2 (reliable with restrictions)		
Rationale for reliability	A GLP compliant study undertaken to EU test guidelines. The nominal test concentration of 100 mg/L was not confirmed by direct analytical measurement of achieved value.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1996-05-13

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD guideline 202 Part II (1984)	no

Principles of method if other than guideline

Following the development of new EC specifications for the 21-day Daphnia study, the test conditions departed from the OECD 202 Part II guideline. The study was likewise harmonized with the EC specification. No influence on the test result was expected. No details on the departures are specified, but the duration of the test was 21 days and there were 10 parents held individually per treatment group, which is an enhancement.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): VESTINOL 9 - Substance type: Technical substance - Physical state: Colorless liquid - Stability under test conditions: Stable (Stability control 26.09.1994) - Storage condition of test material: No data

Analytical monitoring

yes

Details on sampling

The analysis of test concentrations was determined in another test, using the same concentrations of DINP, prior to the subject test. No other details on the sampling procedure reported.

Vehicle

yes

Details on test solutions

A stock solution of 1 g of test substance and 1 g MARLOWET R 40/1 (Ricinusoil+40 EO: castor oil ethoxylate) was prepared in deionized water. Appropriate quantities of stock solution were diluted with synthetic fresh water (Elendt-1990) to provide the required test concentration. The solubilizing agent (MARLOWET) was considered to be a vehicle for the purposes of this robust summary.

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

TEST ORGANISM - Common name: Daphnia - Strain/clone: Daphnia magna Straus (clone 5) - Source: Bred in-house - Feeding during test - Food type: Senedasmus subspicatus - Amount: 0.5-1.0 x 10⁶ cells per Daphnia - Frequency: Daily

Study design

Test type

semi-static

Water media type

freshwater

Limit test

yes

Total exposure duration

21 d Remarks

Test conditions

Hardness

No data

Test temperature

20.4°C-22.2°C

pH

Control: 7.6-8.1 Test: 7.7-8.0

Dissolved oxygen

Control: 88-102% saturation Test: 87-101% saturation

Salinity

Not applicable

Nominal and measured concentrations

Nominal concentrations: 0 (control), 100 (Test) and 0 (vehicle control) mg/L Measured concentrations: - (control), 101 (Test) and - (vehicle control) mg/L Since the surrogate analytical

value for the test substance was within 20% of the nominal value, the exposure concentration for the subject study was taken as 100 mg/L.

Details on test conditions

TEST SYSTEM - Test vessel: - Type (delete if not applicable): Open - Material, size, headspace, fill volume: 100ml glass beakers, containing 60 ml - Aeration: No - Renewal rate of test solution (frequency/flow rate): 3 times per week - No. of organisms per vessel: 1 - No. of vessels per concentration (replicates): 10 - No. of vessels per control (replicates): 10 - No. of vessels per vehicle control (replicates): 10 - Biomass loading rate: 1 organism per 60 ml TEST MEDIUM / WATER PARAMETERS No data OTHER TEST CONDITIONS - Adjustment of pH: No - Photoperiod: 16/8 hours : light/dark - Light intensity: 250/50 Lux EFFECT PARAMETERS MEASURED (with observation intervals if applicable) : Number of offspring counted 3 times per week VEHICLE CONTROL PERFORMED: Yes RANGE-FINDING STUDY: No data

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	NOEC	> 101 mg/L	meas. (arithm. mean)	test mat.	reproduction	

Reported statistics and error estimates

Students t-test was used to compare the reproduction rates of test and control Daphnia

Remarks on results including tables and figures

Mortality and reproduction rate of surviving females exposed to DINP:

Concentration mg/L	Female			Reproduction rate		
	Surviving	Dead	Dead (%)	Young/female±SD	% inhibition	t-test(p = 0.05)
Control	10	0	0	78±2		
Test 100 mg/L	9	1	10	84±14	-20	-

Vehicle control	9*	0	0	103±14	-32	-
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* one male animal eliminated on Day 9

The first broods were produced on Days 7, 9 or 10, for individual parents in the test or control groups.

Applicant's summary and conclusion

Validity criteria fulfilled

yes 1) The mortality of the parent Daphnids did not exceed 20% 2) The mean number of live offspring per parent was >60

Conclusions

Di-isononyl phthalate ester (DINP) did not affect reproductive capacity in *Daphnia magna* after a 21-day exposure at a concentration achieved with a solubilizing agent, 101 mg/L (100 mg/L nominal), which is well above its solubility limit.

Executive summary

The chronic toxicity of di-isononyl phthalate ester (DINP) as measured by reproductive success to the water flea (*Daphnia magna*) was evaluated in freshwater. No significant effect was observed after a 21-day exposure at a concentration of 101 mg/L (100 mg/L nominal). This concentration was achieved with a solubilizing agent and is well above the water solubility of DINP.

These chronic invertebrate (*Daphnia magna*) toxicity data are consistent with valid data for several high molecular weight phthalate esters as summarized by Staples et al. (1997). These data together with the current study results show that high molecular weight phthalate esters, including diisononyl phthalate ester, do not produce chronic toxicity to *Daphnia magna*.

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[Dissemination Dossier](#)

[Chondrophniatox Endpoint.002](#)

[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	May - July 1997
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed an OECD standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1998							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 211 (Daphnia magna Reproduction Test)	yes : minor temperature deviation; it was not determined if 3 broods per female were produced, however, it is believed that a sufficient number of young per adult were produced in the controls of each study to evaluate the study.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Samples were removed from each treatment and the control ("old" solutions) on transfer days 3 or 2 times a week. Samples were also removed at termination.

Vehicle

no

Test organisms**Test organisms (species)**

Daphnia magna

Details on test organisms

Organism age = <24 hours. Organisms used in the study were from an in-house culture.

Study design**Test type**

semi-static

Water media type

freshwater

Limit test

yes

Total exposure duration

21 d Remarks

Test conditions

Hardness

Water hardness = 146 to 184 mg/L (as CaCO₃) Alkalinity = 51 to 56 mg/L (as CaCO₃)

Test temperature

Temperature = 19.8 degrees C (sd = 0.4)

pH

pH = 7.2 to 8.1

Dissolved oxygen

Dissolved oxygen = 8.0 to 8.9 mg/L

Details on test conditions

Test chambers were glass cylinders with Nitex mesh over the bottom. The mesh allowed the water accommodated fraction to circulate throughout the test chambers. The test chambers were suspended in the mixing vessels at a sufficient height to produce an approximate replicate volume of 250mL. The mixing vessels were approximately 13L glass battery jars with a glass and Teflon sampling port at the bottom. The test chambers were covered with plexiglass sheets to minimize contamination. Prior to the addition of the test substance, four replicate chambers were suspended into a glass mixing vessel containing 12.0L of dilution water. This was done to prevent the daphnids from becoming trapped by the test substance floating at the surface. The test substance was then added, volumetrically by glass syringe, approximately 24 hours prior to the addition of the test organisms. The mixing vessels were stirred continuously at a low speed (no vortex) on a magnetic stirplate with a Teflon coated stirbar. The test substance was observed floating on the surface of the mixing vessels. Treatments were prepared every 2 to 3 days (Sun, Tues, Thurs), for renewal, approximately 24 hours prior to the addition or transfer of test organisms. After the transfer of adults and the removal of neonates, all glassware was rinsed with dialyzed water to remove food residue. Observations for mortality/immobilization, abnormal behavior and appearance of the daphnids were performed on all replicate chambers at 24-hour intervals (+/- 1 hour). Adults were transferred, via pipette, to "new" solution every 2 to 3 days (Mon, Wed, Fri) at time of observation. Water quality measurements (pH, dissolved oxygen and temperature) were performed in each "new" and "old" treatment on transfer days and at termination. Once neonates were observed they were counted every 2 to 3 days (Mon, Wed, Fri) and then discarded. Daphnia were fed *Selenastrum capricornutum* (approx. 0.5 mL / replicate /

day days 0 to 6 and approx 1.0 mL / replicate / day days 7 to 21) and yeast/salmon starter/cereal leaves mixture (approx 3.0 mL / replicate / day) during testing. Daphnids were fed once per day during study 1 and the same amount of food was split over two feedings during study 2. This was done to increase the availability of the food to the daphnids and appeared to increase reproduction across all the treatments and controls as seen in the neonate production data.

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	EC50	> 0.0036 mg/L	meas. (arithm. mean)	test mat.	reproduction	
21 d	NOEC	0.0036 mg/L	meas. (arithm. mean)	test mat.	reproduction	

Details on results

Daphnia percent parent (Po) survival and percent reduction in reproduction were evaluated as the biological endpoints. Diisononyl phthalate ester (DINP) showed no statistical effect on survival or reproduction at its water solubility limit, achieved from a loading of 2.0 mg/L under the conditions of this test. Day 21 results from study 1: Po % Mortality % Reduction in Reproduction (a) Control 10 na DIDP 5 0 Day 21 results from study 2: Po % Mortality % Reduction in Reproduction (a) Control 18 na DIDP 13 15 (a) - percent reduction relative to control na - not applicable DINP showed varied results in neonate production between the two studies. However, neither value was statistically significant, based on a one-tailed t-test at a confidence of 95%, when compared to the control. Daphnids were fed once per day during study 1 and the same amount of food was split over two feedings during study 2. This was done to increase the availability of the food to the daphnids and appeared to increase reproduction across all the treatments and controls as seen in the neonate production data. Neonate production study 1, day 21 Control average = 46.9 (replicates were 44.3, 48.9, 43.7, 50.6) DINP treatment average = 49.5 (replicates were 51.0, 47.7, 45.6, 53.7) Neonate production study 2, day 21 Control average = 71.8 (replicates were 78.8, 79.7, 62.4, 66.3) DINP treatment average = 61.2 (replicates were 66.3, 62.1, 56.1, 60.1)

Reported statistics and error estimates

No significant effect on daphnid reproduction, when compared to the control, was observed for the test substance based on a one-tailed t-test at a 95% confidence level.

Remarks on results including tables and figures

Two trials were performed for the first study. The first trial was terminated on Day 8 due to 23% mortality/immobilization in the control. The guideline requires control mortality not to exceed 20% for the 21-day period. Excessive control mortality/immobilization is not infrequent in *Daphnia magna* and is unrelated to exposure to the test substance.

Two trials were also performed for the second study. The first trial was terminated on Day 4 due to 90% mortality/immobilization in a single replicate. No other mortality/immobilization was observed in the treatment. The data and results of the second trial were included in the report.

The test substance showed varied results in neonate production between the two studies. However, neither value was statistically significant, based on a one-tailed t-test at a confidence of 95%, when compared to the control.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) did not affect reproductive capacity in *Daphnia magna* at the maximum water soluble concentration achievable under the test conditions, 0.0036 mg/L.

Executive summary

The chronic toxicity of di-isononyl phthalate ester (DINP; CAS #68515-48-0) as measured by reproductive success to the water flea (*Daphnia magna*) was evaluated in freshwater. No significant effect was observed at the maximum DINP water solubility under the conditions of the test, which measured 0.0036 mg/L.

These chronic invertebrate (*Daphnia magna*) toxicity data are consistent with valid data for several high molecular weight phthalate esters as summarized by Staples et al. (1997). These data together with the current study results show that high molecular weight phthalate esters, including diisononyl phthalate ester, do not produce chronic toxicity to *Daphnia magna*.

Dossier > Document

 Dissemination Dossier

 Chondaphniatox Endpoint.003

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed an OECD standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		1998							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 211 (Daphnia magna Reproduction Test)	no

Principles of method if other than guideline

The test method followed the Daphnia chronic testing procedure described in OECD guideline 202 (1984) with the use of a dispersant, castor oil 40-ethoxylate (Marlowet 40), in accordance with guideline specifications.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Test substance analyses were performed on new and old exposure solutions.

Vehicle

yes

Test organisms

Test organisms (species)

Daphnia magna

Study design

Test type

semi-static

Water media type

freshwater

Limit test

yes

Total exposure duration

21 d Remarks

Test conditions

Hardness

Water hardness = >140 mg/L (as CaCO₃) Alkalinity = >100 mg/L (as CaCO₃)

Test temperature

Temperature = 20 +/- 1.0 degree C

pH

pH = approximately 8

Dissolved oxygen

Dissolved oxygen = 8-9 mg/L

Nominal and measured concentrations

The mean measured test substance concentrations were 1.0 mg/L in new exposure solutions and 1.0 mg/L in the old exposure solutions, which represents 100 and 100%, respectively, of the nominally added test substance.

Details on test conditions

Test substance exposure solutions were prepared using stock dispersions prepared by adding 100 mg substance and 1000 mg dispersant (castor oil 40-ethoxylate; Marlowet 40), then bringing the test solution to 1 L by adding dilution medium. The dilution medium was Elendt's medium (Elendt and Bias, 1990), which was pH adjusted to 8 and aerated for >2 hours prior to use. Ten replicate test systems with 1 daphnid each (< 24 hours old) were prepared in glass beakers with loose fitting lids. Each beaker contained 80 ml of exposure solution with a depth of approximately 5 cm. The photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period. The exposure solution was renewed every Monday, Wednesday, and Friday. On each renewal day the parent organism (Po) was transferred to a new exposure solution and neonates (F1) were counted. Water quality measurements including dissolved oxygen concentration and pH were determined at every renewal for the new and old exposure and control solutions. Standard daily feeding rates with the cultured alga, *Chlorella vulgaris*, was supplemented with microencapsulated food, "Frippak Booster".

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	EC50	> 1.1 mg/L	meas. (arithm. mean)	test mat.	reproduction	
21 d	NOEC	1.1 mg/L	meas. (arithm. mean)	test mat.	reproduction	

Details on results

Daphnia parent (Po) survival, reproduction (cumulative number of offspring, F1, per live parent), and parent length were evaluated as the biological endpoints. Diisononyl phthalate ester showed no effect on survival, reproduction, and length at a loading of 1.1 mg/L test substance and 10 mg/L dispersant under the conditions of this test. Po % Mortality Mean F1/Surviving Po Mean Length Po Test Substance 10 100 (sd=12) 4.2 (sd=0.18) Control 0 93 (sd=9) 4.1 (sd=0.17)

Remarks on results including tables and figures

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) did not produce reproductive effects in *Daphnia magna* at a test concentration in excess of its water solubility (1.1 mg/L), which was achieved using a dispersant.

Executive summary

The chronic toxicity of di-isononyl phthalate ester (DINP) as measured by reproductive success to the water flea (*Daphnia magna*) was evaluated in freshwater. No significant effect was observed under the conditions of the test, which evaluated DINP, measured at 1.1 mg/L, with a surfactant. These chronic invertebrate (*Daphnia magna*) toxicity data are consistent with valid data for several high molecular weight phthalate esters as summarized by Staples et al. (1997). These data together with the current study results show that high molecular weight phthalate esters, including di-isononyl phthalate ester, do not produce chronic toxicity to *Daphnia magna*.

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 Dissemination Dossier

 Algaetox Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The study is rated 1 because the test procedure followed an accepted test guideline and was conducted under GLP. The data are consistent with known toxicological properties of similar high molecular weight phthalate ester substances. Control chlorophyll or cell counts were not reported.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1984							
publication	Adams W, Biddinger G, Robillard K and Gorsuch J	1995	A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms	Environ. Toxicol. Chem. 14(9): 1569-1574					
publication	Staples C, Adams W, Parkerton T, Gorsuch J, Biddinger G and	1997	Aquatic toxicity of eighteen phthalate esters	Environ Toxicol Chem 16(5):875-891					

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Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA 600/9-78-018, Printz Algal Assay Bottle Test	yes Control chlorophyll or cell counts were not reported.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Test organisms

Test organisms (species)

Selenastrum capricornutum (new name: *Pseudokirchnerella subcapitata*)

Details on test organisms

Algal culture stock was obtained from University of Texas at Austin, TX, USA.

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

120 h

Remarks

Test conditions

Test temperature

Test temperature = 22+/-2 Degrees C

pH

pH = 7.4 at test initiation

Details on test conditions

Algal Growth Medium was used as the control and diluent. 10 uL of test substance was added to 1.0 L of sterile water to form a saturated phthalate solution. This solution was sonicated for 1 minute and allowed to settle for 4 hours. After settling, the water soluble fraction (WSF) was removed for testing. Initial algal concentration was 2.0 E4 cells/ml. Only one treatment level was evaluated (100% WSF) because earlier phthalate testing suggested that toxic effects were not expected with higher molecular weight phthalate esters with low water solubility. Lighting = 4,700 lux

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
5 d	EC50	> 1.8	meas. (arithm.	test mat.	other: biomass and	

		mg/L	mean)		growth rate
5 d	NOEC	1.8	meas. (arithm.	test mat.	other: biomass and
		mg/L	mean)		growth rate

Details on results

120 hr (5 day) EC50 >1.8 mg/L (based upon time zero analytical samples). Value was recalculated as per U.S. EPA current practices using mean of measured initiation and termination samples as reported in Staples et al. (1997). Mean measured values were used in the final EC50 calculation. Nominal test concentration as a percent of a saturated solution: 0 (control) and 100.0%. Mean measured test concentrations of time 0 and 120 hr values: <0.10 and 1.8 mg/L (detection limit was 0.10 mg/L). Analytical samples taken at time zero and on a composite of replicates at termination. In-vivo chlorophyll a, measured until less than 5% change. Both cell number and in-vivo chlorophyll a, measured at termination. Control chlorophyll a or cell counts were not reported. Analytical samples were taken at time zero and on a composite of replicates at termination. Chlorophyll a percent change relative to control on sampling days and cell number on day 5 results: Conc. Chlorophyll a Percent Change From Control (mg/L) Day 1 Day 2 Day 3 Day 4 Day 5 Cell # Day 5 1.8 -6 -10 -72 -16 -12 -34

Reported statistics and error estimates

Moving average angle, Probit or Bionomial

Remarks on results including tables and figures

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) does not produce toxicity (growth inhibition) to *Selenastrum capricornutum* at its maximum water solubility (1.8 mg/L) under the conditions of this study.

Executive summary

The toxicity of di-isononyl phthalate ester (DINP) as measured by biomass and growth rate to the green alga (*Selenastrum capricornutum*) was evaluated in freshwater. No significant effect was observed at the maximum DINP water solubility under the conditions of this study, which measured 1.8 mg/L.

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 Dissemination Dossier

 Algaetox Endpoint.002

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	13.12.1994-16.12.1994
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed an EU standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1995-01-21

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method C.3 (Algal Inhibition test) conducted according to 92/69/EEC-(test method C.3 not explicitly stated but can be inferred)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): VESTINOL 9 - Substance type: Technical material - Physical state: Colorless liquid

Analytical monitoring

yes

Details on sampling

For analytical determination of the test material concentrations, samples were prepared in the same way as for the test, but without algae. Assays were undertaken at 0 h and 72 h. Stock solution and vehicle control samples were also analyzed for test material concentration.

Vehicle

yes

Details on test solutions

The stock solution was prepared by adding 1 g test substance and 1 g MARLOWET R 40 (Rucinusol +40 EO castor oil ethoxylate) to 1 L deionized water. The test and control solutions were prepared by appropriate dilution in growth medium as described in EEC test guidelines (92/69/EEC). The stability of the test substance in the test medium was demonstrated during analysis for achieved concentration of the test substance at 0 h and 72 h exposure (see below).

Test organisms

Test organisms (species)

Desmodesmus subspicatus (reported as *Scenedesmus subspicatus*)

Details on test organisms

TEST ORGANISM - Common name: Green Algae - Strain: *Scenedesmus subspicatus* (CHORDAT (86.81 SAG)) - Source (laboratory, culture collection): Water, Soil and Air hygiene Institute, Berlin and subsequent in-house culture - Age of inoculum (at test initiation): 3 days

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

72 h

Remarks

Test conditions**Test temperature**

22°C

pH

8.1-8.6 at the beginning of the test 7.2-9.4 at the end of the test

Nominal and measured concentrations

Nominal concentrations: 0 (control), 100 (test substance) and 0 (vehicle control) mg/L Measured concentrations: <30 (control), 88 (test substance) and <30 (vehicle control) mg/L. The mean analytical value in the test group after 0 h (88 mg/L) and 72 h (88 mg/L) exposure did not deviate by more than 20% from the nominal value. The nominal value was therefore taken as the exposure value.

Details on test conditions

TEST SYSTEM - Test vessel: - Type (delete if not applicable): Open - Material, size, headspace, fill volume: Erlenmeyer flasks - Aeration: Yes - Type of flow-through (e.g. peristaltic or proportional diluter): static - Initial cells density: 20,000 cells/ml - Control end cells density: 510,000 cells/ml - No. of vessels per concentration (replicates): 5 - No. of vessels per control (replicates): 8 - No. of vessels per vehicle control (replicates): 8 GROWTH MEDIUM - Standard medium used: Yes OTHER TEST CONDITIONS - Sterile test conditions: Yes - Adjustment of pH: No - Photoperiod: Continuous light - Light intensity and quality: 8,000 lux (approximate), white EFFECT PARAMETERS MEASURED (with observation intervals if applicable) : - Determination of cell concentrations: Photometric absorbance at 685 nm and conversion by calibration to cell numbers/ml.

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
72 h	EC50	> 88 mg/L	meas. (geom. mean)	test mat.	growth rate	Not applicable
72 h	EC50	> 88 mg/L	meas. (geom. mean)	test mat.	biomass	Not applicable
72 h	NOEC	88 mg/L	meas. (geom. mean)	test mat.	cell number	Not applicable

Details on results

- Exponential growth in the control (for algal test): Yes

Remarks on results including tables and figures

Mean values of cell numbers in relation to the exposure time and the test substance concentration

Concentration (mg/L)	Cell number x10 ⁴ cells/ml			
	Test period (h)			
	0 h	24 h	48 h	72 h
Control	2	5	17	51
Test 100 mg/L	2	6	15	62
Vehicle control	2	5	17	65

Applicant's summary and conclusion

Validity criteria fulfilled

yes The biomass of the control cultures increased by more than 16 fold within the 72 h period

Conclusions

Di-isononyl phthalate ester (DINP) does not produce toxicity (growth inhibition) to *Desmodesmus subspicatus* at its maximum water solubility with the use of a dispersant (88

mg/L) under the conditions of this study.

Executive summary

In a 72 hour growth inhibition study, green algae (*Scenedesmus subspicatus*) were exposed to DINP at a nominal concentration of 100 mg/L (measured 88 mg/L) under static conditions. The 72 hour E_rC_{50} and 72 hour E_bC_{50} were >88 mg/L. The NOEC was also 88 mg/L.

The toxicity of di-isononyl phthalate ester (DINP) as measured by biomass and growth rate to the green alga (*Selenastrum capricornutum*) was evaluated in freshwater. No significant effect was observed at the maximum DINP water solubility with a dispersant under the conditions of this study, which measured 88 mg/L.

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 Dissemination Dossier

 Bactox Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	April 1997
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the study procedure followed an accepted test guideline, but was not conducted under GLP. The data are consistent with known toxicological properties of similar high molecular weight phthalate ester substances.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1998							1998-02-05

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	no

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Details on sampling

Samples for analysis were taken from a 1:10 dilution (100 mg/L) of the stock solution (1000 mg/L) used for the activated sludge inhibition test.

Vehicle

yes

Details on test solutions

A stock dispersion of DINP was prepared by weighing 1.0 g of the DINP and 10 g of Tween 20 into a one liter volumetric flask, adding approximately 200 ml of glass distilled water, and mixing for an hour. The stock dispersion was then diluted to 1 liter with additional glass distilled water, resulting in a stock concentration of 1000 mg/L.

Test organisms

Test organisms (species)

activated sludge of a predominantly domestic sewage

Details on inoculum

Soluble organic material was removed by washing the sludge solids with three consecutive tap water rinses, and the final volume adjusted to achieve an approximate total suspended solids of 4 g/l. The solids concentration of the respiration inhibition test system were approximately 1.6 g/l after dilution with the remaining components. The inoculum was fed with synthetic substrate combining 16 grams peptone, 11 grams beef extract, 3 grams urea, 0.7 grams sodium chloride, 0.4 grams calcium chloride, 0.2 grams magnesium sulphate heptahydrate and 2.8 grams potassium hydrogen sulphate. The mixture was diluted to a 1.0 liter final volume with glass distilled water.

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

30 min

Remarks

Test conditions

Test temperature

Approximately 22 Degrees C

Dissolved oxygen

Dissolved oxygen was 7.71, 7.41, and 7.04 in the DIDP test systems at time 0. Dissolved oxygen was 7.63, 7.83, and 7.62 in the control systems at time 0. After a 30-minute contact time, the contents of each test system were poured into a 300 mL BOD bottle and DO was measured at one minute intervals over a 10 minute period, or until the DO level was approximately 2.5 mg /L, which ever occurred first. Control samples were analyzed at the initiation, the mid point, and the termination of the inhibition testing.

Nominal and measured concentrations

Nominal concentration = 100 mg/L. Measured concentration = 83.9 mg/L (mean of two replicates, 84.8 and 83.0 mg/L)

Details on test conditions

Diisodecyl phthalate ester (DIDP) was obtained from Exxon Chemical, New Milford, CT. Due to the very low water solubility of this substance, a surfactant, sorbitan monolaurate 20 ethoxylate (Tween 20), was used. The Tween 20 was obtained from Mallinckrodt (US), and had a purity of 99+%. DIDP was tested at a nominal concentration of approximately 100 mg/L. A positive control, 3,5-dichlorophenol (DCP), was also tested at 5, 15, and 30 mg/L, which is the expected range for the EC50 concentration (OECD test guideline 209). Preliminary testing of Tween 20 at

1000 mg/L (i.e., the concentration of Tween 20 included in the phthalate dispersions tested) indicated neither stimulation or inhibition of respiration rates. Fresh activated sludge, used as the source of microbial inoculum in the respiration inhibition test, was obtained from a domestic wastewater treatment facility operated by the Pike Brook Sewage Treatment Plant in Belle Mead, New Jersey, USA. Soluble organic material was removed by washing the sludge solids with three consecutive tap water rinses and the final volume adjusted to achieve an approximate total suspended solids (TSS) of 4 g/L. The solids concentration of the respiration inhibition test systems were approximately 1.6 g/L after dilution with the remaining components. A synthetic sewage solution containing an excess of biodegradable substrate was used to feed the fresh activated sludge inoculum in order to stimulate a high metabolic rate. The synthetic substrate solution was prepared by combining the following: 16 grams peptone, 11 grams beef extract, 3 grams urea, 0.7 grams sodium chloride, 0.4 grams calcium chloride, 0.2 grams magnesium sulfate heptahydrate, and 2.8 grams potassium hydrogen phosphate. The mixture was diluted to a 1.0 liter final volume with glass distilled water. Respiration inhibition was assessed by monitoring dissolved oxygen concentration (DO mg/L) in each test system using an Orion pH/selective ion meter, model 720A, coupled to an Orion dissolved oxygen probe, model 97-0899. Triplicate test systems were evaluated. Each test system was prepared in a 500 mL beaker. Each 500 mL beaker received the following additions (not in order of actual sequence followed): 200 mL of fresh activated sludge (TSS 4 g/L), 16 mL of synthetic sewage, 50.0 mL of the stock phthalate dispersion solution, and a sufficient amount of water to bring the total volume of the system to 500 mL. Triplicate control systems were also prepared which contained sludge, synthetic sewage, 50.0 mL of a 10,000 mg/L Tween 20 solution and sufficient water to bring the total volume of the system to 500 mL. The DCP test systems were prepared in 500 mL beakers by pipetting 5, 15, and 30 mL of a 500 mg/L stock solution to individual test systems, followed by addition of 50.0 mL of the 10,000 mg/L Tween 20 solution, sludge, synthetic sewage, and water. After a 30-minute contact time, the contents of each test system were poured into a 300 mL BOD bottle and DO was measured at one minute intervals over a 10 minute period, or until the DO level was approximately 2.5 mg /L, which ever occurred first. Control samples were analyzed at the initiation, the mid point, and the termination of the inhibition testing.

Reference substance (positive control)

yes 3,5-dichlorophenol

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
30 min	EC50	> 83.9 mg/L	meas. (initial)	test mat.	respiration rate	
30 min	NOEC	83.9 mg/L	meas. (initial)	test mat.	respiration rate	

Details on results

No appreciable inhibition of respiration was measured at the highest dose tested, 100 mg/L (nominal), which measured 83.9 mg/L. Therefore, an EC50 value was not calculable. Oxygen consumption rate for one test system replicate was slightly lower than the mean of the controls. Since the respiration rate was within $\pm 15\%$ of the mean control respiration rate, there is no appreciable inhibition at this concentration. The respiration rate of control replicate 1, was slightly greater than the limit recommended by the guideline. The guideline recommends that control respiration rates be within $\pm 15\%$ of their mean. The respiration rate of control replicate 1 (of 2 replicates) was approximately 19% higher than the mean. The cause of this may have been drift by the probe or the probe may not have been fully saturated for the first replicate reading. However, since the EC50 for the positive control was within the range specified by the guideline, the difference in respiration rate exhibited by one of the controls was not considered to have invalidated the study.

Results with reference substance (positive control)

In order for the test to be considered valid, the OECD Guideline 209 requires that the EC50 for the positive control (3,5-DCP) be in the range of 5-30 mg/L. The EC50 obtained for 3,5-DCP was 12.7 mg/L, which satisfied the guideline requirement.

Reported statistics and error estimates

Probit analysis was used to determine the EC50 of 3, 5 dichlorophenol.

Remarks on results including tables and figures

The measured mean concentration of diluted stock solution was 83.9 mg/L (84.8 and 83.0 mg/L for replicates 1 and 2 respectively). The result was considered to be in reasonable agreement with the expected concentration of 100 mg/L.

Activated sludge respiration inhibition: dissolved oxygen concentrations, oxygen consumption rate and % inhibition:

Sample	% inhibition	O ₂ consumption Rate mg/L/hr = *Slope of line x 60	Time, minutes, for dissolved oxygen concentration, mg/L							
			0	1	2	3	4	5	6	7
Control 1		-17.38	7.83	6.11	5.76	5.46	5.16	4.87	4.60	4.37
DINP R1	-1.52	-21.06	6.59	2.96	2.61	2.25	1.90	1.58		
DINP R2	-2.88	-21.34	7.70	5.05	4.75	4.38	4.01	3.65	3.30	2.94
DINP R3	3.98	-19.92	7.32	4.59	4.25	3.94	3.59	3.26	2.94	2.61
3,5 DCP R1	23.37	-21.06	6.59	2.98	2.61	2.25	1.90	1.58		
3,5 DCP R2	60.56	-21.34	7.70	5.05	4.75	4.38	4.01	3.65	3.30	2.94
3,5 DCP R3	69.92	-19.92	7.32	4.59	4.25	3.94	3.59	3.26	2.94	2.61
Control 2		-24.11	7.62	6.28	5.92	5.53	5.14	4.73	4.30	3.90

Control 3	23.17	7.63	5.19	4.81	4.45	4.06	3.68	3.28	2.88	2.49
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* Slope of line = (Y2 -Y1)/(X2 -X1): Y2 = last DO value of line, Y1 = DO at 1 minute . X2 - last minute of reading, X1 = first minute

Time zero value not included in slope of line

% Inhibition = $(1 - [(2 \times \text{test material rate}) / (\text{control 1 rate} + \text{control 2 rate})]) \times 100$

The oxygen consumption for DINP replicate 3 was slightly lower than the mean of the controls, equivalent to a percentage inhibition of 3.98. Since the respiration rate was within $\pm 15\%$ of the mean control respiration rate, there is no appreciable inhibition at this concentration.

The EC₅₀ obtained for 3,5 DCP was 12.7 mg/L which satisfied the guideline requirement.

The respiration rate of control replicate No.1, was slightly greater than the limit recommended by the guideline. The guideline recommends that control respiration rates be within $\pm 15\%$ of their mean. The respiration rate of control replicate No.1 was approximately 19% higher than the mean. The cause of this may have been drift by the probe, or the probe may not have been fully saturated for the first replicate reading. However, since the EC, for the positive control was within the range specified by the guideline, the difference in respiration rate exhibited by the first control was not considered to have had an adverse effect on the integrity of the data.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) was not inhibitory to sludge respiration at a high test material/sludge loading of 100 mg/L (83.9 mg/L measured). Therefore, DINP is not expected to adversely impact microbial activity in a wastewater treatment plant.

Executive summary

Di-isononyl phthalate ester did not inhibit microbial respiration in a sludge sample at a concentration that exceeds its water solubility, 83.9 mg/L. Therefore, DINP is not expected to adversely impact microbial activity in a wastewater treatment plant.

Dossier > Document

 **Dissemination Dossier**

 **Bactox Endpoint.002**

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	April 1997
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the study procedure followed an accepted test guideline, but was not conducted under GLP. The data are consistent with known toxicological properties of similar high molecular weight phthalate ester substances.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1998							1998-02-05

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: Microtox(tm) Assay	no

Principles of method if other than guideline

The assay measures photo-luminescence response of the marine bacterium *Photobacterium phosphoreum*. Adverse effects are measured as a decrease in photo-luminescence.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product

Analytical monitoring

yes

Vehicle

yes

Test organisms

Test organisms (species)

Photobacterium phosphoreum

Study design

Test type

static

Water media type

saltwater

Limit test

yes

Total exposure duration

15 min

Remarks

Test conditions

Test temperature

Approximately 22 Degrees C

Details on test conditions

A nominal concentration of 100 mg/l test substance with Tween 20 dispersant was prepared in lab dilution water contained in a glass vessel that included a teflon stir bar. The vessel was then placed on a stir plate and agitated for 24 hrs. A sample of the water accommodated fraction was then removed from a port at the bottom of this vessel for microtox testing. Serial dilutions representing 100%, 50%, 25%, 12.5% and 6.25% were prepared and assayed with the Microtox assay according to procedures specified by the manufacturer. The 100% test material concentration was analytically verified as 83 mg/L.

Reference substance (positive control)

yes

Results and discussions

Effect concentrations

Duration Endpoint		Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
5 min	NOEC	83.9 mg/L	meas. (initial)	test mat.	other: Photo-luminescence	
15 min	NOEC	83.9 mg/L	meas. (initial)	test mat.	other: Photo-luminescence	

Details on results

The study included two exposure intervals, 5 and 15 minutes. The test material did not demonstrate inhibition to photo-luminescence at either exposure interval.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) did not inhibit photo-luminescence in the bacterium, *Photobacterium phosphoreum*, at a high test material loading of 100 mg/L (83.9 mg/L measured). Therefore, the DINP is not expected to adversely impact microbial activity.

Executive summary

Di-isononyl phthalate (DINP) ester did not inhibit photo-luminescence in the bacterium, *Photobacterium phosphoreum*, at a concentration that exceeds its water solubility, 83.9 mg/L. Therefore, the DINP is not expected to adversely impact microbial activity.

Dossier > Document

 Dissemination Dossier

 Aquatic other Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the study applied valid scientific testing principles, and the results were reviewed for reliability and assessed as valid. However, the study was not conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2001							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available	other guideline: No standard guideline	

Principles of method if other than guideline

The study was designed to assess the toxicity of diisononyl phthalate ester (DINP), initially added to sediment, to the early life stage of the moor frog (*Rana arvalis*) in a test system composed of natural sediment over which water was added to create a two-phase test system. The exposure included an approximately 14 day period during which newly fertilized moor frog eggs were exposed in the test system. Exposure of newly hatched tadpoles continued for a total of 35 days during which the test organisms were observed for mortality and measured for growth.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

99.9% obtained from EXXON Chemicals Holland Inc (Botlek Rt Nederland).

Analytical monitoring

yes

Details on sampling

Prior to and at the end of exposure.

Vehicle

no

Details on test solutions

The sediment for the experiments was collected in Lake Stensjiin during March 2000. Sediment was collected both from shallow and deep parts of the lake using a Ponar sediment sampler (a small Van Ween sampler). Because of the thick ice cover (100 cm) the shore sediment representing a coarse type could only be sampled as shallow as, 1.2m depth. Each grab sample of app. 4 litre sediment represent 0-5 cm of the sediment layer. Sediment samples from each location were mixed in the field and transferred to stainless steel containers for transportation to the laboratory. The sediment from the shore (1.2-m depth) was of a coarse type containing non-degraded materials i.e. birch leaves, pine needles, small bark pieces, grass and small twigs at the surface. Sediment from the deepest part (8-m depth) consisted mostly of degraded material and represented a fine type of sediment. The phthalate ester is dissolved in acetone and carefully mixed with established uncontaminated, homogenized air-dried sediment. The solvent is carefully evaporated under reduced pressure at 40°C in an evaporator. This sediment is added to the fresh uncontaminated test sediment in glass jars sealed with PTFE- or aluminum foil lined cap and thoroughly blended to achieve the nominal concentration targeted. To facilitate homogeneity and equilibrium, the sediment samples were agitated on a shaking board or roller mill at 50C and 100C respectively, until equilibrium was established. The agitation was performed low agitation speeds to prevent erosion of the sediment particles that may increase pore water TOC concentration and thereby alter the equilibrium.

Test organisms

Test organisms (species)

Rana arvalis

Details on test organisms

Newly fertilized eggs of the moor frog (*Rana arvalis*) were field collected in Sweden and were determined to be in the morula or blastula development stage. Collected eggs were transported to the lab in a cooling bath with charcoal filtered aeration. Transport temperature was between approximately 8 to 10 degrees C. To avoid rapid development of the eggs once in the lab, the holding temperature was lowered to approximately 4 degrees C until the start of the study.

Study design

Test type

static

Water media type

freshwater

Limit test

no

Total exposure duration

35 d

Remarks

Test conditions

Test temperature

Approximately 10 degrees C. The experiments took place in water baths with a continuous heating system constructed with rubber and copper pipes to maintain 10°C in the water.

pH

pH = approximately 5.5 to 7.0

Dissolved oxygen

Dissolved oxygen = approximately 80 to 100%

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	NOEC	858 mg/kg sediment dw	meas. (arithm. mean)	test mat.	other: frog-egg hatching	
35 d	NOEC	858 mg/kg sediment dw	meas. (arithm. mean)	test mat.	other: tadpole survival and growth	

Details on results

Neither hatching success nor median hatching time was statistically significantly influenced by DINP in the sediment. There are no significant differences in mortality or deformities at any of the DINP levels compared to control. No significant difference in growth based on weight measurements could be established for any of the treatments or sediment type.

Reported statistics and error estimates

Moorfrog egg hatchability data were first log-transformed and compared with a one way ANOVA (analysis of variance) for heterogeneity (Microsoft Excel 7.0). Each treatment was then compared to the relevant control group with a two-sided Dunnett's test (SPSS). The statistical evaluation of growth, mortality and deformations were made with log-transformed data using Student's T-test (Microsoft Excel 7.0). The median hatching time was calculated using probit analysis (Litchfield and Wilcoxon, 1949).

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester does not effect the egg hatching followed by tadpole survival and growth of the moor frog (*Rana arvalis*) at a very high test material/soil loading, 858 mg/kg

sediment (dw).

Executive summary

The toxicity of di-isononyl phthalate ester (DINP) as measured by moor frog (*Rana arvalis*) egg hatching followed by tadpole survival and growth was evaluated in a natural sediment. No significant effect on frog egg hatching following a 21-day exposure and tadpole survival and growth following a 35-day exposure was demonstrated in the sediments dosed with DINP at a measured concentration of 858 mg/kg sediment (dw).

Dossier > *Document*

 Dissemination Dossier

 Sedimentdwellingtox Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a USEPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		2001							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA/600/R-94/024 Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Purity: 99.7% Diisononyl phthalate ester (DINP) was obtained from Exxon Chemical, New Milford, CT. Due to the very low water solubility of this substance, a surfactant, sorbitan monolaurate 20 ethoxylate (Tween 20), was used. The Tween 20 was obtained from Mallinckrodt (US), and had a purity of 99+%.

Analytical monitoring

yes

Details on sampling

During mixing, samples of sediment were collected at periodic intervals for analysis of PE concentrations in the bulk sediment and pore water. Homogeneity of mixing was determined from multiple sediment samples usually collected on day 6. The degree to which an equilibrium in pore-water concentrations had been achieved was determined from samples of pore water collected on two occasions, usually days 3 and 6. During the test, samples of bulk sediment, pore water, and overlying water were sampled on days 0 and 10 and at two intermediate times for measurement of test substance concentrations. Overlying water samples (500 ml) were collected with an Eppendorf pipettor approximately 1 to 2 cm above the sediment. Samples were analyzed by high performance liquid chromatography. The overlying water was then siphoned out of the beaker to obtain sediment samples. Sediment cores were collected using a glass tube (13-mm inner diameter). Pore water was defined as the supernatant liquid obtained from centrifuging wet sediment at 10,000 g for 30 minutes. Following centrifugation, an aliquot of the pore water was pipetted into a vial and a 1:1 mixture of acetonitrile and deionized water added to bring the sample volume to 1 ml. The vial was capped and mixed, after which the contents were centrifuged to remove any precipitate present. The remainder of the pore water was removed from the sample tube and the weight of the bulk sediment was determined. Sediments were extracted by adding 5 ml of acetonitrile to the centrifuged pellet (from the pore-water preparation step), mixing the pellet and acetonitrile with a stainless steel spatula, and sonicating for 15 minutes in a 35 degrees C water bath. Samples were mixed with a clean spatula and then sonicating for an additional 15 minutes. The tube was centrifuged in a bench-top centrifuge for 5 minutes and a sample of the supernatant diluted with a mixture of acetonitrile and water (1:1, v/v). The remaining supernatant was removed from the sediment and the sediment dried overnight at 27 degrees C for dry weight (%) estimates.

Vehicle

yes

Details on sediment and application

Uncontaminated, natural sediment samples for this study were collected from Airport Pond (St. Louis County, MN, USA) and West Bearskin Lake (Cook County, MN, USA). Samples were

collected with a Ponar dredge, placed into clean polyethylene containers, and stored at 4 degrees C until used. For the test, the sediments were homogenized in a 119-L stainless steel container using a commercial drill with a stainless steel mortar-mixing paddle. To achieve a desired medium total organic carbon (TOC) sediment level, aliquots from Airport Pond and West Bearskin Lake were blended. A summary of TOC content and particle size distribution for the two blended sediments follows (+/- standard deviation in parentheses): the mean TOC content was 4.8% (0.65) ; sand content was 46.9% (4.09); silt content was 30.2% (3.59); coarse clay content was 2.3% (1.47); and fine clay content was 20.5% (1.86). The test substance was dissolved in acetone and coated onto a 20% aliquot of a wet sediment sample. The sediment aliquot was dried and then placed into a 4-L glass jar, which was rotated in an air stream to evaporate the acetone. Deionized water equal to the volume lost in drying the 20% portion of wet sediment was then added to the dried sediment aliquot, mixed, and added back into the jar containing the remaining 80% wet sediment sample. The jar was sealed with a Teflon-lined cap and rotated on a roller mill in a cold room (approximately 4 degrees C) for approximately 6 days at a speed of approximately 8 rpm.

Test organisms

Test organisms (species)

Chironomus tentans

Details on test organisms

Groups of 50 (five replicates of 10 organisms)

Study design

Study type

laboratory study

Test duration type

long-term toxicity

Test type

static

Water media type

freshwater

Type of sediment

natural sediment

Limit test

yes

Total exposure duration

10 d

Remarks

Test conditions

Hardness

Measured, but not reported; was likely to have met guideline requirements.

Test temperature

Measured, but not reported; was likely to have met guideline requirements.

pH

Measured, but not reported; was likely to have met guideline requirements.

Dissolved oxygen

Measured, but not reported; was likely to have met guideline requirements.

Ammonia

Measured, but not reported; was likely to have met guideline requirements.

Nominal and measured concentrations

Nominal test substance loading = 3000 mg/kg sediment dry weight Measured test substance concentration = 2680 mg/kg sediment dry weight (range = 2280-3020 mg/kg sediment dry weight)

Details on test conditions

Sediment testing proceeded in two phases. The first phase evaluated the effectiveness of the mixing process in achieving a homogeneous distribution of test substance in bulk sediment, the

establishment of equilibrium concentrations of test chemicals in the pore water, and the stability of the test substance in pore water under simulated toxicity test conditions. This phase was used to determine, in part, if meaningful toxicity tests could be performed (i.e., to assess if stable exposure concentrations could be maintained during the test period). The second phase evaluated the toxicity of the test substance in spiked sediments toward the test species. During the mixing process, samples of bulk sediment were collected on days 1, 3, and 6 after test substance amendment and pore water samples were separated by centrifugation. Duplicate bulk sediment samples were analyzed on day 6 to evaluate homogeneity. Mean pore-water concentrations were compared between days 3 and 6 to determine the extent to which equilibrium had been achieved. Approximately 100 ml of test substance-amended wet sediment was then transferred into 300-ml high-form beakers and placed into the toxicity testing system described below. Samples of sediment, overlying water, and pore water were collected at intervals over 9 to 10 days. The PE stability in pore water was evaluated by calculating half-lives, using log-transformed concentrations in simple linear regression analyses with time. Five replicates of a single nominal concentration of 3,000 mg/kg dry sediment and five control replicates were used to enhance statistical resolution. The rationale for a spiking limit of 3,000 mg/kg was based on several factors. First, the application of true water solubility limits for di-n-hexyl phthalate (DHP) and diethylhexyl phthalate (DEHP) of 0.05 and 0.003 mg/L in EqP calculations resulted in sediment spiking limits of approximately 2,400 and 1,700 mg/kg, respectively, for a low TOC sediment. Second, the sediment concentration of 3,000 mg/kg is well in excess of environmental exposures since it exceeds by at least two orders of magnitude recently reported field concentrations of DEHP as well as maxima for any of the other high molecular weight PEs. Third, preliminary experiments at a DEHP spiking level of 30,000 mg/kg appeared to adversely affect the dissolved oxygen concentration. Based on published aquatic toxicity data and water-only toxicity test results (Call et al., 2001b), the target dose of 3,000 mg/kg dry sediment was not anticipated to exhibit acute toxicity. For the second phase (i.e., toxicity testing), 100 ml of test substance-amended test sediment was added to each beaker and allowed to equilibrate in the flow-through test system for approximately 24 hours before test organisms were added. Ten *Hyalella azteca* (7-14 days old) or ten *Chironomus tentans* larvae (2nd-3rd instars, 10-12 days old) were then added to each exposure beaker. The daily feeding regimes utilized for the chambers containing *H. azteca* and *C. tentans* consisted of 1.0 to 1.5 ml of a yeast-trout chow-Cerophyll mixture and 1.5 ml of a 4 g/L Tetrafin[®] slurry (TetraWerke, Melle, Germany), respectively. Tests were conducted in an intermittent water renewal system, with screened 300-ml high-form beakers as the primary exposure chambers. They were conducted at a nominal temperature of 23 degrees C, with a 16:8 light:dark photoperiod. The beakers contained approximately 100 ml of sediment and 100 to 175 ml of overlying water, dependent on the stage in the siphoning and renewal cycle. Overlying water, was replaced at a rate of four to eight volume additions daily. The water in the tanks holding the primary exposure chambers was aerated throughout the exposure period to ensure that an adequate level of dissolved oxygen was maintained. General observations were made daily, with counts of survivors obtained after the 10-day exposure period. At that time, the sediment was sieved, survivors collected, cleaned of debris, oven-dried for approximately 24 hours at 105 degrees C, and weighed. Weights were determined to 0.01 mg for the pooled survivors from each replicate. Toxicity tests (48 hours) with KCl as a reference toxicant were performed regularly throughout the testing program with the test species to ensure that organisms used in tests were healthy and that LC50 values were within acceptable limits for performing the toxicity tests. The reference toxicant tests were conducted within one month of the test substance

toxicity test. Control animal survival acceptability criteria of 80 and 70% were used for 10-day toxicity tests with *H. azteca* and *C. tentans*, respectively. A silica sand performance control also accompanied each toxicity test as a check on the performance of animals in the test system. Tests were performed using documentation consistent with good laboratory practices. Temperature, dissolved oxygen, and pH were recorded in all test chambers on days 0 and 10. Conductivity was measured in all test chambers on days 1 and 9. Hardness, alkalinity, and ammonia were measured on days 1 and 9 in at least one sediment control chamber. On days 2 to 9, temperature, dissolved oxygen, and pH were measured in at least one sediment control chamber and one exposure chamber.

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
10 d	LC50	> 2680 mg/kg sediment dw	meas. (arithm. mean)	test mat.	other: mortality; growth	
10 d	NOEC	2680 mg/kg sediment dw	meas. (arithm. mean)	test mat.	other: mortality; growth	

Details on results

Chironomus tentans survival and growth were evaluated as the biological endpoints. The test substance showed no effect on survival or growth at a test substance loading of 3000 mg/kg sediment dry weight (measured as 2680 mg/kg sediment dry weight). There were five control and five exposure systems each containing 10 organisms and two sand controls with 10 organisms each. The means (ranges) of test substance concentrations in pore water and bulk sediment, and mean number of survivors (mean organism dry weights) were as follows: Pore water: control mean conc. = <0.004 mg/L (<0.004-0.004), exposure mean conc. = 1.18 mg/L (0.766-1.80) Bulk sediment: control conc. = <1.89 mg/kg sediment dry weight (<1.89-<1.89), exposure mean conc. = 2630 mg/kg sediment dry weight (2340-3320) Control survivors = 47 (1.60 mg dry weight) Exposure survivors = 45 (1.77 mg dry weight) Sand survivors = 18 (0.99 mg dry weight)

Reported statistics and error estimates

Survival data from toxicity tests were summarized using the trimmed Spearman-Kärber method.

Dry weight data were analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStatt Program (SPSS, Chicago, IL, USA).

Remarks on results including tables and figures

Although the test substance was not tested for stability, it was assumed from the measured half-life of DHP (di-n-hexylphthalate) and DIDP (diisodecylphthalate) and the apparent stability of DEHP (diehtylhexylphthalate) that the test substance would be sufficiently stable to conduct toxicity tests (DHP and DEHP were also evaluated in this study). DEHP was recently reported to have a half-life of 15 d in aqueous solution but >70 d in soil. Overall, the results from the stability study indicated that the high molecular weight phthalate esters were sufficiently stable to proceed with 10-day sediment toxicity tests. Means and ranges of DINP concentrations in pore water (mg/L) and bulk sediment (mg/kg/dry weight) tested at 3,000 mg/kg/dry weight, and mean number of survivors and mean organism dry weight (mg):

Test organism	Matrix	Control Mean concentration mg/L (range)	Control survivors* (dry weight, mg)	High exposure Mean (range)	High exposure survivors (dry weight, mg)
Chironomus tentans	Pore water	<0.043	-	0.869	-
		(<0.043 - <0.043)		(0.356-1.55)	
	Sediment	<2.58	53	2680	46
		(<2.58 - <2.58)	(1.90)	(2280-3020)	(1.80)

* 5 replicates of ten organisms each

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) was not toxic to *Chironomus tentans* at a high test material/sediment loading of 3000 mg/kg sediment dry weight (measured as 2680 mg/kg sediment dry weight).

Executive summary

The sediment toxicity data for *Chironomus tentans* reported for di-isononyl phthalate ester (DINP) are consistent with the sediment and aqueous exposure toxicity data for several high molecular weight phthalate esters for two freshwater invertebrates as summarized by the authors. These data clearly showed that high molecular weight phthalate esters, including DINP, did not produce toxicity, as measured by survival rate and growth, to freshwater sediment invertebrates at a sediment loading rate (3000 mg/kg sediment dry weight) that far exceed expected field levels, based on field measurements of similar higher molecular weight phthalate esters.

Dossier > *Document*

 Dissemination Dossier

 Sedimentdwellingtox Endpoint.002

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	Not applicable
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a USEPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		2001							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: USEPA/600/R-94/024 Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Purity: 99.7% Diisononyl phthalate ester (DINP) was obtained from Exxon Chemical, New Milford, CT. Due to the very low water solubility of this substance, a surfactant, sorbitan monolaurate 20 ethoxylate (Tween 20), was used. The Tween 20 was obtained from Mallinckrodt (US), and had a purity of 99+%.

Analytical monitoring

yes

Details on sampling

During mixing, samples of sediment were collected at periodic intervals for analysis of PE concentrations in the bulk sediment and pore water. Homogeneity of mixing was determined from multiple sediment samples usually collected on day 6. The degree to which an equilibrium in pore-water concentrations had been achieved was determined from samples of pore water collected on two occasions, usually days 3 and 6. During the test, samples of bulk sediment, pore water, and overlying water were sampled on days 0 and 10 and at two intermediate times for measurement of test substance concentrations. Overlying water samples (500 ml) were collected with an Eppendorf pipettor approximately 1 to 2 cm above the sediment. Samples were analyzed by high performance liquid chromatography. The overlying water was then siphoned out of the beaker to obtain sediment samples. Sediment cores were collected using a glass tube (13-mm inner diameter). Pore water was defined as the supernatant liquid obtained from centrifuging wet sediment at 10,000 g for 30 minutes. Following centrifugation, an aliquot of the pore water was pipetted into a vial and a 1:1 mixture of acetonitrile and deionized water added to bring the sample volume to 1 ml. The vial was capped and mixed, after which the contents were centrifuged to remove any precipitate present. The remainder of the pore water was removed from the sample tube and the weight of the bulk sediment was determined. Sediments were extracted by adding 5 ml of acetonitrile to the centrifuged pellet (from the pore-water preparation step), mixing the pellet and acetonitrile with a stainless steel spatula, and sonicating for 15 minutes in a 35 degrees C water bath. Samples were mixed with a clean spatula and then sonicated for an additional 15 minutes. The tube was centrifuged in a bench-top centrifuge for 5 minutes and a sample of the supernatant diluted with a mixture of acetonitrile and water (1:1, v/v). The remaining supernatant was removed from the sediment and the sediment dried overnight at 27 degrees C for dry weight (%) estimates.

Vehicle

yes

Details on sediment and application

Uncontaminated, natural sediment samples for this study were collected from Airport Pond (St. Louis County, MN, USA) and West Bearskin Lake (Cook County, MN, USA). Samples were

collected with a Ponar dredge, placed into clean polyethylene containers, and stored at 4 degrees C until used. For the test, the sediments were homogenized in a 119-L stainless steel container using a commercial drill with a stainless steel mortar-mixing paddle. To achieve a desired medium total organic carbon (TOC) sediment level, aliquots from Airport Pond and West Bearskin Lake were blended. A summary of TOC content and particle size distribution for the two blended sediments follows (+/- standard deviation in parentheses): the mean TOC content was 4.8% (0.65) ; sand content was 46.9% (4.09); silt content was 30.2% (3.59); coarse clay content was 2.3% (1.47); and fine clay content was 20.5% (1.86). The test substance was dissolved in acetone and coated onto a 20% aliquot of a wet sediment sample. The sediment aliquot was dried and then placed into a 4-L glass jar, which was rotated in an air stream to evaporate the acetone. Deionized water equal to the volume lost in drying the 20% portion of wet sediment was then added to the dried sediment aliquot, mixed, and added back into the jar containing the remaining 80% wet sediment sample. The jar was sealed with a Teflon-lined cap and rotated on a roller mill in a cold room (approximately 4 degrees C) for approximately 6 days at a speed of approximately 8 rpm.

Test organisms

Test organisms (species)

Hyalella azteca

Study design

Study type

laboratory study

Test duration type

long-term toxicity

Test type

static

Water media type

freshwater

Type of sediment

natural sediment

Limit test

yes

Total exposure duration

10 d

Remarks

Test conditions

Hardness

Measured, but not reported; was likely to have met guideline requirements.

Test temperature

Measured, but not reported; was likely to have met guideline requirements.

pH

Measured, but not reported; was likely to have met guideline requirements.

Dissolved oxygen

Measured, but not reported; was likely to have met guideline requirements.

Ammonia

Measured, but not reported; was likely to have met guideline requirements.

Nominal and measured concentrations

Nominal test substance loading = 3000 mg/kg sediment dry weight
Measured test substance concentration = 2900 mg/kg sediment dry weight (range = 2670-3170 mg/kg sediment dry weight)

Details on test conditions

Sediment testing proceeded in two phases. The first phase evaluated the effectiveness of the mixing process in achieving a homogeneous distribution of test substance in bulk sediment, the establishment of equilibrium concentrations of test chemicals in the pore water, and the stability of the test substance in pore water under simulated toxicity test conditions. This phase was used to determine, in part, if meaningful toxicity tests could be performed (i.e., to assess if stable exposure concentrations could be maintained during the test period). The second phase evaluated the toxicity of the test substance in spiked sediments toward the test species. During the mixing

process, samples of bulk sediment were collected on days 1, 3, and 6 after test substance amendment and pore water samples were separated by centrifugation. Duplicate bulk sediment samples were analyzed on day 6 to evaluate homogeneity. Mean pore-water concentrations were compared between days 3 and 6 to determine the extent to which equilibrium had been achieved. Approximately 100 ml of test substance-amended wet sediment was then transferred into 300-ml high-form beakers and placed into the toxicity testing system described below. Samples of sediment, overlying water, and pore water were collected at intervals over 9 to 10 days. The PE stability in pore water was evaluated by calculating half-lives, using log-transformed concentrations in simple linear regression analyses with time. Five replicates of a single nominal concentration of 3,000 mg/kg dry sediment and five control replicates were used to enhance statistical resolution. The rationale for a spiking limit of 3,000 mg/kg was based on several factors. First, the application of true water solubility limits for di-n-hexyl phthalate (DHP) and diethylhexyl phthalate (DEHP) of 0.05 and 0.003 mg/L in EqP calculations resulted in sediment spiking limits of approximately 2,400 and 1,700 mg/kg, respectively, for a low TOC sediment. Second, the sediment concentration of 3,000 mg/kg is well in excess of environmental exposures since it exceeds by at least two orders of magnitude recently reported field concentrations of DEHP as well as maxima for any of the other high molecular weight PEs. Third, preliminary experiments at a DEHP spiking level of 30,000 mg/kg appeared to adversely affect the dissolved oxygen concentration. Based on published aquatic toxicity data and water-only toxicity test results (Call et al., 2001b), the target dose of 3,000 mg/kg dry sediment was not anticipated to exhibit acute toxicity. For the second phase (i.e., toxicity testing), 100 ml of test substance-amended test sediment was added to each beaker and allowed to equilibrate in the flow-through test system for approximately 24 hours before test organisms were added. Ten *Hyalella azteca* (7-14 days old) or ten *Chironomus tentans* larvae (2nd-3rd instars, 10-12 days old) were then added to each exposure beaker. The daily feeding regimes utilized for the chambers containing *H. azteca* and *C. tentans* consisted of 1.0 to 1.5 ml of a yeast-trout chow-Cerophyll mixture and 1.5 ml of a 4 g/L Tetrafin[®] slurry (TetraWerke, Melle, Germany), respectively. Tests were conducted in an intermittent water renewal system, with screened 300-ml high-form beakers as the primary exposure chambers. They were conducted at a nominal temperature of 23 degrees C, with a 16:8 light:dark photoperiod. The beakers contained approximately 100 ml of sediment and 100 to 175 ml of overlying water, dependent on the stage in the siphoning and renewal cycle. Overlying water, was replaced at a rate of four to eight volume additions daily. The water in the tanks holding the primary exposure chambers was aerated throughout the exposure period to ensure that an adequate level of dissolved oxygen was maintained. General observations were made daily, with counts of survivors obtained after the 10-day exposure period. At that time, the sediment was sieved, survivors collected, cleaned of debris, oven-dried for approximately 24 hours at 105 degrees C, and weighed. Weights were determined to 0.01 mg for the pooled survivors from each replicate. Toxicity tests (48 hours) with KCl as a reference toxicant were performed regularly throughout the testing program with the test species to ensure that organisms used in tests were healthy and that LC50 values were within acceptable limits for performing the toxicity tests. The reference toxicant tests were conducted within one month of the test substance toxicity test. Control animal survival acceptability criteria of 80 and 70% were used for 10-day toxicity tests with *H. azteca* and *C. tentans*, respectively. A silica sand performance control also accompanied each toxicity test as a check on the performance of animals in the test system. Tests were performed using documentation consistent with good laboratory practices. Temperature, dissolved oxygen, and pH were recorded in all test chambers on days 0 and 10. Conductivity was

measured in all test chambers on days 1 and 9. Hardness, alkalinity, and ammonia were measured on days 1 and 9 in at least one sediment control chamber. On days 2 to 9, temperature, dissolved oxygen, and pH were measured in at least one sediment control chamber and one exposure chamber.

Reference substance (positive control)

yes 3,5-dichlorophenol

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
10 d	LC50	> 2900 mg/kg sediment dw	meas. (arithm. mean)	test mat.	other: mortality; growth	
10 d	NOEC	2900 mg/kg sediment dw	meas. (arithm. mean)	test mat.	other: mortality; growth	

Details on results

Hyalella azteca survival and growth were evaluated as the biological endpoints. The test substance showed no effect on survival or growth at a test substance loading of 3000 mg/kg sediment dry weight (measured as 2900 mg/kg sediment dry weight). There were five control and five exposure systems each containing 10 organisms and two sand controls with 10 organisms each.

Reported statistics and error estimates

Survival data from toxicity tests were summarized using the trimmed Spearman-Kärber method. Dry weight data were analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStat Program (SPSS, Chicago, IL, USA).

Remarks on results including tables and figures

Although the test substance was not tested for stability, it was assumed from the measured half-lives of DHP (di-n-hexyl phthalate) and DIDP (diisononyl phthalate) and the apparent stability of DEHP (diethylhexyl phthalate) that the test substance would be sufficiently stable to conduct toxicity tests (DHP and DEHP were also evaluated in this study). DEHP was recently reported to have a half-life of 15 d in aqueous solution but >70 d in soil. Overall, the results from the stability study indicated that the high molecular weight phthalate esters were sufficiently stable to proceed with 10-day sediment toxicity tests.

Means and ranges of DINP concentrations in pore water (mg/L) and bulk sediment (mg/kg/dry weight) tested at 3,000 mg/kg/dry weight, and mean number of survivors and mean organism dry weight (mg):

Test organism	Matrix	Control Mean (range)	Control survivors* (dry weight, mg)	High exposure Mean (range)	High exposure survivors* (dry weight, mg)
Hyalella azteca	Pore water	<0.043	-	0.442	-
		(<0.043 - <0.043)		(0.289-0.560)	
	Sediment	<2.58	47	2900	44
		(<2.58 - <2.58)	(0.14)	(2670-3170)	(0.11)

* 5 replicates of ten organisms each

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) was not toxic to *Hyalella azteca* at a high test material/sediment loading of 3000 mg/kg sediment dry weight (measured as 2900 mg/kg sediment dry weight).

Executive summary

The sediment toxicity data for *Hyalella azteca* reported for di-isononyl phthalate ester (DINP) are consistent with the sediment and aqueous exposure toxicity data for several high molecular weight phthalate esters for two freshwater invertebrates as summarized by the authors. These data clearly showed that high molecular weight phthalate esters, including DINP, did not produce toxicity, as measured by survival rate and growth, to freshwater sediment invertebrates at a sediment loading rate (3000 mg/kg sediment dry weight) that far exceed expected field levels, based on field measurements of similar higher molecular weight phthalate esters.

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[Data source](#)

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Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	April 1996
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study procedure followed an accepted test guideline and was conducted under GLP. The data are consistent with known toxicological properties of similar high molecular weight phthalate ester substances.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1996							1996-07-08

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 207 (Earthworm, Acute Toxicity Tests)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product Physical state: Colourless liquid Storage condition of test material: Room temperature

Analytical monitoring

yes

Details on sampling

Test soils dosed with diisodecyl phthalate ester (DIDP) were analyzed at test initiation and termination, days -1 and 14, respectively. The worm tissues were also analyzed after completion of treatment.

Vehicle

no

Details on preparation and application of test substrate

Test soil was homogenized prior to use by placing soil into 4L size plastic (HDPE) containers and mixing on a jar mill for approximately 15 minutes. After homogenizing, a sample was removed to determine the moisture fraction. The moisture fraction was measured by placing soil into a crystallizing dish and weighing it (initial wt.). The sample and crystallizing dish were then placed in an oven at 100 degrees C for 23 hours. After drying and cooling in a desiccator, the final weight of the sample and crystallizing dish was measured. Treatments were prepared by adding the appropriate amount of test material to a soil sample. The treatment soils were stirred copiously in stainless steel bowls with stainless steel spoons for 15 to 30 minutes and held overnight at room temperature. On day 0, each treatment was divided into 5 replicates. The test chambers were one pint glass canning jars containing approximately 200g of a soil control or treatment and the appropriate amount of hydration water to bring the soils up to 75% of their water holding capacity. The worms were then transferred to randomized test chambers, which were capped with a lid containing a 1/8 inch hole in the center.

Test organisms

Test organisms (species)

Eisenia fetida

Animal group

annelids

Details on test organisms

Worms, *Eisenia fetida*, were obtained from Carolina Biological, Burlington, NC, USA. Species were verified based on: Earthworms of Ontario, J.W. Reynolds, Life Sciences Miscellaneous Publishing, The Royal Ontario Museum, 15 June 1977 Average weight of worms was 0.393g (sd = 0.090), based on a subsample from the stock culture after the study was completed (n=20) of worms. The organism loading rate during the study was 10 worms/~200 g soil. Age at test initiation (mean and range, SD): Adults with clitellum Acclimation period: At least 14 days

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Substrate type

other: Two soils were tested: natural soil and artificial soil

Limit test

yes

Total exposure duration

14 d

Remarks

Test conditions

Test temperature

Temperature = 18.2 to 20.6 degrees C

pH

pH = 6.9 to 7.2 The artificial and natural soils measured prior to addition of DINP. Artificial soil pH 6.9, Natural soil pH 7.2. The values are slightly outside the OECD guideline recommendation

of 6.0 ± 0.5 . The pH of the test soils was not measured after addition of DINP. These deviations were not considered to have had any effect on the outcome of the study.

Moisture

Control and test soils were hydrated with the appropriate amount of reverse osmosis water to achieve 75% of the water holding capacity of the soil.

Nominal and measured concentrations

Soil samples were dosed with 10,000 mg test substance/kg soil. Soil were analyzed for DIDP concentration at test initiation and termination. The soil analytical results are based on the mean of duplicate samples for each soil sampled: DIDP Conc. Day -1 DIDP Conc. Day 14 Soil Sample (mg/kg soil, dry wt.) (mg/kg soil, dry wt.) Snyder Soil 7664 7994 Artificial Soil 8435 9180 Snyder soil was a natural soil.

Details on test conditions

Two soils were tested, a natural soil and an artificial soil. Natural soil was obtained from Snyder Research Farm located in Pittstown, NJ, USA, which was managed by Rutgers University. The soil was sieved through a 10 mesh sieve prior to use to remove large particles. Artificial soil was prepared using 70% sand, 20% clay, 10% peat, and CaCO_3 to adjust pH. The water holding capacity of each soil was determined as follows: 25g of the dried sample was placed into a 100mL glass beaker with 25mL of reverse osmosis water and mixed thoroughly. This slurry was added to a pre-weighed, wetted filter paper and funnel (initial weight = wt. of funnel, wetted paper, + 25 g soil). The filter paper (#113 Whatman 150mm) was wetted with 9mL of reverse osmosis water. The glass funnel measured 10cm top diameter with an approximately 30mm stem. The slurry, filter and funnel were covered with aluminum foil and allowed to stand for 3 hours at which time a final weight was measured. The water holding capacity, expressed in mL water/100g of soil, is the difference between the final and initial weights multiplied by 4 to achieve 100g equivalence. Test temperature ranged from 18.2 to 20.6 degrees C as measured continuously and recorded by computer for the first 6 days, then measured daily thereafter. Light intensity ranged from 721 to 1185 Lux. Lighting was measured continuously and recorded by computer for the first 6 days, then measured daily thereafter. Soil pH was measured at test initiation (day 0) of the study. Ten grams of each treatment were mixed with 20mL of distilled water. The pH value of the slurry was measured using a soil pH probe. Soil pH ranged from 6.9 to 7.2. Observations for worm mortality were performed on all replicate chambers on day 7 and 14. Worms not found were assumed to have died and decomposed. TEST SYSTEM - Test container (material, size): 1 pint glass canning jars - Amount of soil or substrate: 200 g - No. of organisms per container (treatment): 10 - No. of replicates per treatment group: 5 - No. of replicates per control: 5 SOURCE AND PROPERTIES OF SUBSTRATE (if soil) - Geographic location: Natural soil from Snyder Research Farm, Rutgers University, Pittstown, NJ, USA - Collection procedures: pH adjusted and 10 mesh sieved - Composition (if artificial substrate): 70% sand, 20% clay, 10% peat and CaCO_3 to raise pH - Organic carbon (%): 10% (dry weight) - Maximum water holding capacity (in % dry weight): 75% OTHER TEST CONDITIONS -

Photoperiod: Continuous - Light intensity: approximately 721 to 1185 Lux. This was slightly above the guideline recommendation of 400-800 Lux but was not considered to have had any effect on the outcome of the study EFFECT PARAMETERS MEASURED (with observation intervals if applicable): Mortality on Days 7 and 14

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
14 d	LC50	> 7270 mg/kg natural soil dw	meas. (initial)	test mat.	mortality	
14 d	NOEC	7270 mg/kg natural soil dw	meas. (initial)	test mat.	mortality	
14 d	LC50	> 7372 mg/kg artificial soil dw	meas. (initial)	test mat.	mortality	
14 d	NOEC	7372 mg/kg artificial soil dw	meas. (initial)	test mat.	mortality	

Details on results

Diisononyl phthalate ester (DINP) demonstrated no effect on survival in the two soils tested at a soil loading rate of 10,000 mg/kg soil (dry wt.) (7270 mg/kg natural soil (dry wt.) and 7372 mg/kg artificial soil (dry wt.); measured concentrations reflect initial measured concentrations). The following are the survival results: Earthworm Mean % Soil Sample Survival* Survival Snyder Soil Control 10,10,10,10,10 100 Artificial Soil Control 10,10,10,10,10 100 Snyder Soil + DINP 10,10,10,10,10 100 Artificial Soil + DINP 10,10,10,10,10 100 *10 earthworms were added per replicate. There were five replicates per control and treatment.

Reported statistics and error estimates

None reported.

Remarks on results including tables and figures

	Number of worms surviving at 7 and 14 Days	Mean percentage survival
--	--	--------------------------

Replicates	1	2	3	4	5	
Artificial soil control	10	10	10	10	10	100
Artificial soil with DINP at 10,000 mg/kg	10	10	10	10	10	100
Natural (Snyder) soil control	10	10	10	10	10	100
Natural(Snyder soil) with DINP at 10,000 mg/kg	10	10	10	10	10	100

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) does not effect earthworm (*Eisenia fetida*) survival in soil, based on 14-day limit studies at very high concentrations, 7270 mg/kg natural soil (dw) and 7372 mg/kg artificial soil (dw).

Executive summary

The toxicity of di-isononyl phthalate ester (DINP) as measured by mortality to the earthworm (*Eisenia fetida*) was evaluated in 14 -day studies using natural and artificial soils. No significant mortality was observed in the soils dosed with DINP at a nominal loading rate of 10,000 mg/kg soil (dw), which measured 7270 mg/kg natural soil (dw) and 7372 mg/kg artificial soil (dw) (concentrations are from analyses of soils at test initiation).

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 Dissemination Dossier

 Soildwellington Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	April 1996
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study procedure followed an accepted test guideline and was conducted under GLP. The data are consistent with known toxicological properties of similar high molecular weight phthalate ester substances.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1996							1996-07-08

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 207 (Earthworm, Acute Toxicity Tests)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: commercial product Physical state: Colourless liquid Storage condition of test material: Room temperature

Analytical monitoring

yes

Details on sampling

Test soils dosed with diisodecyl phthalate ester (DIDP) were analyzed at test initiation and termination, days -1 and 14, respectively. The worm tissues were also analyzed after completion of treatment.

Vehicle

no

Details on preparation and application of test substrate

Test soil was homogenized prior to use by placing soil into 4L size plastic (HDPE) containers and mixing on a jar mill for approximately 15 minutes. After homogenizing, a sample was removed to determine the moisture fraction. The moisture fraction was measured by placing soil into a crystallizing dish and weighing it (initial wt.). The sample and crystallizing dish were then placed in an oven at 100 degrees C for 23 hours. After drying and cooling in a desiccator, the final weight of the sample and crystallizing dish was measured. Treatments were prepared by adding the appropriate amount of test material to a soil sample. The treatment soils were stirred copiously in stainless steel bowls with stainless steel spoons for 15 to 30 minutes and held overnight at room temperature. On day 0, each treatment was divided into 5 replicates. The test chambers were one pint glass canning jars containing approximately 200g of a soil control or treatment and the appropriate amount of hydration water to bring the soils up to 75% of their water holding capacity. The worms were then transferred to randomized test chambers, which were capped with a lid containing a 1/8 inch hole in the center.

Test organisms

Test organisms (species)

Eisenia fetida

Animal group

annelids

Details on test organisms

Worms, *Eisenia fetida*, were obtained from Carolina Biological, Burlington, NC, USA. Species were verified based on: Earthworms of Ontario, J.W. Reynolds, Life Sciences Miscellaneous Publishing, The Royal Ontario Museum, 15 June 1977 Average weight of worms was 0.393g (sd = 0.090), based on a subsample from the stock culture after the study was completed (n=20) of worms. The organism loading rate during the study was 10 worms/~200 g soil. Age at test initiation (mean and range, SD): Adults with clitellum Acclimation period: At least 14 days

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Substrate type

other: Two soils were tested: natural soil and artificial soil

Limit test

yes

Total exposure duration

14 d

Remarks

Test conditions

Test temperature

Temperature = 18.2 to 20.6 degrees C

pH

pH = 6.9 to 7.2 The artificial and natural soils measured prior to addition of DINP. Artificial soil pH 6.9, Natural soil pH 7.2. The values are slightly outside the OECD guideline recommendation

of 6.0 ± 0.5 . The pH of the test soils was not measured after addition of DINP. These deviations were not considered to have had any effect on the outcome of the study.

Moisture

Control and test soils were hydrated with the appropriate amount of reverse osmosis water to achieve 75% of the water holding capacity of the soil.

Nominal and measured concentrations

Soil samples were dosed with 10,000 mg test substance/kg soil. Soil were analyzed for DIDP concentration at test initiation and termination. The soil analytical results are based on the mean of duplicate samples for each soil sampled: DIDP Conc. Day -1 DIDP Conc. Day 14 Soil Sample (mg/kg soil, dry wt.) (mg/kg soil, dry wt.) Snyder Soil 7664 7994 Artificial Soil 8435 9180 Snyder soil was a natural soil.

Details on test conditions

Two soils were tested, a natural soil and an artificial soil. Natural soil was obtained from Snyder Research Farm located in Pittstown, NJ, USA, which was managed by Rutgers University. The soil was sieved through a 10 mesh sieve prior to use to remove large particles. Artificial soil was prepared using 70% sand, 20% clay, 10% peat, and CaCO_3 to adjust pH. The water holding capacity of each soil was determined as follows: 25g of the dried sample was placed into a 100mL glass beaker with 25mL of reverse osmosis water and mixed thoroughly. This slurry was added to a pre-weighed, wetted filter paper and funnel (initial weight = wt. of funnel, wetted paper, + 25 g soil). The filter paper (#113 Whatman 150mm) was wetted with 9mL of reverse osmosis water. The glass funnel measured 10cm top diameter with an approximately 30mm stem. The slurry, filter and funnel were covered with aluminum foil and allowed to stand for 3 hours at which time a final weight was measured. The water holding capacity, expressed in mL water/100g of soil, is the difference between the final and initial weights multiplied by 4 to achieve 100g equivalence. Test temperature ranged from 18.2 to 20.6 degrees C as measured continuously and recorded by computer for the first 6 days, then measured daily thereafter. Light intensity ranged from 721 to 1185 Lux. Lighting was measured continuously and recorded by computer for the first 6 days, then measured daily thereafter. Soil pH was measured at test initiation (day 0) of the study. Ten grams of each treatment were mixed with 20mL of distilled water. The pH value of the slurry was measured using a soil pH probe. Soil pH ranged from 6.9 to 7.2. Observations for worm mortality were performed on all replicate chambers on day 7 and 14. Worms not found were assumed to have died and decomposed. TEST SYSTEM - Test container (material, size): 1 pint glass canning jars - Amount of soil or substrate: 200 g - No. of organisms per container (treatment): 10 - No. of replicates per treatment group: 5 - No. of replicates per control: 5 SOURCE AND PROPERTIES OF SUBSTRATE (if soil) - Geographic location: Natural soil from Snyder Research Farm, Rutgers University, Pittstown, NJ, USA - Collection procedures: pH adjusted and 10 mesh sieved - Composition (if artificial substrate): 70% sand, 20% clay, 10% peat and CaCO_3 to raise pH - Organic carbon (%): 10% (dry weight) - Maximum water holding capacity (in % dry weight): 75% OTHER TEST CONDITIONS -

Photoperiod: Continuous - Light intensity: approximately 721 to 1185 Lux. This was slightly above the guideline recommendation of 400-800 Lux but was not considered to have had any effect on the outcome of the study EFFECT PARAMETERS MEASURED (with observation intervals if applicable): Mortality on Days 7 and 14

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
14 d	LC50	> 7270 mg/kg natural soil dw	meas. (initial)	test mat.	mortality	
14 d	NOEC	7270 mg/kg natural soil dw	meas. (initial)	test mat.	mortality	
14 d	LC50	> 7372 mg/kg artificial soil dw	meas. (initial)	test mat.	mortality	
14 d	NOEC	7372 mg/kg artificial soil dw	meas. (initial)	test mat.	mortality	

Details on results

Diisononyl phthalate ester (DINP) demonstrated no effect on survival in the two soils tested at a soil loading rate of 10,000 mg/kg soil (dry wt.) (7270 mg/kg natural soil (dry wt.) and 7372 mg/kg artificial soil (dry wt.); measured concentrations reflect initial measured concentrations). The following are the survival results: Earthworm Mean % Soil Sample Survival* Survival Snyder Soil Control 10,10,10,10,10 100 Artificial Soil Control 10,10,10,10,10 100 Snyder Soil + DINP 10,10,10,10,10 100 Artificial Soil + DINP 10,10,10,10,10 100 *10 earthworms were added per replicate. There were five replicates per control and treatment.

Reported statistics and error estimates

None reported.

Remarks on results including tables and figures

	Number of worms surviving at 7 and 14 Days	Mean percentage survival
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Replicates	1	2	3	4	5	
Artificial soil control	10	10	10	10	10	100
Artificial soil with DINP at 10,000 mg/kg	10	10	10	10	10	100
Natural (Snyder) soil control	10	10	10	10	10	100
Natural(Snyder soil) with DINP at 10,000 mg/kg	10	10	10	10	10	100

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester (DINP) does not effect earthworm (*Eisenia fetida*) survival in soil, based on 14-day limit studies at very high concentrations, 7270 mg/kg natural soil (dw) and 7372 mg/kg artificial soil (dw).

Executive summary

The toxicity of di-isononyl phthalate ester (DINP) as measured by mortality to the earthworm (*Eisenia fetida*) was evaluated in 14 -day studies using natural and artificial soils. No significant mortality was observed in the soils dosed with DINP at a nominal loading rate of 10,000 mg/kg soil (dw), which measured 7270 mg/kg natural soil (dw) and 7372 mg/kg artificial soil (dw) (concentrations are from analyses of soils at test initiation).

- Dissemination Dossier
- Honeybeestox Endpoint.001

Administrative Data

Data waiving study scientifically unjustified

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 Dissemination Dossier

 Planttox Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	May 1996
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a USEPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP. Additionally, seed germination (mean % germinated) in the controls met guideline requirements, which validates the test results.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1996							1996-07-09

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: U.S. EPA-600/3-88/029 Protocols for Short Term Toxicity Screening of Hazardous Waste Sites (NTIS / PB88-235510/AS)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: technical product

Analytical monitoring

yes

Details on sampling

Samples of each test substance treatment were taken on Day 0 and at termination on a composite of the 5 replicates.

Vehicle

no

Details on test substrate

Two soils were tested, a natural soil and an artificial soil. Natural soil was obtained from Snyder Research Farm located in Pittstown, NJ, USA, which was managed by Rutgers University. The soil was sieved through a 10 mesh sieve prior to use to remove large particles. Artificial soil was prepared using 70% sand, 20% clay, and 10% peat. The treatments were prepared by adding the appropriate amount of DINP to the Artificial or Snyder soil. The treatments were stirred for approximately 15 minutes. The treatments were then prepared by diluting the stock with the appropriate amount of carrier soil to prepare the 1000mg/kg and 3000mg/kg treatments. The two diluted treatments were mixed on a jar mill for approximately 15 minutes. The test treatments were divided into five replicates of approximately 100g in plastic petri dishes (150mm diameter x 15mm high). The replicates were then hydrated with the appropriate amount of reverse osmosis water to achieve 85% of the water holding capacity of the soil. Due to an error in the soil hydration calculation, three ml more of water per replicate was added to the sand/artificial soil treatments than was required to achieve 85% of the water holding capacity. It is not believed that this minimal extra amount of water had an adverse effect on the study.

Test organisms**Test organisms**

Species	Lactuca sativa
Plant group	Dicotyledonae (dicots)
Details on test	- Common name: Lettuce - Plant family: Asteraceae - Variety: Lot

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Test type

seedling emergence toxicity test

Substrate type

other: Two soils were tested: natural soil and artificial soil

Limit test

no

Total exposure duration

5 d

Remarks

Test conditions

Test temperature

Test temperature: 24.0 degrees C (sd = 0.4 degrees C) as monitored continuously by computer.

pH

Ranged from 6.0 to 6.1 for natural soil and was 6.9 for artificial soil.

Moisture

Soil moisture was adjusted by adding the appropriate amount of reverse osmosis water to achieve 85% of the water holding capacity of the soil.

Nominal and measured concentrations

Soil samples were dosed with 1000, 3000 and 10000 mg test substance/kg soil.

Details on test conditions

TEST SYSTEM - Testing facility: Exxon Biomedical Sciences, Inc. - Test container (type, material, size): Bottom half of 150 mm (Diam.) x 15 mm (High) plastic Petri dishes - Amount of soil: 190 g - Method of seeding: Seeds were spaced about 0.5 inches from the edge of the test chamber and covered with approximately 90 g of appropriate soil - No. of seeds per container: 40 - No. of replicates per treatment group: 5 - No. of replicates per control: 5 SOURCE AND PROPERTIES OF SUBSTRATE (if soil) - Geographic location: Natural soil was from Rutgers University, Snyder Research Farm, Pittstown, NJ, USA - Composition (if artificial substrate): 20-mesh sieved sand with 10% artificial soil (70% sand, 20% clay, 10% peat) - Maximum water holding capacity (in % dry weight): 24/48 (artificial soil) or 59 (natural soil) ml/100g % GROWTH CONDITIONS - Photoperiod: 48 hours dark, followed by 16 hours of light and 8 hours of dark until termination of the test - Light intensity and quality: 4300 +/- 430 Lux during daylight periods - Relative humidity (%): Held in polythene bags ACCLIMATION PERIOD: None EFFECT PARAMETERS MEASURED (with observation intervals if applicable): percentage germination

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Species	Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
Lactuca sativa	5 d	NOEC	1018 mg/kg artificial soil (d.w.)	meas. (arithm. mean)	test mat.	germination	
Lactuca sativa	5 d	NOEC	1080 mg/kg natural soil (d.w.)	meas. (arithm. mean)	test mat.	germination	

Details on results

At test termination, after 5 days of exposure, the number of germinated seeds was determined by counting each seedling that protruded above the surface of the soil. The germination results are located in the remarks field as a table.

Reported statistics and error estimates

The germination results were evaluated using Dunnett's procedure (one tailed T test, $\alpha=0.05$) of SAS [SAS/STAT® User's Guide. Version 6, Fourth Edition, Volumes 1 and 2. 1989. SAS Institute, Inc., Cary, NC, USA]. A random numbers computer generated table was used for test chamber placement in the test area.

Remarks on results including tables and figures

Germination data- Lettuce (*Lactuca sativa*)

Replicates	Number of seeds germinated (out of 40)					Mean ger
	1	2	3	4	5	
Artificial soil-control	36	38	40	40	40	
Artificial soil - 1000 mg/kg	38	37	36	37	39	
Artificial soil - 3000 mg/kg	34	32	35	33	33	
Artificial soil - 10000 mg/kg	30	26	31	34	33	
Natural (Snyder) soil-control	40	40	40	39	38	
Natural Soil - 1000 mg/kg	37	40	38	40	32	
Natural Soil - 3000 mg/kg	32	33	23	36	29	
Natural Soil - 10000 mg/kg	35	22	31	32	16	

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Di-isononyl phthalate ester does not effect the germination of lettuce (*Lactuca sativa*) seeds at high test material/soil concentrations, 1080 mg/kg natural soil (dw) and 1018 mg/kg artificial soil (dw).

Executive summary

The toxicity of di-isononyl phthalate ester (DINP) as measured by the germination of lettuce (*Lactuca sativa*) seeds was evaluated in natural and artificial soils. Germination in the control soils ranged from 97 to 99%. Germination in soils dosed with DINP at 1000 mg/kg was 94%. No significant effect on germination was demonstrated in the soils dosed with DINP at a nominal loading rate of 1000 mg/kg soil (dw) (measured at 1080 mg/kg natural soil (dw) and 1018 mg/kg artificial soil (dw)).

Dossier > Document

 Dissemination Dossier

 Planttox Endpoint.002

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	12.10.2000-2.11.2000
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2001							2001-02-23

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 208 (Terrestrial Plants Test: Seedling Emergence and Seedling Growth Test)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): VESTINOL 9 - Substance type: Technical substance - Physical state: Clear liquid

Analytical monitoring

no

Vehicle

no

Details on test substrate

The base substrate was standard soil (sp 2.3) of LUFA Speyer, sieved over mesh size 0.5 cm. Organic carbon content was <1.5% , particle size <20µm was 10-20% and pH 5-7.5. VESTINOL 9 was blended with quartz sand and admixed with base substrate to provide the required test concentrations as mg/kg dry weight.

Test organisms

Test organisms

Species Lepidum sativum

Plant group Dicotyledonae (dicots)

Details on test organisms - Common name: cress - Plant family: brassicaceae

Species Triticum aestivum

Plant group Monocotyledonae (monocots)

Details on test organisms - Common name: wheat - Plant family: Poaceae

Species Lactuca sativa

Plant group Dicotyledonae (dicots)

Details on test organisms - Common name: Lettuce - Plant family: Asteraceae

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Test type

seedling emergence toxicity / vegetative vigour test

Substrate type

artificial soil

Limit test

no

Total exposure duration

22 d

Remarks

Post exposure observation period

Not applicable

Test conditions

Test temperature

Day: 21-26 °C Night: approximately 18 °C

pH

6.6-6.7

Moisture

Maximum water holding capacity during the course of the test 19-86%

Nominal and measured concentrations

Nominal test concentrations: 0 (control), 10, 30, 100, 300, 1000 mg/kg Measured concentrations:
Not performed

Details on test conditions

TEST SYSTEM - Testing facility: Infracor GmbH - Test container (type, material, size): Black plastic flower pots 8-6 cm (Diam) x 6 cm (High) - Amount of soil: Not specified - Method of seeding: Not specified - No. of seeds per container: 5 - No. of plants (retained after thinning): 5 - No. of replicates per treatment group: 4 - No. of replicates per control: 4 SOURCE AND PROPERTIES OF SUBSTRATE (if soil) - Composition (if artificial substrate): Sieved over 0.5 mesh - Organic carbon (%): <1.5 % - Maximum water holding capacity (in % dry weight): 37 g/100g soil GROWTH CONDITIONS - Photoperiod: 16 hours light 2 hours dawn/dusk, 6 hours dark - Light intensity and quality: 60-71 Watts/m² light; 1 Watt/m² dawn and dusk - Day/night temperatures: 21-26 °C day; approximately 16-18 °C night - Relative humidity (%): 44-75% day; approx 80% night - Air exchange rate: 2000 L/hour through test chamber - Watering regime and schedules: - Interval of applications: Once daily VEHICLE CONTROL PERFORMED: no

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Species	Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on effect	Basis for Remarks (e.g. 95% CL)
Lepidum sativum	22 d	NOEC	1000 mg/kg soil d.w.	nominal	test mat.	
Triticum aestivum	22 d	NOEC	1000 mg/kg soil d.w.	nominal	test mat.	
Lactuca sativa	22 d	NOEC	1000 mg/kg soil d.w.	nominal	test mat.	

Remarks on results including tables and figures

Effect of DINP on the sprouting seedlings until the sixth day, and number of healthy plants at the test end (Day 22)

	Concentration (mg/kg)	Planted (number)	Emerged (number)	Healthy plants (number)
Cress	Control	20	19	19
	10	20	19	19
	30	20	17	18
	100	20	19	20

	300*	15	15	15
	1000	20	15	16
Wheat	Control	20	18	19
	10	20	20	20
	30	20	20	20
	100	20	17	17
	300	20	19	19
	1000	20	19	19
Lettuce	Control	20	19	20
	10	20	17	19
	30	20	18	20
	100	20	15	16
	300	20	14	17
	1000	20	17	20

* one pot only two plants (Day 19), data not included in evaluation

Applicant's summary and conclusion

Validity criteria fulfilled

yes seedling emergence >70%. No visible phototoxic effects. Control survival >90%.

Conclusions

There was no adverse effect on sprouting or growth at 1000 mg/kg, for cress, wheat or lettuce.

Executive summary

In a 22-day terrestrial plant growth test, cress (*Lepidium sativum*), wheat (*Triticum aestivum*) and lettuce (*Lactuca sativa*) were exposed to di-isononyl phthalate at a nominal concentration of 1000 mg/kg. There was no effect on sprouting of the seeds or growth of the plants for any of the three species. The NOEL was 1000 mg/kg soil (d.w.).

The plant growth study is considered acceptable for classification, and satisfies the guideline requirements for terrestrial plant growth inhibition studies.

Dossier > Document

 Dissemination Dossier

 Planttox Endpoint.003

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	April 1996
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a USEPA standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP. Additionally, seed germination (mean % germinated) in the controls met guideline requirements, which validates the test results.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1996							1996-07-08

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: U.S. EPA-600/3-88/029 Protocols for Short Term Toxicity Screening of Hazardous Waste Sites (NTIS / PB88-235510/AS)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: technical product Physical state: colorless liquid Purity: 100% commercial substance Storage condition of test material: Room temperature

Analytical monitoring

yes

Details on sampling

Soil samples dosed with 10,000 mg test substance/kg soil were analyzed for test substance at test initiation.

Vehicle

no

Details on test substrate

Two soils were tested, a natural soil and an artificial soil. Natural soil was obtained from Snyder Research Farm located in Pittstown, NJ, USA, which was managed by Rutgers University. The soil was sieved through a 10 mm mesh sieve prior to use to remove large particles. Artificial soil was prepared using 70% sand, 20% clay, and 10% peat. The treatments were prepared by adding the appropriate amount of DINP to the Artificial or Snyder soil. The treatments were stirred in stainless steel bowls with stainless steel spoons for at least 15-30 minutes and held overnight at room temperature. On Day 0, each treatment was divided into five replicates of about 100 g each. The replicates were then hydrated with the appropriate amount of reverse osmosis water to achieve 85% of the water holding capacity of the soil. Due to an error in the soil hydration calculation, three ml more of water per replicate was added to the sand/artificial soil treatments than was required to achieve 85% of the water holding capacity. It is not believed that this minimal extra amount of water had an adverse effect on the study.

Test organisms

Test organisms

Species other: Lolium species

Plant group Monocotyledonae (monocots)

Details on test organisms Seeds were obtained from Carolina Biological Supply Co., Burlington, NC, USA. - Common name: Ryegrass - Plant family: Poaceae - Variety: Lot 119

Species	Lactuca sativa
Plant group	Dicotyledonae (dicots)
Details on test organisms	- Common name: Lettuce - Plant family: Asteraceae - Variety: Lot 121 - Source of seed: Carolina Biological Supply Co., Burlington, NC, USA

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Test type

seedling emergence toxicity test

Substrate type

other: Two soils were tested: natural soil and artificial soil

Limit test

yes

Total exposure duration

5 d

Remarks

Test conditions

Test temperature

Test temperature = 24.5 degrees C (sd = 0.3 degrees C)

pH

pH = 6.4 to 7.0 Artificial soil control 6.7, Artificial soil with DINP 7.0, Natural (Snyder) soil control 6.9 and Natural (Snyder) soil with DINP 6.9

Moisture

Soil moisture was adjusted by adding the appropriate amount of reverse osmosis water to achieve 85% of the water holding capacity of the soil.

Nominal and measured concentrations

Soil samples were dosed with 10,000 mg test substance/kg soil. The soil analytical results are based on the mean of duplicate samples for each soil sampled: DINP Conc. Day -1 Soil Sample (mg/kg soil, dry wt.) Snyder Soil 8062 Artificial Soil 8473 Snyder soil was a natural soil.

Details on test conditions

Seeds were inspected and sized. Ryegrass (*Lolium* species) seeds selected for the test passed through a 1/6 x 1/28 inch screen. Test soils were homogenized prior to use by placing soil into 4L size plastic (HDPE) containers and mixing on a jar mill for approximately 15 minutes. After homogenizing, a sample was removed to determine the moisture fraction. The moisture fraction was measured by placing soil into a crystallizing dish and weighing it (initial wt.). The sample and crystallizing dish were then placed in an oven at 100C for 23 hours. After drying and cooling in a desiccator, the final weight of the sample and crystallizing dish was measured. The water holding capacity of each soil was determined as follows: 25g of the dried sample were placed into a 100mL glass beaker with 25mL of reverse osmosis water and mixed thoroughly. This slurry was added to a pre-weighed, wetted filter paper and funnel (initial weight = wt. of funnel, wetted paper, + 25 g soil). The filter paper (#113 Whatman 150mm) was wetted with 9mL of reverse osmosis water. The glass funnel measured 10cm top diameter with an approximately 30mm stem. The slurry, filter and funnel were covered with aluminum foil and allowed to stand for 3 hours at which time a final weight was measured. The water holding capacity, expressed in mL water/100g of soil, is the difference between the final and initial weights multiplied by 4 to achieve 100g equivalence. The test chambers were bottom halves of 150mm (diameter) x 15mm (high) plastic petri dishes placed in approximately 11" x 11" Ziploc bags. Each dish contained approximately 190g of test soil including the cover soil. Soil treatments included: control (no test material) and a nominal 10,000 mg test substance per kg soil (dry wt.). Soil was hydrated at 85% of water holding capacity. Five replicates each of the two soils were tested, each with a control soil. Prior to seed distribution, all treatment replicates were randomly positioned, then 40 seeds were distributed to each chamber. The seeds were distributed about the surface, but not closer than approximately 0.5 inches from the edge of the test chamber. Approximately 90g of cover soil was then evenly distributed on top of each hydrated treatment and control soils. Each chamber was placed directly into a resealable polypropylene (Ziploc) bag, centered over the bottom of the bag. The sides were raised to a vertical position over the chamber and the plastic covers were removed. The bags were then sealed leaving headspace. Test temperature: 24.5C (sd = 0.3C), as measured continuously and recorded by computer. Test photoperiod was as follows: Initial 48 hours dark, followed by 16 hours of light and 8 hours of dark until termination of the test. Light intensity ranged from 4300+/- 430 Lux during daylight periods, which was measured daily. Soil pH was measured at test initiation (day 0) of the study. Ten grams of each treatment were mixed with 20mL of distilled water. The pH value of the slurry was measured using a soil

pH probe. Soil pH ranged from 6.4 to 7.0. At test termination, after 5 days of exposure, the number of germinated seeds in each dish was determined by counting each seedling that protruded above the surface of the soil. TEST SYSTEM - Testing facility: Exxon Biomedical Sciences, Inc. - Test container (type, material, size): Bottom half of 150 mm (Diam.) x 15 mm (High) plastic Petri dishes - Amount of soil: 190 g - Method of seeding: Seeds were spaced about 0.5 inches from the edge of the test chamber and covered with approximately 90 g of appropriate soil - No. of seeds per container: 40 - No. of replicates per treatment group: 5 - No. of replicates per control: 5 SOURCE AND PROPERTIES OF SUBSTRATE (if soil) - Geographic location: Natural soil was from Rutgers University, Snyder Research Farm, Pittstown, NJ, USA - Composition (if artificial substrate): 20-mesh sieved sand with 10% artificial soil (70% sand, 20% clay, 10% peat) - Maximum water holding capacity (in % dry weight): 24/48 (artificial soil) or 59 (natural soil) ml/100g % GROWTH CONDITIONS - Photoperiod: 48 hours dark, followed by 16 hours of light and 8 hours of dark until termination of the test - Light intensity and quality: 4300 +/- 430 Lux during daylight periods - Relative humidity (%): Held in polythene bags ACCLIMATION PERIOD: None EFFECT PARAMETERS MEASURED (with observation intervals if applicable): percentage germination

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Species	Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
other: Lolium species	5 d	EC50	> 8062 mg/kg natural soil (d.w.)	meas. (initial)	test mat.	germination	
other: Lolium species	5 d	NOEC	8062 mg/kg natural soil (d.w.)	meas. (initial)	test mat.	germination	
other: Lolium species	5 d	EC50	> 8473 mg/kg artificial soil (d.w.)	meas. (initial)	test mat.	germination	
other: Lolium species	5 d	NOEC	8473 mg/kg artificial soil (d.w.)	meas. (initial)	test mat.	germination	
Lactuca sativa	5 d	EC50	> 8062 mg/kg	meas. (initial)	test mat.	germination	

Lactuca sativa 5 d EC50 natural soil (d.w.) > 8473 mg/kg artificial soil (d.w.) meas. (initial) test mat. germination

Details on results

At test termination, after 5 days of exposure, the number of germinated seeds was determined by counting each seedling that protruded above the surface of the soil. The germination results are located in the remarks field as a table.

Reported statistics and error estimates

The germination results were evaluated using Dunnett's procedure (one tailed T test, $\alpha=0.05$) of SAS [SAS/STAT® User's Guide. Version 6, Fourth Edition, Volumes 1 and 2. 1989. SAS Institute, Inc., Cary, NC, USA]. A random numbers computer generated table was used for test chamber placement in the test area.

Remarks on results including tables and figures

Germination data-Ryegrass (*Lolium* sp.)

	Number of seeds germinated (out of 40)					Mean
Replicates	1	2	3	4	5	germ
Artificial soil- control	38	38	38	38	35	
Artificial soil –DINP 10,000 mg/kg	39	37	36	36	38	
Natural (Snyder) soil-control	34	40	34	33	40	
Natural (Snyder) soil-DINP-10,000 mg/kg	40	40	38	40	40	

The germination of ryegrass was considered to have been unaffected by DINP in either soil.

Germination data- Lettuce (*Lactuca sativa*)

	Number of seeds germinated (out of 40)					Mean
Replicates	1	2	3	4	5	germ
Artificial soil-control	39	40	40	40	35	
Artificial soil-DINP 10,000 mg/kg	30	27	30	31	23	
Natural (Snyder) soil-control	39	36	39	38	35	
Natural (Snyder) soil-DINP-	30	33	28	33	31	

10,000 mg/Kg					
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The mean percentage germination of lettuce exposed to DINP in artificial or natural soil was about 75%. The difference with control values was statistically significant for both soils ($\alpha = 0.05$).

Applicant's summary and conclusion

Validity criteria fulfilled

yes Control seed germination >85%.

Conclusions

Di-isononyl phthalate ester does not effect the germination of ryegrass (*Lolium* species) seeds at very high test material/soil concentrations, 8062 mg/kg natural soil (dw) and 8473 mg/kg artificial soil (dw). Di-isononyl phthalate ester showed an effect on the germination of lettuce (*Lactuca sativa*) seeds at very high test material/soil concentrations, 8062 mg/kg natural soil (dw) and 8473 mg/kg artificial soil (dw). A 50% effect level was not established. The mean percentage germination was 71 and 78% in artificial and natural soils, respectively.

Executive summary

The toxicity of di-isononyl phthalate ester (DINP) as measured by the germination of ryegrass (*Lolium* species) seeds was evaluated in natural and artificial soils. Germination in the control soils ranged from 94 to 97%. Germination in soils dosed with DINP ranged from 93 to 99%. No significant effect on germination was demonstrated in the soils dosed with DINP at a nominal loading rate of 10,000 mg/kg soil (dw) (measured at 8062 mg/kg natural soil (dw) and 8473 mg/kg artificial soil (dw)).

The toxicity of di-isononyl phthalate ester (DINP) as measured by the germination of lettuce (*Lactuca sativa*) seeds was evaluated in natural and artificial soils. Germination in the control soils ranged from 94 to 97%. Germination in soils dosed with DINP ranged from 71 to 78%. A significant effect on germination was demonstrated in the soils dosed with DINP at very high test material/soil concentrations and a nominal loading rate of 10,000 mg/kg soil (dw) (measured at 8062 mg/kg natural soil (dw) and 8473 mg/kg artificial soil (dw)). In a long-term germination and growth test, no inhibition up to a concentration of 1,500 mg/kg dw was observed.

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 Dissemination Dossier

 Planttox Endpoint.004

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	1999
Reliability	1 (reliable without restriction)		
Rationale for reliability	The reliability is rated 1 because the study followed a standard guideline, which describes a procedure designed to evaluate this endpoint, the results were reviewed for reliability and assessed as valid, and the study was conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1996							2000-06-14

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 208 (Terrestrial Plants Test: Seedling Emergence and Seedling Growth Test)	yes One plant species with one seed per replicate to calculate at NOEC. This was determined to give an adequate evaluation. Carbon slightly exceeded 1.5% which did not affect the outcome.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

Substance type: technical product

Analytical monitoring

yes

Details on sampling

Samples of the test substance mixed in Snyder soil were taken in quadruplicate on Day -1 (pre-test) and at termination (composite of replicates).

Vehicle

no

Details on test substrate

Natural soil was obtained from Snyder Research Farm located in Pittstown, NJ, USA, which was managed by Rutgers University. The soil was sieved through a 2 mm mesh sieve prior to use to remove large particles. Soil Texture: Loam (34% Sand, 48% Silt, 18% Clay) pH: 6.1 Organic Carbon: 1.66% Organic Matter: 2.86% Macronutrients (lbs/acre) Micronutrients (ppm)
Phosphorous 181 Zinc 4.6 Potassium 441 Copper 25.0 Magnesium 405 Manganese 92.0 Calcium 1730 Boron 0.9

Test organisms

Test organisms

Species	Lactuca sativa
Plant group	Dicotyledonae (dicots)
Details on test organisms	- Common name: Lettuce - Plant family: Asteraceae - Variety: Lot 125

Study design

Study type

laboratory study

Test duration type

long-term toxicity

Test type

other: Seed Germination and Growth

Substrate type

natural soil

Limit test

no

Total exposure duration

28 d

Remarks

Test conditions**Test temperature**

The test was conducted at $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Ambient relative humidity was between 30 and 50%. The test area was monitored continuously with a temperature and humidity chart recorder.

pH

Ranged from 6.1 to 6.2 at test initiation.

Moisture

On Day 0, each treatment was hydrated to 30% of the dry soil weight using reverse osmosis dialyzed water and mixed thoroughly. To prevent drying of the soil, each replicate was hydrated with 1 to 4ml of reverse osmosis dialyzed water as needed.

Nominal and measured concentrations

Nominal: 375, 750, 1500 mg/kg dry weight Mean Measured: 374, 691, 1387 mg/kg dry weight

Details on test conditions

After germination, the test chambers were observed daily. At termination, after 28 days (± 1 hr) of exposure, the number of germinated seeds in each treatment was determined by counting each seedling that protruded above the surface of the soil. The plants were then cut at the soil level and individually weighed (wet weight).

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Species	Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
Lactuca sativa	28 d	NOEC	1387 mg/kg soil d.w.	meas. (arithm. mean)		test mat. germination	
Lactuca sativa	28 d	NOEC	1387 mg/kg soil d.w.	meas. (arithm. mean)		test mat. growth	

Reported statistics and error estimates

The germination results were evaluated using Fisher's Exact Test ($p=0.05$). The results for plant growth were evaluated using Bonferroni's t-Test ($p=0.05$), with the underlying assumptions for normality and homogeneity of variance formally tested using the Chi-square Test ($\forall=0.01$) and Bartlett's Test ($\forall=0.01$, $\forall=0.05$). All statistics were performed using TOXSTAT version 3.4 software.

Remarks on results including tables and figures

Germination Data

Concentration (mg/kg)	Control	375	750	1500
# Germinated	25	25	27	23
% Germinated	83	83	90	77

MEAN PLANT WEIGHTS (per pack)

Pack #	Treatment (mg/kg)			
	Control	375	750	1500

1	0.28403	0.29287	0.21211	0.35963
2	0.30041	0.28297	0.21648	0.26441
3	0.33827	0.24538	0.33199	0.18016
4	0.23401	0.27386	0.21541	0.30900
5	0.32586	0.22303	0.20126	0.18560
Mean	0.29652	0.26362	0.23545	0.25976
Std. Dev.	0.04087	0.02879	0.05430	0.07787

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

In a long term (28 day) test, di-isononyl phthalate ester does not effect the seed germination or growth of lettuce (*Lactuca sativa*) seeds at high test material/soil concentrations, 1387 mg/kg natural soil (dw).

Executive summary

The toxicity of di-isononyl phthalate ester (DINP) as measured by the germination and growth of lettuce (*Lactuca sativa*) seeds was evaluated in natural soils in a 28 day test. No significant effect on germination or growth was demonstrated in the soils dosed with DINP at a nominal loading rate of 1500 mg/kg soil (dw) (measured at 1387 mg/kg natural soil (dw)).

Dossier > Document

 Dissemination Dossier

 Soil micro tox Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	1999
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The reliability is rated 2 because the study procedure followed an accepted test guideline, but was not conducted under GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Study report; company data		1999							

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	other guideline: OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test)	

Principles of method if other than guideline

Method adapted to measure soil respiration:

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

substance type: technical product

Analytical monitoring

yes

Details on sampling

At the conclusion of testing a sample from each test substance flask was analyzed by GC/FID.

Vehicle

no

Details on preparation and application of test substrate

Testing was performed with fresh soil obtained from Rutgers Extension Snyder Research Farm in Quakertown, NJ. The test substance was added to the soil, mixed thoroughly and analyzed by gas chromatography/flame ionization detection (GC/FID) to verify the test substance concentration.

Test organisms

Test organisms (species)

soil

Study design

Total exposure duration

33 d

Remarks

Test conditions

Moisture content

65% of water holding capacity

Organic carbon content (% dry weight)

Not reported

Nitrogen content (% dry weight)

Not reported

Nominal and measured concentrations

Nominal 1000 mg/kg, measured initial 946 mg/kg, 1010 mg/kg (mean: 978 mg/kg) Nominal 10000 mg/kg, measured initial 9728 mg/kg, 9503 mg/kg (mean: 9616 mg/kg)

Details on test conditions

Evaluation of the test substance in soil with 500 mg glucose/kg (200 mg Carbon/kg) was performed at two concentrations (1000 and 10,000 mg/kg). Five replicates were set up for each test substance concentration along with soil only blanks.

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
33 d	NOEC	9616 mg/kg soil d.w.	meas. (initial)	test mat.	respiration rate	

Remarks on results including tables and figures

TABLE 1			
Sample Description		Glucose Percent Biodegradation (SD)	
		Day 28	Day 33
		47.25 (6.26)	50.19 (7.07)

500 ppm Glucose /kg Mercuric Chloride		0	0
500 ppm Glucose/kg 1000 mg DINP/Kg		82.02 (8.80)	88.03 (9.41)
500 ppm Glucose /kg 10000 mg DINP/Kg		73.63 (10.97)	78.91 (12.85)

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

DINP did not inhibit soil respiration during a 33-day exposure at a test material/soil loading of 10,000 mg/kg (d.w.) (9616 mg/kg (d.w. measured)). Therefore, DINP is not expected to adversely impact soil microbial activity.

Executive summary

DINP did not inhibit soil respiration during a 33-day exposure at a test material/soil loading of 10,000 mg/kg (d.w.) (9616 mg/kg (d.w. measured)). Therefore, DINP is not expected to adversely impact microbial activity in soil.

-  Dissemination Dossier
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Administrative Data

Data waiving study scientifically unjustified

 Dissemination Dossier

 Data tox Summary

Administrative Data

Results and discussions

Workers

Acute / short-term exposure - systemic effects

Dermal DN(M)EL in mg/kg bw/day not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Acute / short-term exposure - local effects

Dermal DN(M)EL in mg/cm² not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Long-term exposure - systemic effects

Dermal DN(M)EL in mg/kg bw/day DNEL 366 Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ DNEL 51.72 Assessment factor Dose descriptor starting point Most sensitive endpoint

Long-term exposure - local effects

Dermal DN(M)EL in mg/cm² not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

General population

Acute / short-term exposure - systemic effects

Dermal DN(M)EL in mg/kg bw/day not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Oral DN(M)EL in mg/kg bw/day not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Acute / short-term exposure - local effects

Dermal DN(M)EL in mg/cm² not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Long-term exposure - systemic effects

Dermal DN(M)EL in mg/kg bw/day DNEL 220 Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ DNEL 15.3 Assessment factor Dose descriptor starting point Most sensitive endpoint

Oral DN(M)EL in mg/kg bw/day DNEL 4.4 Assessment factor Dose descriptor starting point Most sensitive endpoint

Long-term exposure - local effects

Dermal DN(M)EL in mg/cm² not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Inhalation DN(M)EL in mg/m³ not quantifiable Assessment factor Dose descriptor starting point Most sensitive endpoint

Administrative Data

Purpose flag	key study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	Acceptable, well-documented study report which meets basic scientific principles: non-GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Koch HM, Angerer J.	2007	Di-isobutylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP.	Int J Hyg Environ Health. 2007 Jan;210(1):9-19. Epub 2006 Dec 19.					

Materials and methods

Type of method

in vivo

Objective of study

metabolism

excretion

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

The metabolism and elimination of DINP were studied in a single male volunteer after a single oral DINP dose of 1.27 mg/kg body-weight. To avoid interference by omnipresent background exposure, deuterium labeled DINP was used. The urinary excretion of the simple monoester mono-iso-nonylphthalate (MINP) and oxidised isomers with hydroxy (OH-MINP), oxo (oxo-MINP) and carboxy (carboxy-MINP) functional groups were investigated.

GLP compliance

no data

Test material equivalent to submission substance identity

no

Radiolabelling

other: Deuterated

Test materials

Details on test material

- Name of test material (as cited in study report): di-iso-nonylphthalate-3,4,5,6 D4 (D4-DINP)

Test animals

Species

other: human

Sex

male

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: butter

Duration and frequency of treatment / exposure

Single exposure

Doses / concentrations

1.27 mg/kg-bw (less than one-tenth the lowest NOAEL for DINP of 15 mg/kg/day).

No. of animals per sex per dose

Single adult male aged 63, 175 cm tall, and weighing 77 kg (senior author in reference).

Control animals

no

Details on study design

Consecutive and complete urine samples, one sample prior to dosing and 22 samples throughout the following 48 h, were collected. Samples were analyzed via LC-MS/MS.

Results and discussions

Pharmacokinetic studies

Absorption

Not assessed

Distribution in tissues

Not assessed

Excretion

Within 48 h, 43.6% of the applied dose was recovered in urine as the DINP metabolites, 20.2%

as OH-MINP, 10.7% as carboxy-MINP, 10.6% as oxo-MINP and 2.2% as MINP. Other oxidised DINP metabolites not determined in this study may increase the amount of the DINP dose excreted via urine.

Metabolite characterisation studies

Metabolites identified

yes

Details on metabolites

The urinary excretion of the simple monoester mono-iso-nonylphthalate (MINP) and oxidised isomers with hydroxy (OH-MINP), oxo (oxo-MINP) and carboxy (carboxy-MINP) functional groups were assessed using isomeric MINP and three specific oxidised isomer standards for quantification: mono-(4-methyl-7-hydroxyoctyl) phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxyheptyl)phthalate (7carboxy-MMeHP).

Applicant's summary and conclusion

Interpretation of results

other: DINP is rapidly metabolized and eliminated.

Executive summary

This describes human metabolism and elimination of DINP in a male volunteer after a single oral deuterium labeled DINP dose of 1.27 mg/kg body-weight. Urinary excretion of the simple monoester mono-iso-nonylphthalate (MINP) and oxidized isomers with hydroxy (OH-MINP), oxo (oxo-MINP) and carboxy (carboxy-MINP) functional groups was investigated using isomeric MINP and three specific oxidized isomer standards for quantification: mono-(4-methyl-7-hydroxyoctyl) phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxyheptyl)phthalate (7carboxy-MMeHP). Within 48 h, 43.6% of the applied dose in urine was recovered as the above DINP metabolites: 20.2% as OH-MINP, 10.7% as carboxy-MINP, 10.6% as oxo-MINP and 2.2% as MINP. Elimination followed a multi-phase pattern; elimination half-lives in the second phase (beginning 24 h post-dose) can only roughly be estimated to be 12 h for the OH- and oxo-MINP-metabolites and 18 h for carboxy-MINP metabolites. After 24 h, the carboxy-MINP metabolites replaced the OH-MINP metabolites as the major urinary metabolites.

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 Dissemination Dossier

 Meta mam Endpoint.002

[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussions](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability	Acceptable, well-documented study report which meets basic scientific principles: pre-GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1972							1972-09-25

Materials and methods

Type of method

in vivo

Objective of study

metabolism

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

Animals received "cold" compound daily for five days. On the sixth day, each animal received a

single dose of "hot" compound and placed in metabolism cages for 72 hours.

GLP compliance

no

Test material equivalent to submission substance identity

yes

Radiolabelling

yes C14 labeled DINP, 105 uCi/g

Test materials

Details on test material

[14C] DINP (CAS 68515 -48 -0)

Test animals

Species

rat

Strain

no data

Sex

male

Administration / exposure

Route of administration

oral: gavage

Vehicle

no data

Duration and frequency of treatment / exposure

Animals received "cold" compound daily for five days. On the sixth day, each animal received a single dose of "hot" compound and placed in metabolism cages for 72 hours.

Doses / concentrations

0.5 ml ("cold" and "hot")

No. of animals per sex per dose

5 male animals

Control animals

no

Results and discussions

Pharmacokinetic studies

Distribution in tissues

No tissue studied had over 0.001% per gram of the administered dose. On a total sample basis, residual amounts of radioactivity were found in the gastrointestinal tract of each animal. Of the selected organs which were analyzed, the liver contained the most radioactivity, 0.010% (mean). Most of the radioactivity retained by the animals was found in the carcass and remaining viscera. Each animal retained an amount of the administered dose in the carcass and remaining viscera which was close to the average value of 0.060%. The value for the average amount retained after 72 hours is 0.09% of the administered dose.

Excretion

The amount excreted in the urine within 72 hours ranged from a high of 18.34% to a low of 8%. The majority of radioactivity found was excreted in the first collection (12 hr). Thereafter, the amount of radioactivity found fell off rapidly so that by the 72-hours collection, less than 0.1% of the dose was found. In all test animals, over 80% of the administered dose was excreted in the feces, and most of that radioactivity was excreted within 24 hours after dosing. Between the 60 and 72-hour post dosage period, no animal excreted over 0.2% of the administered dose.

Applicant's summary and conclusion

Interpretation of results

no bioaccumulation potential based on study results

Executive summary

This study was designed to assess the extent of gastrointestinal absorption, the distribution in selected tissues, and the rate and routes of excretion of [14C] DINP (CAS 68515 -48 -0) following oral administration to male albino rats weighing approximately 200 grams each (4 treated rats, 2 controls). Four animals received 0.5 ml of "cold" compound daily for five days and on the sixth day, they were dosed via oral intubation with 0.5 ml of "hot" compound (about 2,500 mg/kg/d). Expired gases, fecal and urinary excreta were collected separately, at 12 hours intervals, during the entire study period (72 hours). Recordings were made of animal appearance and behaviour, body weights, volumes of urine excreted, weights of feces excreted, and amounts of food consumed. Seventy-two hours after intubation, the animals were sacrificed and gross pathological observations were made. Blood and urine remaining in the bladder were collected. The hearts, livers, kidneys, intestines, stomachs and aliquots of fat and muscle were removed and weighed. The contents of the intestines and stomach were separated from their respective walls. The remaining viscera and carcass from each animal were combined and weighed.

During the six-day period, all the animals exhibited a normal behaviour. All animals gained weight during the five-day period when they were given the cold compound. During the 72 hours after the administration of the "hot" compound, one test animal and one control animal lost weight, and the test animals consumed more food and excreted more feces than the controls, but the controls excreted more urine than the test animals.

The amount of compound excreted in the urine within 72 hours ranged from 8% to 18.34% of the administered dose. The majority of the radioactivity found was excreted in the first collection (12 hours). Thereafter, the amount of radioactivity found fell off rapidly so that by the 72-hour collection period less than 0.1% of the dose was found in all animals except in one animal. Over 80% of the administered dose was excreted in the feces and most of the radioactivity was excreted within 24-hours after dosing. Between the 60- and 72-hour postdosage period no animal excreted over 0.2% of the administered dose.

At sacrifice the tissues and organs of all animals appeared normal when examined for gross pathological conditions. No tissue studied had over 0.001% per gram of the administered dose. On a total sample basis, residual amounts of radioactivity were found in the gastrointestinal tract of each animal. Of the selected organs, the liver contained the most radioactivity (i.e. 0.010% mean). Most of the radioactivity retained by the animals was found in the carcass and remaining viscera (i.e. 0.060% of the administered dose).

No expired [14C] DINP was detected.

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 Dissemination Dossier

 Meta mam Endpoint.003

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag supporting study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, Brock JW, Needham LL, and Calafat AM	2004	Urinary Levels of Seven Phthalate Metabolites in the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2000	Environmental Health Perspectives (112): 331-338					

Materials and methods

Type of method

other: Measurement of urinary metabolites

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

GLP compliance

no data

Test material equivalent to submission substance identity

no

Test materials

Details on test material

monoethyl phthalate (MEP), monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), and mono-(2-ethylhexyl) phthalate (MEHP) were detected in urine samples

Test animals

Species

other: human urine samples

Sex

male/female

Administration / exposure

Route of administration

other: urinary metabolites

Vehicle

other: not applicable

Duration and frequency of treatment / exposure

Not applicable

Doses / concentrations

Not applicable

No. of animals per sex per dose

Not applicable

Control animals

no

Statistics

Statistical analyses were performed using the statistical software packages SAS and SUDAAN. SUDAAN incorporates the sample population weights and calculates variance estimates that account for the complex design of NHANES. The survey-specific sample weights were designed specifically for the one-third subset of the full survey. Parametric statistics were computed only for analytes for which the frequency of detection was 60%. The geometric means (GMs) and distribution percentiles for both the volume-based and creatinine-corrected concentrations using the survey sampling weights, which take into account the unequal selection probabilities caused by the cluster design and the planned oversampling of certain subgroups were calculated. The analytical limits of detection (LODs) were MEP, 1.0 µg/L; MBP, 0.6 µg/L; MBzP, 0.8 µg/L; MEHP, 1.2 µg/L; mono-cyclohexyl phthalate (MCHP), 0.7 µg/L; mono-n-octyl phthalate (MOP), 0.9 µg/L; and mono-3-methyl-5-dimethylhexyl phthalate [monoisononyl phthalate (MINP)], 0.8 µg/L. For concentrations below the LOD, a value equal to the LOD divided by the square root of 2 was used. An analysis of covariance model was used to study the effects of several covariates (i.e., sex, race/ethnicity, age, and urinary creatinine) on the log-transformed urinary phthalate metabolite concentrations. This model enabled the comparison of the expected least square geometric mean (LSGM) phthalate metabolite levels for selected demographic groups (e.g., male vs. female), which were adjusted statistically so that subjects had comparable levels of all other covariates.

Results and discussions

Pharmacokinetic studies

Absorption

Not applicable

Distribution in tissues

Not applicable

Excretion

Infrequently detection of monoisononyl phthalate.

Metabolite characterisation studies

Metabolites identified

yes

Details on metabolites

Infrequently detection of monoisononyl phthalate.

Applicant's summary and conclusion

Interpretation of results

other: Infrequent detection of MINP in urine samples

Executive summary

Dossier > Document

 Dissemination Dossier

 Meta mam Endpoint.004

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	July 1981-February 1982
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered equivalent to OECD test guidelines.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1983							1983-12-19
publication	McKee RH, El-Hawari M, Stoltz M, Pallas F, Lington AW.	2002	Absorption, disposition and metabolism of diisononyl phthalate (DINP) in F-344 rats.	J Appl Toxicol. 2002 Sep-Oct;22(5):293-302.					

Materials and methods

Type of method

in vivo

Objective of study

absorption

excretion

distribution

metabolism

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 417 (Toxicokinetics)	no

Principles of method if other than guideline

Although there is no statement concerning guidelines in the report, the study was undertaken to a standard considered to be equivalent to at least OECD guideline 417.

GLP compliance

yes

Test material equivalent to submission substance identity

yes

Radiolabelling

yes

Test materials

Details on test material

- Name of test material (as cited in study report): Diisononylphthalate - Substance type: Technical material - Physical state: No data - Radiochemical material: Amersham Corporation Batch No. S42334 - Radiochemical purity (if radiolabelling): 97-98% (TLC) - Specific activity (if radiolabelling): 53 mCi/mmol - Locations of the label (if radiolabelling): ¹⁴C carboxyl - Expiration date of radiochemical substance (if radiolabelling): No data - Stability under test conditions: No data - Storage condition of test material: No data

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Breeding laboratory - Age at study initiation: 60-79 days - Weight at study initiation: 143-219 g males and 122-132 g females - Fasting period before study: No data - Housing: polycarbonate cages - Individual metabolism cages: individual stainless steel metabolism cages - Diet (e.g. ad libitum): Zeigler NIH-07 rat chow ad libitum - Water (e.g. ad libitum): tap water ad libitum - Acclimation period: At least 7 days
ENVIRONMENTAL CONDITIONS - Temperature (°C): 70 ± 2 °F - Humidity (%): 50 ± 10 % - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): 12 hours dark/ 12 hours light
IN-LIFE DATES: From: July 1981 To: February 1982

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Details on exposure

PREPARATION OF DOSING SOLUTIONS: Appropriate amounts of the ¹⁴C-labelled and non-labelled test material were mixed to supply the required amounts of the ¹⁴C label for each dose level. These mixtures were dissolved in corn oil and administered by oral gavage. Aliquots of each dosing solution were counted to determine the amounts of radioactivity
VEHICLE - Justification for use and choice of vehicle (if other than water): Test material dissolves in corn oil - Concentration in vehicle: No data - Amount of vehicle (if gavage): 2 ml/kg body weight - Lot/batch no. (if required): No data - Purity: No data
HOMOGENEITY AND STABILITY OF TEST MATERIAL: No data

Duration and frequency of treatment / exposure

A single administration, or once daily for five days.

Doses / concentrations

50 and 500 mg/kg for single administration and 50, 150 and 500 mg/kg/day for repeated dose exposure

No. of animals per sex per dose

40 males (20 per dose group) and 8 females (4 per dose group) for single administration. 45 males (15 per dose group) for repeat dose administration

Control animals

no

Positive control

Not applicable

Details on study design

- Dose selection rationale: No data, other than 'selected by Sponsor'. - Rationale for animal assignment (if not random): No data

Details on dosing and sampling

PHARMACOKINETIC STUDY (Absorption, distribution, excretion) - Tissues and body fluids sampled (delete / add / specify): urine, faeces, blood, plasma, tissues (liver, kidneys, lungs, brain, spleen, testes/ovaries, skin, skeletal muscle, fat and GI tract plus contents), and cage washes - Time and frequency of sampling: 1, 4, 8, 24 and 72 hours after dosing for male rats (four animals per time point for the single dose phase and three animals per time point for the multiple dose phase). After 24 hours for female rats. METABOLITE CHARACTERISATION STUDIES - Tissues and body fluids sampled (delete / add / specify): urine, faeces, GI tract, liver, testes and fat - Method type(s) for identification (e.g. GC-FID, GC-MS, HPLC-DAD, HPLC-MS-MS, HPLC-UV, Liquid scintillation counting, NMR, TLC)

Statistics

The means $\pm$ standard errors were calculated for each test group with a programmable calculator. The significance of the data in the single dose study was determined by the two-tailed Student's t-test. Data from the multiple dosing study were subjected to analysis of variance. Significant F-ratios were then analysed by Dunnett's procedure. Significant differences were indicated when $p < 0.05$.

Results and discussions

Preliminary studies

Not reported in the robust summary as they have no significant bearing on the results presented for the main study.

Pharmacokinetic studies

Absorption

See 'remarks on results' window below A. Absorption and Tissue Distribution In male rats receiving one dose of ¹⁴C-DINP, levels of radioactivity in blood and most tissues were highest at 1 hr following administration of both doses. The highest levels were demonstrated in liver, followed by kidneys, then blood; other tissues examined had lower ¹⁴C contents. ¹⁴C levels in fat were low and showed no particular increase following administration of the high dose. At all times examined, most of the blood radioactivity was recovered in the plasma, while liver radioactivity was localized primarily in the cytosol. The lower recovery (percent of the administered dose) in livers of rats receiving the high dose may be due to a limited absorption of DINP, but the higher recovery in other tissue (e.g., skin and muscle) and the lower liver-to-blood ratios in rats receiving the high dose suggest a limited or saturated liver uptake of DINP and/or its metabolites. Radioactivity rapidly disappeared from blood, liver, and kidney, reaching low levels at 72 hr. Rates of ¹⁴C disappearance from other tissues were slower. Radioactivity in some tissues was below the level of accurate detection at 72 hr and, occasionally, at 24 hr. Although blood ¹⁴C levels in female rats sacrificed at 24 hr following the high dose were lower than in the males, the levels in tissues were generally similar in the males and females treated at either dose level. Concentrations of radioactivity in blood and most tissue of rats sacrificed after five daily doses were highest at 1 hr following the last dose. The highest levels were demonstrated in liver, followed by kidney, blood, then skin. Other tissues including fat and testes contained lower ¹⁴C levels. Most of the liver radioactivity (50-60%) was localized in the cytosol. Blood radioactivity was recovered primarily in plasma. Recovery (percent of administered dose) in liver of rats receiving the high dose was lower than in rats receiving the mid and low doses, which reflect limited absorption and/or saturated liver uptake of ¹⁴C-DINP or its metabolites with the increase in dose level. Rapid disappearance of radioactivity was demonstrated for blood, liver, and kidney. Radioactivity disappeared from other tissues at slower rates. Recoveries in blood and tissue at 1 hr amounted to 7 to 8% of the administered doses, but declined to 0.3 to 0.5% by 72 hr. Retention or accumulation of the diester or its metabolites in tissues was not evident. B. Urinary and Fecal Excretion See 'remarks on results' window below

Distribution in tissues

See 'remarks on results' window below

Excretion

See 'remarks on results' window below

Metabolite characterisation studies

Metabolites identified

yes

Details on metabolites

See 'remarks on results' window below

Remarks on results including tables and figures

A. Absorption and Tissue Distribution

In male rats receiving one dose of ¹⁴C-DINP, levels of radioactivity in blood and most tissues were highest at 1 hr following administration of both doses. The highest levels were demonstrated in liver, followed by kidneys, then blood; other tissues examined had lower ¹⁴C contents. ¹⁴C levels in fat were low and showed no particular increase following administration of the high dose. At all times examined, most of the blood radioactivity was recovered in the plasma, while liver radioactivity was localized primarily in the cytosol. The lower recovery (percent of the administered dose) in livers of rats receiving the high dose may be due to a limited absorption of DINP, but the higher recovery in other tissue (e.g., skin and muscle) and the lower liver-to-blood ratios in rats receiving the high dose suggest a limited or saturated liver uptake of DINP and/or its metabolites. Radioactivity rapidly disappeared from blood, liver, and kidney, reaching low levels at 72 hr. Rates of ¹⁴C disappearance from other tissues was slower. Radioactivity in some tissues was below the level of accurate detection at 72 hr and, occasionally, at 24 hr. Although blood ¹⁴C levels in female rats sacrificed at 24 hr following the high dose were lower than in the males, the levels in tissues were generally similar in the males and females treated at either dose level.

Concentrations of radioactivity in blood and most tissues of rats sacrificed after five daily doses were highest at 1 hr following the last dose. The highest levels were observed in liver, followed by kidney, blood, and then skin. Other tissues including fat and testes contained lower ¹⁴C levels. Most of the liver radioactivity (50-60%) was localized in the cytosol. Blood radioactivity was recovered primarily in plasma. Recovery (percent of administered dose) in liver of rats receiving the high dose was lower than in rats receiving the mid and low doses, which reflects limited absorption and/or saturated liver uptake of ¹⁴C-DINP or its metabolites with the increase in dose level. Rapid disappearance of radioactivity was observed for blood, liver, and kidney. Radioactivity disappeared from other tissues at lower rates. Recoveries in blood and tissue at 1 hr amounted to 7 to 8% of the administered doses, but declined to 0.3 to 0.5% by 72 hr. Retention or accumulation of the diester or its metabolites in tissues was not evident.

B. Urinary and Fecal Excretion

In male and female rats receiving a single dose of ¹⁴C-DINP, the entire administered radioactivity was essentially eliminated in urine and feces within 72 hr after dosing; the major portions were eliminated within the first 24 hr. After the low dose, excretion in urine was

initially higher than in feces, but by 72 hr equal amounts were recovered in each. Following the high dose, elimination in urine and feces was initially equal, but excretion in feces at 72 hr exceeded that in urine. In female rats, urinary and fecal excretion following the low or high doses was similar to that demonstrated for male rats.

Following administration of five doses of ^{14}C -DINP, almost all of the administered radioactivity was recovered in urine and feces within 72 hr following the last dose; the major portions were eliminated within the first 48 hr. Excretion in urine was higher than in feces at the three dose levels. Studies on DINP metabolic profiles in feces indicated that fecal excretion represents a balance of unabsorbed DINP and the metabolites eliminated in bile.

C. Urinary Metabolites

HPLC analyses of urine collected from male rats between 0-8 hr following a single dose of ^{14}C -DINP showed a major portion of the radioactivity (up to 28%) co-eluting with phthalic acid (PA) and a minor amount (up to 7%) eluting at the origin of the chromatogram (polar component).

Most of the radioactivity in urine (58-83%) eluted as a broad peak more polar than the monoester, monoisononyl phthalate (MINP). This peak, which was tentatively identified as side chain oxidation products of the monoester, was present in higher amounts in urine collected between 8 and 24 hr. The peak corresponding to PA was lower while that of the polar component remained the same. Elimination of PA was dose-dependent and decreased in urine of rats treated with the high dose. Urine collected from female rats showed similar profiles to those of males. In all urine samples, the monoester and diester were absent or present only in trace amounts.

In urine of rats treated with five daily doses, the major portions of urinary radioactivity (79 to 91%) eluted as broad peaks corresponding to the side-chain oxidation products of the monoester. The quantities of this metabolite in urine collected between 0 and 8 hr, or 8 and 24 hr were similar, but were slightly higher in urine of rats receiving the high dose. Smaller amounts (up to 13%) of PA were recovered in urine; PA excretion following repeated dosing showed no dose-dependence. The polar component was recovered in trace amounts following the high dose level but represented up to 6% of urinary radioactivity following the low dose. The diester and the monoester were absent or present only in trace amounts.

D. Fecal Metabolites

In feces, 8 and 41% of the radioactivity was associated with the diester following treatment with the single low and high doses, respectively. The remainder eluted in the areas of the monoester and monoester oxidation products; PA was absent (high dose) or present in small amounts (low dose). No radioactivity was associated with the polar component. Fecal samples collected from female rats treated with the low dose showed minor quantitative differences from those of male rats, but following the high dose, the feces of female rats showed higher amounts of the parent compound and lower amounts of the oxidative metabolites. In feces of rats treated with five doses of ^{14}C -DINP, the fecal radioactivity was divided between the diester, monoester, and the oxidation products; higher amounts of the diester were recovered following the high dose. Only trace amounts of PA and the polar component were recovered.

E. GI tract Metabolites

The GI tract of male rats treated with a single low dose showed minor peaks of DINP and the polar component; the majority of radioactivity (83%) eluted as the oxidation products of the monoester. After the high dose, more DINP and less oxidation products were recovered. The GI tracts of female rats contained significantly higher amounts of the diester and the monoester, and lower amounts of the oxidation products compared to their male counterparts. In rats receiving five doses, the profiles of the GI tract showed primarily the oxidation products of the monoester

and small amounts of the diester and monoester. Only trace amounts of PA and the polar component were detected. Recovery of the diester and the monoester in the GI tract showed minor quantitative differences following repeated administration of the low and high doses of ¹⁴C-DINP.

F. Tissue Metabolites

The livers of male rats receiving the single low doses contained major peaks of the monoester and its oxidation products. The monoester and PA decreased with time of sampling, and by 24 hr, almost all of the liver radioactivity eluted as the oxidation products, with only traces of the diester and none of the polar component being detected. On the other hand, small amounts of the diester were recovered in livers of rats treated with the high dose and sacrificed at early times (1 and 4 hr). At 1 hr, the monoester and its oxidation products constituted the two major peaks; only 1% of the radioactivity coeluted with the PA. At 4, 8, and 24 hr, the oxidation products increased and the monoester decreased. No differences were apparent in the liver profiles of male and female rats sacrificed at 24 hr.

The livers of rats receiving five doses of ¹⁴C-DINP contained major amounts of the monoester and its oxidation products. At later sacrifice times most liver radioactivity (71 to 90%) eluted as oxidation products. Only small amounts of PA and the polar component were detected, while the diester was absent or present only in trace amounts. The metabolic profiles in the liver were similar irrespective of dose.

The major metabolites recovered in the testes of rats receiving one dose of ¹⁴C-DINP include the monoester, its oxidation products, and the acid; small amounts of the polar component were also present. Slightly higher amounts of the monoester and lower amounts of the acid were recovered in testes of rats receiving the high dose. The parent compound was not detected from either dose level. In rats treated with five daily doses, the profiles from testes showed primarily oxidation products present in amounts up to 89% of the radioactivity following the high dose; small amounts of the monoester and PA were recovered. The diester and the polar component were absent or only occasionally present.

Fat collected from rats receiving a single low dose showed peaks corresponding to the monoester, its oxidation products, and traces of the polar component. The diester was detected only in fat collected from rats receiving the high dose.

Applicant's summary and conclusion

Interpretation of results

other: Biological half-life is approximately 7 hours.

Conclusions

These data indicate that a single low dose of ¹⁴C-DINP administered orally to rats was readily absorbed and distributed to major tissues, particularly the liver. Absorption, however, was incomplete following a high dose of ¹⁴C-DINP and after repeated dosing of the compound at all dose levels. DINP metabolites were readily excreted in urine and to a lesser extent in feces. Prolonged retention of DINP and/or its metabolites in blood and tissues was not apparent. DINP was de-esterified to the mono-ester which was further metabolized by side-chain oxidation of the

ester group or by hydrolysis to phthalic acid; the formation of oxidation products appeared to increase following the high dose while hydrolysis to phthalic acid decreased. A major sex difference was demonstrated in the recovery of lower amounts of monoisononylphthalate-oxidation products in the GI tracts and feces of female rats. Repeated dosing caused no accumulation of DINP and/or its metabolites in blood and tissue but resulted in increased formation and elimination of the monoester-oxidation products. Irrespective of dose or the number of doses administered, most of the liver radioactivity was localized in the cytosol.

Executive summary

The pharmacokinetic and metabolic behavior of ¹⁴C-diisononyl phthalate (¹⁴C-DINP) were investigated in male and female Fischer 344 rats treated with a single oral dose of 50 or 500 mg/kg (low and high, respectively) and in male rats treated with five daily oral doses of 50, 150, and 500 mg/kg (low, mid, and high, respectively). Elimination of radioactivity in urine and feces was examined for up to 72 hrs after the last dose. Blood and tissue levels of radioactivity were determined in male rats sacrificed at 1, 4, 8, 24, and 72 hr and in females sacrificed at 24 hr. Liver tissues were subjected to subcellular fractionation and ¹⁴C contents in different fractions were determined. The metabolic profiles of urine, feces, GI tract, and selected tissues (liver, testes, and fat) were examined by high performance liquid chromatography (HPLC). In addition, selected metabolites were isolated and subjected to chemical characterization.

These data indicate that a single low dose of ¹⁴C-DINP administered orally to rats was readily absorbed and distributed to major tissues, particularly the liver. Absorption, however, was incomplete following a high dose of ¹⁴C-DINP and after repeated dosing of the compound at all dose levels. DINP metabolites were readily excreted in urine and to a lesser extent in feces. Prolonged retention of DINP and/or its metabolites in blood and tissues was not apparent. DINP was de-esterified to the mono-ester which was further metabolized by side-chain oxidation of the ester group or by hydrolysis to phthalic acid; the formation of oxidation products appeared to increase following the high dose while hydrolysis to phthalic acid decreased. A major sex difference was demonstrated in the recovery of lower amounts of monoisononyl phthalate-oxidation products in the GI tracts and feces of female rats. Repeated dosing caused no accumulation of DINP and/or its metabolites in blood and tissue but resulted in increased formation and elimination of the monoester-oxidation products. Irrespective of dose or the number of doses administered, most of the liver radioactivity was localized in the cytosol. DINP was therefore considered to have low bioaccumulation potential.

The pharmacokinetic study is considered acceptable and satisfies the guideline requirements for rat toxicokinetic studies.

Dossier > Document

 Dissemination Dossier

 Meta mam Endpoint.005

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag supporting study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Silva MJ, Reidy JA, Preau Jr, Needham LL, Calafat AM.	2006	Oxidative metabolites of diisononyl phthalate as biomarkers for human exposure assessment.	Environ Health Perspect. 2006 Aug;114(8):1158-61.					

Materials and methods

Type of method

other: urinary metabolite screen

Objective of study

metabolism

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

The urinary concentrations of mono-3-methyl-5-dimethylhexyl phthalate (MINP), mono(carboxyisooctyl) phthalate (MCIOP), mono(oxoisooctyl) phthalate (MOINP), and mono(hydroxyisooctyl) phthalate (MHINP) were measured in 129 adult anonymous human volunteers with no known exposure to DINP.

GLP compliance

no data

Test material equivalent to submission substance identity

no

Test animals

Species

other: human

Sex

male/female

Administration / exposure

Route of administration

other: not administered

Duration and frequency of treatment / exposure

not applicable

Doses / concentrations

not applicable

No. of animals per sex per dose

not applicable

Control animals

no

Statistics

Samples with values below the LOD were assigned a concentration equal to the LOD divided by the square root of 2 for the statistical analyses. Statistical significance was set at $p < 0.05$.

Results and discussions

Pharmacokinetic studies

Absorption

not applicable

Distribution in tissues

not applicable

Excretion

MHINP was present in all samples tested at concentrations ranging from 1.4 to 202.7 ng/ml, with 5% of the samples having > 43.7 ng/ml. Similarly, MCIOP was detected in 97% of the samples tested at levels ranging from $< \text{LOD}$ to 310.8 ng/ml. MOINP was detected in 87% of the samples at levels ranging from $< \text{LOD}$ to 201.7 ng/mL. The geometric mean concentrations of MHINP, MCIOP, and MOINP were 11.4, 8.6, and 1.2 ng/ml, respectively. MINP was not detected in any of the samples analyzed.

Metabolite characterisation studies

Metabolites identified

yes

Details on metabolites

The urinary concentrations of mono-3-methyl-5-dimethylhexyl phthalate (MINP), mono(carboxyisooctyl) phthalate (MCIOP), mono(oxoisooctyl) phthalate (MOINP), and mono(hydroxyisooctyl) phthalate (MHINP) were measured

Applicant's summary and conclusion

Interpretation of results

other: Metabolites of DINP can be identified in urine

Dossier > Document

 Dissemination Dossier

 Meta mam Endpoint.006

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag supporting study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Silva MJ, Kato K, Wolf C, Samandra E, Silva SS, Gray EL, Needham LL, Calafat AM.	2006	Urinary biomarker Toxicology. s of di- isononyl phthalate in rats.	2006 Jun 1;223(1-2):101-12.					

Materials and methods

Type of method

in vitro

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

The urinary excretion of DINP metabolites was monitored in adult female Sprague-Dawley rats after oral administration of a single dose (300 mg/kg) of commercial DINP.

Test material equivalent to submission substance identity

no

Results and discussions

Pharmacokinetic studies

Excretion

Excretion toxicokinetics of the DiNP metabolites in urine followed a biphasic pattern with initial rapid decay in concentration.

Metabolite characterisation studies

Metabolites identified

yes

Details on metabolites

Mono(carboxy-isooctyl)phthalate (MCiOP) was identified as the major metabolite of DiNP; in addition, mono(hydroxy-isononyl)phthalate (MHiNP) and mono(oxo-isononyl)phthalate (MOiNP) were present. Furthermore, metabolites of di-isooctyl phthalate (DiOP) and di-isodecyl phthalate (DiDP) were also detected.

- Dissemination Dossier
- Dermal absorption Endpoint.001

Administrative Data

Purpose flag supporting study

Data source

Reference			
Reference type	Author	Year Title	Bibliographic source
publication	Elsisi AE, Carter DE, Sipes IG.	1989 Dermal absorption of phthalate diesters in rats.	Fundam Appl Toxicol. 1989 Jan;12(1):70-7.

Dossier > Document

 Dissemination Dossier

 Dermal absorption Endpoint.002

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	06.12.1982 to 01.02.1983
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered equivalent to the OECD 427 test guidelines		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1983							1983-08-04
publication	McKee RH, El-Hawari M, Stoltz M, Pallas F, Lington AW.	2002	Absorption, disposition and metabolism of diisononyl phthalate (DINP) in F-344 rats.	J Appl Toxicol. 2002 Sep-Oct;22(5):293-302.					

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 427 (Skin Absorption: In Vivo Method)	no

GLP compliance

yes

Test materials

Radiolabelling

yes

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate - Physical state: No data
- Radiochemical purity (if radiolabelling): 97-98% (TLC) - Specific activity (if radiolabelling): 53 mCi/mmol - Locations of the label (if radiolabelling): ¹⁴C carboxyl - Expiration date of radiochemical substance (if radiolabelling): No data - Stability under test conditions: No data - Storage condition of test material: No data

Test animals

Species

rat

Strain

Fischer 344

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Breeding Laboratories - Age at study initiation: 63 to 64 days - Weight at study initiation: 140 to 182 g - Fasting period before study: Not applicable - Housing: polycarbonate cages - Individual metabolism cages: Stainless steel metabolism cages -

Diet (e.g. ad libitum): Commercial rat chow ad libitum - Water (e.g. ad libitum): Tap water ad libitum - Acclimation period: 7 days ENVIRONMENTAL CONDITIONS - Temperature (°C): 70 +/- 2°F - Humidity (%): 50 +/- 10% - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): 12-hrs light / 12 hrs dark EXPERIMENTAL WORK PERFORMED: from: 6 December 1982 To: 1 February

Administration / exposure

Type of coverage

occlusive

Vehicle

other: Diethyl ether

Duration of exposure

up to 7 days

Doses

0.2 ml conditioned (Group1), 0.2 ml nonconditioned (Group2) or 0.1 ml nonconditioned (Group3). A single dose of ¹⁴C labelled DINP was applied dermally to the shaved skin on the back of each rat. Animals in Group 1 were pre-treated with nonlabelled DINP which was kept on the skin for three days and then blotted off prior to application of radio-labelled dose.

No. of animals per group

Six Group 1, six Group 2, or three Group 3.

Control animals

no

Details on study design

DOSE PREPARATION - Method for preparation of dose suspensions: A mixture of ¹⁴C-labelled and nonlabelled DINP was prepared as a stock solution in diethyl ether - Method of storage: No data APPLICATION OF DOSE: VEHICLE - Justification for use and choice of vehicle (if other than water): No data - Amount(s) applied (volume or weight with unit): 0.2 or 0.1 ml/rat TEST SITE - Preparation of test site: Freshly shaven dorsal skin - Area of exposure: 3X4 cm - % coverage: No data - Type of cover / wrap if used: see below - SITE PROTECTION / USE OF RESTRAINERS FOR PREVENTING INGESTION: yes: Styrofoam cup lined with

aluminium foil and secured in place with adhesive tape ANALYSIS - Method type(s) for identification (e.g. GC-FID, GC-MS, HPLC-DAD, HPLC-MS-MS, HPLC-UV, Liquid scintillation counting, NMR, TLC): Scintillation counting

Details on in vitro test system (if applicable)

Not applicable

Results and discussions

Signs and symptoms of toxicity

no data

Dermal irritation

no data

Percutaneous absorption rate

Time point 7 days post treatment Dose 0.2 ml Absorption (%) > 2 — < 4

Remarks

Conversion factor human vs. animal skin

Not applicable

Remarks on results including tables and figures

A summary of the recovery of radioactivity following dermal application of ¹⁴C-DINP is appended in the 'Overall remarks, and attachments' window below.

Absorption of ¹⁴C-DINP was slow as indicated by the recovery of only ~ 0.3% of applied doses in urine, feces, GI tract, and tissue at 24 hr following treatment. The rates of absorption increased slightly between 3 and 7 days following treatment, but the total absorption did not exceed 4% of the administered doses in rats receiving the high dose. Similar amounts (~3%) were absorbed during a 7-day period following treatment with the lower dose. Most of the applied radioactivity was recovered from the application areas (skin and cover). Blood and tissue contained low levels of radioactivity. Skin from non-application areas demonstrated the highest levels followed by variable amounts in livers, muscle, and fat. Tissue Levels were slightly higher at 3 and 7 days than at 1 day following treatment. No major differences were demonstrated in tissue levels or excretion rates of ¹⁴C-DINP in rats treated under the conditions used in this study.

Applicant's summary and conclusion

Conclusions

Dermal absorption of ^{14}C -DINP was slow but occurred at a steady rate as evidenced by the increased amounts of radioactivity recovered in urine, faeces, and tissues examined at the later time periods. However, the total amounts absorbed during the 7-day period ranged from 2 to 4% of the applied doses. Essentially all of the remaining doses were recovered from the skin application areas.

Executive summary

In a 7-day dermal disposition study, ^{14}C -DINP was applied to freshly shaven areas on the backs of three groups of 3 or 6 male rats (Fischer 344) at 0.1 or 0.2 ml/rat (~ 1.2 ml/kg). Rats were sacrificed at 1, 3, or 7 days after treatment, and levels of radioactivity determined in excreta, GI tract, blood and selected tissues. Dermal absorption of ^{14}C -DINP was slow but occurred at a steady rate as evidenced by the increased amounts of radioactivity recovered in urine, faeces, and tissues examined at the later time periods. However, the total amounts absorbed during the 7-day period ranged from 2 to 4% of the applied doses. Dermal absorption was therefore considered to be very low in rats, and most of the unabsorbed dose remained at the dermal application site. Assuming a treatment area of 12 cm² (3 x 4 cm) and a total fraction absorbed of 3% in 7 days, the overall rate of dermal absorption = $0.03 / (7 \text{ days})(24 \text{ hr/day})(12 \text{ cm}^2) = 1.5 \times 10^{-5} \text{ /hr/cm}^2$.

The dermal disposition study is considered acceptable and satisfies the guideline requirements for *in vivo* skin absorption studies.

Dossier > Document

 Dissemination Dossier

 Acute oral Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	acceptable, well documented study report which meets basic scientific principles

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1981							1981-12-21

Materials and methods

Test type

standard acute method

Limit test

yes

Principles of method if other than guideline

In principle, the methods described in OECD Guideline 401 were used. Young adult laboratory rats were purchased from a breeder. 5 rats per sex were treated simultaneously by gavage without a vehicle. Documentation of clinical signs was performed over a 14 day period. Body weight was determined before the start of the study only, as it was needed for determination of dose. The clinical signs and findings were reported.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

purity >99%

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Administration / exposure

Route of administration

oral: gavage

Vehicle

unchanged (no vehicle)

Doses

10000 mg/kg

No. of animals per sex per dose

5

Control animals

no

Results and discussions

Effect levels

Sex	Endpoint	Effect level	95% CL	Remarks
male/female	LD50	> 10000 mg/kg bw		

Mortality

no animal died within the observation period

Remarks on results including tables and figures

Mortality after	5 males	5 females
1 h	0	0
1 d	0	0
2 d	0	0
7 d	0	0
14 d	0	0

Symptoms in all animals at 10000 mg/kg:

DYSPNEA, APATHY, SPASTIC MOTION, SCRUBBY FUR, ALOPECIA, BAD GENERAL CONDITION

Applicant's summary and conclusion

Executive summary

No death and no abnormalities observed upon terminal necropsy at a dose level of 10,000 mg/kg Palatinol CE 5250 (CAS 28553-12-0, DINP2) administered undiluted by gavage to ten Sprague-Dawley rats (5 males and 5 females). The observation period was 14 days. Dyspnea, apathy, spastic gait, piloerection, alopecia and poor general state were observed.

Dossier > *Document*

 Dissemination Dossier

 Acute oral Endpoint.002

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	No data
Reliability	2 (reliable with restrictions)		
Rationale for reliability	A 1968 non-GLP study report without guidelines specified. However, the report contains the essential elements for an acute oral rat study and there is sufficient data reported to come to a conclusion. The level of information reported for systemic toxicity parameters was minimal.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1968							1968-05-20

Materials and methods

Test type

standard acute method

Limit test

no

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

Although no guideline appears to have been followed, the study was undertaken as a classical LD50 determination

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

An organic ester; clear, slightly viscous liquid with an unpleasant odour received on 25 March 1968

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS - Source: No data - Age at study initiation: No data - Weight at study initiation: 252-295 g - Fasting period before study: 4 hours - Housing: No data - Diet (e.g. ad libitum): ad libitum - Water (e.g. ad libitum): ad libitum - Acclimation period: No data
ENVIRONMENTAL CONDITIONS - Temperature (°C): No data - Humidity (%): No data - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): No data
IN-LIFE DATES: No data

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Details on oral exposure

VEHICLE - Concentration in vehicle: 10% vol/vol or undiluted - Amount of vehicle (if gavage): volume-dosage must have been variable but no data - Justification for choice of vehicle: No data - Lot/batch no. (if required): No data - Purity: No data

Doses

34.6, 120, 417, 1450, 5,000 and 10,000 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: Immediately after dosing; 1, 4 and 24 hours; and once daily thereafter. Bodyweights initially and terminally - Necropsy of survivors performed: Yes

Statistics

Not necessary

Results and discussions

Preliminary study (if fixed dose study)

No data

Effect levels

Sex	Endpoint	Effect level	95% CL	Remarks
male	LD50	> 10000 mg/kg bw		

Mortality

None

Clinical signs

Depression, laboured respiration, and oily-appearing fur at 5,000 and 10,000 mg/kg. Onset of signs within four hours and recovery within three to seven days No signs at lower dosages

Body weight

Weight loss at 10,000 mg/kg

Gross pathology

None at any dose level

Other findings

None reported

Remarks on results including tables and figures

No tables or appendices of data were presented in the study report.

Applicant's summary and conclusion

Interpretation of results

relatively harmless

Criteria used for interpretation of results

expert judgment

Conclusions

The acute oral LD 50 for male rats was > 10,000 mg/kg,

Executive summary

In an acute oral toxicity study, male rats (Sprague-Dawley) were each given a single oral dose of MRD 68-28 in corn oil at nominal dosages of 34.6, 120, 417, 1450, 5,000 and 10,000 mg/kg.

There were no deaths in the subsequent 14-day observation period. Depression, laboured respiration, and oily-appearing fur were observed at 5,000 and 10,000 mg/kg, with onset within four hours and recovery within three to seven days. Weight loss was also recorded at the highest dosage. Based on these results, the LD₅₀ of MRD 68-28 was greater than 10,000 mg/kg.

The toxicity study is considered acceptable to assess classification, and essentially satisfies the guideline requirements for rat acute oral toxicity studies. The level of information reported for systemic toxicity parameters was minimal.

Dossier > Document

Dissemination Dossier

Acute inhal Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	acceptable, well documented study report which meets basic scientific principles

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1981							1981-12-21

Materials and methods

Test type

other: inhalation hazard test

Limit test

no

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

The test was performed in principle as described in OECD test guideline 403. It assess the

toxicity of an atmosphere saturated with vapors, although more likely an aerosol, of the volatile components of a test substance at 20 °C. Young adult laboratory rats, 3 per sex, were exposed sequentially to the vapors generated by bubbling 200 L/h air through a substance column of about 5 cm above a fritted glass disc in a glass cylinder for 7 h. The exposure was subsequently repeated in the same manner. No analytical determination of the atmosphere concentrations was performed. The nominal concentration was calculated as quotient of the amount of test substance weight loss during exposure, and the amount of air used during exposure. Group-wise documentation of clinical signs was performed over a 14 day study period. Body weight of groups was determined before the start of the study and at the end of the observation period.

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

purity > 99%

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Administration / exposure

Route of administration

inhalation: vapour

Type of inhalation exposure

nose/head only

Vehicle

unchanged (no vehicle)

Details on inhalation exposure

- Temperature, humidity in air chamber: 22+/-2°C, 55+/-5%

Analytical verification of test atmosphere concentrations

no

Duration of exposure

7 h Remarks

Concentrations

No verification of the concentration was performed. Nominal concentration: 0.07 mg/l

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: group wise documentation on working days - Necropsy of survivors performed: yes - Other examinations performed: clinical signs, body weight

Results and discussions

Effect levels

Sex	Endpoint	Effect level	95% CL	Exp. duration	Remarks
male/female	LC50	> 0.07 mg/L air			aerosol

Mortality

no animal died within the observation period

Clinical signs

no effects

Body weight

no effects

Remarks on results including tables and figures

No mortality and no clinical signs after 7 hours exposure in an atmosphere saturated or enriched with the test substance at 20°C (vapor; nominal test substance concentration: 0.07 mg/l).

Applicant's summary and conclusion

Executive summary

No death, no clinical signs, no abnormalities were observed upon terminal necropsy at a mean vapour concentration of 0.07 mg/l DINP (Palatinol CE 5250 CAS 28553-12-0) administered to six Sprague-Dawley rats (3 males and 3 females) during 7 hours. The observation period was 14 days. Two experiments were conducted. No justification was given on the choice of the tested concentrations (only 0.07 mg/l), and the nominal concentration was likely not reached. Moreover no information on analytical concentration was provided. In the test conditions, the test substance was probably administered as an aerosol rather than as a vapour.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentc69a.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-0577b56c-a3a1-4a11-a354-df6216484d32%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Acute inhal Endpoint.002

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability	EU RAR, secondary literature, however, the rapporteur member state (France) has evaluated the study report for the EU RAR

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		1980							1980-12-18

Materials and methods

Test type

standard acute method

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: 43CFR163 (EPA proposed test guideline, 1978)	

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Diisononylphthalate, no information on CAS No available. Note that the different DINP's in the market are regarded as technically exchangeable and have been evaluated together for different reasons as outlined in the EU RAR rationale

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

5 animals per sex

Administration / exposure

Route of administration

inhalation: aerosol

Type of inhalation exposure

no data

Analytical verification of test atmosphere concentrations

yes

Duration of exposure

4 h Remarks

Concentrations

4.40 mg/l (nominal)

No. of animals per sex per dose

5

Control animals

yes

Results and discussions

Effect levels

Sex	Endpoint	Effect level	95% CL	Exp. duration	Remarks
male/female	LC50	> 4.4 mg/L air (nominal)		4 h	aerosol

Remarks on results including tables and figures

LC50 > 4.4 mg/l (nominal concentration) (Hazleton, 1980) DINP (no information on the CAS number) was administered as an aerosol to ten Sprague- Dawley rats (5 males and 5 females) at a nominal concentration of 4.40 mg/l which was according to the testing facility the highest attainable concentration in air during 4 hours (The report has conflicting information as once in the report a nominal concentration of 5.0 -5.5 mg/l is listed, however on two other pages the nominal concentration is indicated to be 4.40 mg/l). Gravimetric evaluation of the aerosol resulted in concentrations of approx. 1.7 mg/l. Particle sizing was also undertaken showing that majority of the non-volatile mass collected by the mini-impactor was in the respirable range (i.e. less than 5 micron). A control group of ten Sprague-Dawley rats (5 males and 5 females) received air. The observation period was 14 days. All treated animals were observed to have slight tearing of the eyes and slight clear nasal discharge starting 40 minutes after exposure was initiated. One male was observed to have blood around both eyes on day 4 and a slight brownish discharge around both eyes on days 5 to 10; from day 8 to 10, this animal had developed an opaque spot on one eye. All other animals exhibited normal behaviour and appearance throughout the exposure period. No animal died during this study, no definite compound-related changes of body weight, no consistent compound-related gross lesions, no compound-related microscopic alterations of lungs, liver and kidneys in the treated animals were observed.

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 Dissemination Dossier

 Acute dermal Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	No data
Reliability	2 (reliable with restrictions)		
Rationale for reliability	A 1968 non-GLP study report without guidelines specified. However, the report contains the essential elements for an acute dermal toxicity study and there were sufficient data reported to come to a conclusion. The level of information reported for systemic toxicity parameters was minimal.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1968							1968-05-20

Materials and methods

Test type

standard acute method

Limit test

no

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

Although no guideline appears to have been followed, the study was undertaken as a classical LD50 determination

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

An organic ester; clear, slightly viscous liquid with an unpleasant odour received on 25 March 1968

Test animals

Species

rabbit

Strain

New Zealand White

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS - Source: No data - Age at study initiation: Adult - Weight at study initiation: 2.0-2.8 kg - Fasting period before study: No evidence of fasting - Housing: No data - Diet (e.g. ad libitum): Purina rabbit chow. ad libitum - Water (e.g. ad libitum): Ad libitum - Acclimation period: No data ENVIRONMENTAL CONDITIONS - Temperature (°C): No data - Humidity (%): No data - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): No data IN-

LIFE DATES: No data

Administration / exposure

Type of coverage

occlusive

Vehicle

corn oil

Details on dermal exposure

TEST SITE - Area of exposure: Abdominal skin; no area reported - % coverage: No data - Type of wrap if used: Rubber dam binder wrapped with gauze and sticky tape REMOVAL OF TEST SUBSTANCE - Washing (if done): Water - Time after start of exposure: 24 hours after exposure TEST MATERIAL - Amount(s) applied (volume or weight with unit): No data-must have been variable volume dosage - Concentration (if solution): 10% vol/vol or undiluted - Constant volume or concentration used: constant concentration or undiluted

Duration of exposure

24 hours

Doses

50, 200, 754 and 3,160 mg/kg body weight

No. of animals per sex per dose

4

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days - Frequency of observations and weighing: Immediately, 1, 4 and 24 hours after dosing, and once daily thereafter. Bodyweights initially and terminally - Necropsy of survivors performed: Yes

Statistics

Not necessary

Results and discussions

Preliminary study (if fixed dose study)

No data

Effect levels

Sex	Endpoint	Effect level	95% CL	Remarks
male/female	LD50	> 3160 mg/kg bw		

Mortality

No deaths

Clinical signs

No evidence of systemic toxicity at any dosage.

Body weight

No data

Gross pathology

No remarkable findings

Other findings

Skin reactions generally consisted of moderate erythema at 24 hours, which diminished and completely subsided at all levels by Day 6, and slight desquamation in two to four animals, which disappeared by Day 12.

Remarks on results including tables and figures

No tables or appendices on mortality, clinical signs, bodyweight or necropsy observations were reported. Dermal reactions at the application site are attached in the 'Overall remarks, and attachments' window below.

Applicant's summary and conclusion

Interpretation of results

practically nontoxic

Criteria used for interpretation of results

expert judgment

Conclusions

The acute dermal LD₅₀ for rabbits of either sex was > 3160 mg/kg. Skin reactions generally consisted of moderate erythema at 24 hours which diminished and completely subsided at all levels by Day 6, and slight desquamation in two to four animals which disappeared by Day 12.

Executive summary

In an acute dermal toxicity study, rabbits of either sex (New Zealand White) were given a single occluded dermal dose of MRD 68-28 in corn oil to the clipped abraded abdominal skin at nominal dosages of 50, 200, 754 or 3160 mg/kg. There were no deaths or no evidence of systemic toxicity observed during the subsequent 14-day observation period. Skin reactions generally consisted of moderate erythema at 24 hours which diminished and completely subsided at all levels by Day 6, and slight desquamation in two to four animals which disappeared by Day 12. Based on these results, the LD₅₀ of MRD 68-28 was greater than 3160 mg/kg.

The toxicity study is considered acceptable for classification, and essentially satisfies the guideline requirements for rabbit acute dermal toxicity studies. The level of information reported for systemic toxicity parameters was minimal.

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 Dissemination Dossier

 Skin irritation Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	acceptable, well documented study report which meets basic scientific principles

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1981							1981-12-21

Materials and methods

Type of method

in vivo

Principles of method if other than guideline

Method: Draize Test

GLP compliance

no

Test material equivalent to submission substance identity

no

Test materials

Details on test material

Palatinol N

Test animals

Species

rabbit

Strain

Vienna White

Details on test animals and environmental conditions

TEST ANIMALS - Source: GAUKLER 6050 OFFENBACH / MAIN - Housing: 1 animal/cage - Diet (e.g. ad libitum): OVATOR SOLIKANIN 4 MM ; MUSKATOR-WERKE 4000 DUESSELDORF-1 (daily about 130 G/animal) - Water (e.g. ad libitum): daily about 250 ML/animal: - Acclimation period: about 8 days before start of study ENVIRONMENTAL CONDITIONS - Temperature (°C): 20 - 26 °C - Humidity (%): 50 - 80 % - Photoperiod (hrs dark / hrs light): 12 H/12 H (6.00 - 18.00 H/18.00 - 6.00 H)

Test system

Type of coverage

occlusive

Preparation of test site

other: clipping of fur

Vehicle

unchanged (no vehicle)

Amount/concentration applied

0.5 ml

Duration of treatment / exposure

24 h

Observation period

8 days

Number of animals

1 male, 5 females

Control animals

other: untreated skin sites of the same animal

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
erythema score	mean	24 - 72 hours	0.9		fully reversible within: 72 hours	
edema score	mean	24 - 72 hours	0.25		fully reversible within: 72 hours	

Remarks on results including tables and figures

Readings	Animal	Erythema	Edema	
24 h	1	1	0	
	2	1	0	
	3	1	0	
	4	2	1	
	5	2	2	
	6	1	0	
72 h	1	1	0	
	2	1	0	
	3	0	0	
	4	0	0	
	5	1	0	
	6	0	0	

8 d	1	0	0	
	2	0	0	
	3	0	0	
	4	0	0	
	5	0	0	
	6	0	0	
Mean	1-6	0.9	0.25	

Applicant's summary and conclusion

Executive summary

Palatinol CE 5250 (CAS 28553-12-0, DINP2) was applied undiluted (0.5 ml) for 24 hours under an occlusive dressing to intact or abraded skin of 6 White Vienna rabbits (5 females and 1 male) and followed by an observation period of 8 days with readings at 15-30 minutes after removal of the test patches and 48 hours, 72 hours and 8 days after the beginning of application. Slight erythema was observed at 48 and 72 hours on intact skin (mean score 1.3 and 0.5, respectively) and on abraded skin (mean score 1.7 and 0.8, respectively). Slight oedema was only observed at 48 hours with a mean score of 0.5 and 1.0 on intact or abraded skin, respectively. All the effects were reversible at day 8.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumenta036.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-dbb8c3bd-2192-4c7d-9644-dccaa57de4f1%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 **Dissemination Dossier**

 **Skin irritation Endpoint.002**

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	15.12.1995 to 26 .12 1995
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered to be equivalent to international test guidelines for a primary skin irritation study.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1996							1996-01-26

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	no

Principles of method if other than guideline

There is no explicit statement in the report that the study was undertaken in accordance with a

specific guideline, although there is a reference to 'OECD 1992 test guideline 404'.

GLP compliance

yes

Test material equivalent to submission substance identity

yes

Test materials

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate - Substance type: Technical material - Physical state: Colourless liquid - pH : 6 (determined by the laboratory) - Stability under test conditions: No data - Storage condition of test material: Room temperature

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS - Source: HRP Inc., Denver, PA, USA - Age at study initiation: Approximately 13-14 weeks - Weight at study initiation: 2.29 to 2.49 kg - Housing: Single housing in suspended stainless steel and wire mesh cages with absorbent paper below. - Diet (e.g. ad libitum): Fixed amount daily - Water (e.g. ad libitum): Ad libitum - Acclimation period: 10 days
ENVIRONMENTAL CONDITIONS - Temperature (°C): 65-70°F - Humidity (%): 40-60% RH - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): Approximately 12 hours dark/12 hours light
IN-LIFE DATES: From: 21 December 1995 To: 26 December 1995

Test system

Type of coverage

semioclusive

Preparation of test site

other: clipped

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL - Amount(s) applied (volume or weight with unit): 0.5 ml per animal -
Concentration (if solution): Undiluted test material

Duration of treatment / exposure

Four hours

Observation period

72 hours

Number of animals

6

Control animals

no

Details on study design

TEST SITE - Area of exposure: 1 inch X 1 inch - Type of wrap if used: gauze patch held in place by non-irritating tape and a semi-occluded dressing, and protected by a neck collar during the exposure period. REMOVAL OF TEST SUBSTANCE - Washing (if done): wiped using peanut oil and paper towel - Time after start of exposure: 4 hours SCORING SYSTEM: Draize 1959

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
erythema score	mean	24 through 72 hours	0.06		fully reversible within: 24 hours	
edema score	mean	24 through 72	0		fully reversible	

hours

Irritant/corrosive response data

Very slight erythema (barely perceptible) (Grade 1) observed in one rabbit at the 6 hour examination and one different rabbit at the 24 hour examination. The scores for erythema and oedema were 'Grade 0' (No response) at all other occasions of examination.

Other effects

There were no other effects of treatment.

Remarks on results including tables and figures

Dermal reactions to treatment were confined to very slight erythema (barely perceptible) in single animals at the 1 or 24 hour examinations. Individual dermal scores are appended in the 'attached background information' window below.

1) The mean erythema and oedema scores for each time interval are:

	1 hr	24 hr	48 hr	72 hr
Erythema	0.17	0.17	0.00	0.00
Oedema	0.00	0.00	0.00	0.00

Mean erythema and oedema scores for 24, 48 and 72 hours are: Erythema: 0.06 Oedema: 0.00

2)The primary irritation index, as calculated by the modified Draize method, is: 0.08

Applicant's summary and conclusion

Interpretation of results

other: very mild skin irritant

Criteria used for interpretation of results

other: Draize method

Conclusions

The test material is an extremely mild skin irritant to rabbits

Executive summary

In a 72 hour primary skin irritation study, 6 rabbits (New Zealand White) were exposed to a 4 hour semi-occluded application of 0.5 ml of undiluted di-isononyl phthalate to 1x1 inch.of

clipped dorsal skin, and the sites examined 1, 24, 48 and 72 hours after treatment. Irritation responses were confined to very slight erythema in single rabbits at the 1 or 24 hour examinations. The primary skin irritation index was 0.08, and the test material was considered to be an extremely mild skin irritant.

The study was considered suitable for classification, and satisfied the guideline requirements for rabbit primary skin irritation studies.

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 Dissemination Dossier

 Eye irritation Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	No data
Reliability	2 (reliable with restrictions)		
Rationale for reliability	A 1968 non-GLP study report without guidelines specified. However, the report contains the essential elements of an acute eye irritation study and there were sufficient data reported to come to a reliable conclusion.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1968							1968-05-20

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

Although no guideline appears to have been followed, the study was undertaken as a standard acute eye irritation study

GLP compliance

no

Test material equivalent to submission substance identity

yes

Test materials

Details on test material

An organic ester; clear, slightly viscous liquid with an unpleasant odour received on 25 March 1968

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS - Source: No data - Age at study initiation: Adult - Weight at study initiation: 1.9-2.5 Kg - Housing: No data - Diet (e.g. ad libitum): Purina rabbit chow ad libitum - Water (e.g. ad libitum): ad libitum - Acclimation period: No data ENVIRONMENTAL CONDITIONS - Temperature (°C): No data - Humidity (%): No data - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): No data IN-LIFE DATES: No data

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): as supplied (undiluted)

Duration of treatment / exposure

Single administration (eyes not washed)

Observation period

72 hours

Number of animals

6

Control animals

not required

Details on study design

REMOVAL OF TEST SUBSTANCE - Washing (if done): No SCORING SYSTEM: Draize (Draize J H 1959) TOOL USED TO ASSESS SCORE: hand-slit lamp / biomicroscope / fluorescein Terminal fluorescein examination. No other data

Results and discussions

Overall irritation / corrosion results

Irritation parameter	Basis	Time point	Max. score	Reversibility	Remarks
conjunctivae score	mean	1 hour	2.7 3		

Irritant/corrosive response data

Irritation was confined to the conjunctivae and consisted of marked redness (Grade 3 in five animals and Grade 2 in the sixth animal) and slight discharge (Grade 1 in four animals) at 1 and 4 hours, and slight redness only, (Grade 1 and in one case Grade 2), at the 24 hour examination. By 24 or 48 hours, the irritation completely subsided in all eyes.

Other effects

No other effects.

Remarks on results including tables and figures

No other remarks on results. Individual ocular scores for the six rabbits are attached in the 'Overall remarks and attachments' window below.

Applicant's summary and conclusion

Interpretation of results

slightly irritating

Criteria used for interpretation of results

expert judgment

Conclusions

MRD 68-28 caused slight transient conjunctival irritation.

Executive summary

In an acute eye irritation study, six rabbits (New Zealand White) were given a single 0.1 ml undiluted ocular instillation of MRD 68 -28 into the left conjunctival sac. Irritation responses were limited to slight conjunctival redness during the first 24 hours. All responses had resolved within 48 hours. Based on these results, MRD 68-28 was considered to cause slight transient conjunctival irritation. Using EC classification criteria, the test material would have been designated 'non-irritant to eyes'.

The irritation study is considered acceptable for classification, and essentially satisfies the guideline requirements for rabbit acute eye irritation studies.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4cdc-1b7a-e044-00144fd73934/showdocument0974.html?treeUuid=DISS-828e03e1-4cdc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4cdc-1b7a-e044-00144fd73934&uuid=AGGR-e47a98dd-30ac-4ae3-a02c-b9b1cc2bcbf3%2FDISS-828e03e1-4cdc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Eye irritation Endpoint.002

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	acceptable, well documented study report which meets basic scientific principles

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1981							1981-12-21

Materials and methods

Type of method

in vivo

Principles of method if other than guideline

Method: Draize Test

GLP compliance

no

Test material equivalent to submission substance identity

no

Test materials

Details on test material

Palatinol N

Test animals

Species

rabbit

Strain

Vienna White

Details on test animals and environmental conditions

TEST ANIMALS - Source: GAUKLER 6050 OFFENBACH / MAIN - Housing: 1 animal/cage - Diet (e.g. ad libitum): OVATOR SOLIKANIN 4 MM ; MUSKATOR-WERKE 4000 DUESSELDORF-1 (daily about 130 G/animal) - Water (e.g. ad libitum): daily about 250 ML/animal: - Acclimation period: about 8 days before start of study ENVIRONMENTAL CONDITIONS - Temperature (°C): 20 - 26 °C - Humidity (%): 50 - 80 % - Photoperiod (hrs dark / hrs light): 12 H/12 H (6.00 - 18.00 H/18.00 - 6.00 H)

Test system

Vehicle

unchanged (no vehicle)

Amount/concentration applied

0.1 ml, single application into the conjunctival sac of the right eye, the substance is not washed off

Observation period

72 h

Number of animals

2 males, 4 females

Control animals

other: untreated eye of the same animal

Results and discussions

Overall irritation / corrosion results

Irritation parameter	Basis	Time point	Max. score	Reversibility	Remarks
cornea score	mean	24-72H	0 4	other: n.a.	
iris score	mean	24-72H	0 2	other: n.a.	
conjunctivae score	mean	24-72H	0.28 3	fully reversible within: 2 days	

Remarks on results including tables and figures

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		op	ar		red	ch	di	
24 h	1	0	0	0	1	0	0	
	2	0	0	0	1	0	0	
	3	0	0	0	1	0	0	
	4	0	0	0	1	0	0	
	5	0	0	0	0	0	0	
	6	0	0	0	1	0	0	
48 h	1	0	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
	4	0	0	0	0	0	0	
	5	0	0	0	0	0	0	
	6	0	0	0	0	0	0	
72 h	1	0	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
	4	0	0	0	0	0	0	
	5	0	0	0	0	0	0	
	6	0	0	0	0	0	0	
Mean 24-72 h	1-6	0	0	0	0.28	0	0	

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument7ac1.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-ac43fc63-2b92-43c5-a70a-5fcfe80cff37%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Sensitization Endpoint.001

[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussion](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale reliability	for Acceptable, well-documented study report equivalent or similar to Directive 84/449EEC, B6: GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1994							1994-11-15

Materials and methods

Type of method

in vivo

Type of study

Buehler test

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method B.6 (Skin Sensitisation)	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Jayflex DINP - Substance type: liquid - Physical state: clear colorless liquid

Test animals

Species

guinea pig

Strain

Dunkin-Hartley

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS - Source: D. Hall, Newchurch, Staffordshire, England - Age at study initiation: 6-7 weeks - Weight at study initiation: 281-341 g - Housing: groups of 5 - Diet (e.g. ad libitum): ad libitum - Water (e.g. ad libitum): ad libitum - Acclimation period: 11 days ENVIRONMENTAL CONDITIONS - Temperature (°C): 21 - Humidity (%): 30-70 - Air changes (per hr): 15 - Photoperiod (hrs dark / hrs light): 12/12

Test system

Traditional sensitisation test

Route of induction exposure

epicutaneous, occlusive

Route of challenge exposure

epicutaneous, occlusive

Vehicle

unchanged (no vehicle)

Concentration

0.5 ml

No. of animals per dose

5

Details on study design (Traditional tests)

MAIN STUDY A. INDUCTION EXPOSURE - No. of exposures: 3 - Exposure period: Days 1, 8, and 15 - Test groups: 1 - Control group: 1 - Site: left shoulder region - Exposure: 6 hours - Frequency of applications: single - Concentrations: 0.5 ml B. CHALLENGE EXPOSURE - No. of exposures: 1 - Day(s) of challenge: 2 weeks after the final induction - Test groups: 1 - Control group: 1 - Site: right flank - Concentrations: 0.5 ml - Exposure: 6 hours - Evaluation (hr after challenge): 24, 48 and 72 hours after exposure

Challenge controls

Induction control - no test compound Challenge control - test compound

Positive control substance(s)

yes Test material

Results and discussion

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours challenge	after	Group	Dose level	No. with reactions	+ Total group	no. in Clinical observations
1st reading	24		test group	0.5 ml	0	5	NA
2nd reading	48		test group	0.5ml	0	5	NA

Applicant's summary and conclusion

Interpretation of results

not sensitising

Executive summary

A Buehler test was carried out in forty female Guinea pigs (20 control and 20 treated animals) with Jayflex DINP (CAS 68515-48-0). The substance was applied neat (0.5 ml) for induction at day 1-8-15 during 6 hours under occlusive bandaging and for challenge at day 29. No evidence of skin sensitisation was observed in any of the treated animals. This test was conducted in conformity with GLP procedures and with EEC Method B6.

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[Administrative Data](#)

[Data source](#)

[Materials and methods](#)

[Results and discussion](#) [Applicant's summary and conclusion](#)

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	13 July 1992-8 October 1992
Reliability	2 (reliable with restrictions)		
Rationale for reliability	The study does not appear to have been undertaken to full GLP standards and there is no GLP compliance statement. The study was undertaken according to a modification of the Buehler method (Buehler, 1965; Ritz and Buehler, 1980). The preliminary irritation test, which may have been helpful in the interpretation of the equivocal test result, is not reported.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1992							1992-10-08

Materials and methods

Type of method

in vivo

Type of study

Buehler test

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 406 (Skin Sensitisation)	no

Principles of method if other than guideline

A modification of the Buehler method (Buehler, 1965; Ritz and Buehler, 1980), which included the use of only female guinea pigs and employed an irritation control group.

GLP compliance

no There is no GLP compliance statement in the report.

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate - Substance type: Technical material - Physical state: Colourless liquid - Expiration date of the lot/batch: No data - Stability under test conditions: No data - Storage condition of test material: Room temperature - Other: Received on 22 May, 1992

Test animals

Species

guinea pig

Strain

Hartley

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Hazleton Research Products, Inc., Denver, PA, USA - Age at study initiation: Approximately 6 weeks - Weight at study initiation: 331-431 grams - Housing: Singly housed, in suspended stainless steel and wire mesh cages, with absorbent paper below. - Diet (e.g. ad libitum): ad libitum - Water (e.g. ad libitum): ad libitum - Acclimation period: 16 days ENVIRONMENTAL CONDITIONS

- Temperature (°C): 18 - 22°C - Humidity (%): 40-70% - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): 12 hrs dark / 12 hrs light IN-LIFE DATES: From: 29 July 1992 To: 4 September 1992

Test system

Traditional sensitisation test

Route of induction exposure

epicutaneous, occlusive

Route of challenge exposure

epicutaneous, occlusive

Vehicle

peanut oil

Concentration

Induction: neat test material Challenge and re-challenge: 5.0% (w/w) in peanut oil

No. of animals per dose

20 females for the test group 20 females for the irritation control group 10 females for the positive control material

Details on study design (Traditional tests)

RANGE FINDING TESTS: -Not reported MAIN STUDY A. INDUCTION EXPOSURE - No. of exposures: 20 test animals; 0 irritation controls - Exposure period: Test groups: 2 weeks Control group: No exposure - Site: Scapular area - Frequency of applications: Days 0, 7 and 14 - Duration: 6 hours - Concentration: 100% (neat test material) B. CHALLENGE EXPOSURE - No. of exposures: 20 test animals; 10 naive irritation controls - Day(s) of challenge: Day 28 - Exposure period: Test groups: 6 hours Control group: 6 hours - Site: Right abdominal flank - Concentration: 5.0% - Evaluation (hr after challenge): 24 and 48 hours after removal of challenge patches C. RE-CHALLENGE EXPOSURE - No. of exposures: 20 test animals; 10 naive irritation controls - Day(s) of challenge: Day 35 - Exposure period: Test groups: 6 hours Control group: 6 hours - Site: Left abdominal flank of test and irritation control animals - Concentration: 5.0% - Evaluation (hr after challenge): 24 and 48 hours after removal of re-challenge patches

Challenge controls

Ten female animals used as irritation controls for challenge and ten used for re-challenge. The animals were untreated during the induction phase.

Positive control substance(s)

yes 1-chloro-2, 4-dinitrobenzene (DNCB)

Results and discussion

Positive control results

Topical application of DNCB to the positive control animals during the induction phase elicited slight to well-defined erythema and slight oedema. Following the challenge phase, signs of sensitisation were observed in nine of the ten positive control animals.

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with reactions	+ Total no. in group	Clinical observations
1st reading	24	test group	5.0%	0	20	None
2nd reading	48	test group	5.0%	0	20	None
rechallenge	24	test group	5.0%	10	20	Grade 1 erythema
rechallenge	48	test group	5.0%	7	20	Grade 1 or 2 erythema and a single observation of grade 2 oedema
1st reading	24	other: irritation control	5.0%	0	10	None
2nd reading	48	other: irritation control	5.0%	0	10	None
rechallenge	24	other: irritation control	5.0%	0	10	None
rechallenge	48	other: irritation control	5.0%	4	10	Grade 1 erythema
1st reading	24	positive control	0.1%	9	10	Grade 1 or 2 erythema and Grade 1 oedema
2nd reading	48	positive control	0.1%	9	10	Grade 1 or 2 erythema and Grade 2 oedema

LLNA

Remarks on results including tables and figures

INDUCTION

Topical application of Di-isononyl phthalate (DINP) during the induction of the treated group animals produced erythema ranging from very slight to well-defined in all twenty animals. Oedema also was noted in all twenty animals during induction and ranged from very slight to slight.

CHALLENGE (See attached table below for incidence of dermal scores)

Following challenge with 5.0% DINP no signs of dermal irritation were noted in the treated or irritation control animals.

RE-CHALLENGE (See attached table below for incidence of dermal scores)

Re-challenge dosing with 5.0% DINP produced dermal irritation in ten of the twenty treated group animals. Ten treated animals were observed with very slight erythema 24 hours following the re-challenge dose. While the incidence of erythema decreased slightly at 48 hours after re-challenge, the severity was slightly increased. On Day 37 four animals displayed very slight erythema and three animals displayed well-defined erythema in the treated group. Very slight oedema was noted in one treated group animal 48 hours after rechallenge.

Four irritation control animals were noted with very slight erythema 48 hours after rechallenge.

POSITIVECONTROL:

Topical application of DNCB to the positive control group animals elicited slight to well-defined erythema and slight edema during the induction phase. Following the challenge phase, signs of sensitisation were observed in nine of the ten positive control animals.

Applicant's summary and conclusion

Conclusions

Based on the results of this study, DINP is either not a potential sensitiser or may have a weak potential for sensitisation.

Executive summary

In a dermal sensitisation study (modified Buehler method), 20 female guinea pigs (Hartley) received three occluded induction applications of di-isononyl phthalate (DINP) as supplied (100%) to the clipped scapular region on Days 0, 7 and 14.

On Day 28, each animal received an occluded challenge application of 5% (w/w) DINP in peanut oil to the clipped right abdominal flank. On Day 35, the animals were re-challenged with the same concentration of test material to the clipped left abdominal flank. Two groups of ten naive female animals received 5% DINP in peanut oil to the right or left abdominal flank on Days 28 or 35 and acted as irritation controls. Dermal responses were evaluated approximately 24 and 48 hours after removal of the bandages. Ten further animals were treated with 1-chloro-2, 4-dinitrobenzene (DNCB) and acted as a positive control.

No signs of dermal irritation were observed in either the treated or irritation control animals

following challenge with 5% DINP. Re-challenge with 5% DINP elicited dermal irritation in ten of the twenty treated animals and four of the ten irritation control animals. Challenge application of DNCB elicited responses in nine of the ten positive control animals. Based on these observations, it was concluded that DINP is either not a potential sensitiser or may have a weak potential for sensitisation.

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 Dissemination Dossier

 Sensitization respiratory Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussion Applicant's summary and conclusion

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability	Acceptable, well-documented study.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory no.	Report Owner company	Report date
publication	Butala JH, David RM, Gans G, McKee RH, Guo TL, Peachee VL, White KL Jr.	2004	Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F1 mice	Toxicology. 2004 Sep 1;201(1-3):77-85.			

Materials and methods

Type of method

in vivo

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

Principles of method if other than guideline

Topical application (and challenge) of test substances to mice followed by measurements of total serum IgE. In addition, auricular lymph nodes were harvested for measurement of IL-4 and IL-13 proteins and their corresponding messenger RNAs. Because skin absorption of high molecular weight phthalates is limited, liver weight increase, a measure of peroxisomal proliferation, was monitored to assure that internal dosing had been achieved.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test animals

Species

mouse

Strain

B6C3F1

Sex

no data

Test system

Route of induction exposure

dermal

Route of challenge exposure

other: application to ear

Vehicle

unchanged (no vehicle)

Concentration

undiluted, 50 ul/flank

No. of animals per dose

10

Positive control substance(s)

trimellitic anhydride (TMA) 25%

Negative control substance(s)

2,4-dinitrochlorobenzene (DNCB) 1%

Results and discussion

Results

The mice tolerated the treatment well; all animals survived, and there were no significant differences in body weight. Liver weights of DINP- treated mice were significantly elevated with respect to control at the $P < 0.05$ level. Total serum IgE levels were significantly increased by treatment with the positive control, TMA. There were also smaller but still significant increases in IgE levels in mice treated with DNCB. The appropriate responses with the reference compounds demonstrated the specificity of the assay system. In contrast to these materials, total IgE levels in serum from mice treated with the DINP were not significantly different from control values. The assessment of IL-4 and IL-13 levels in cultured lymph node cells revealed a large and statistically significant increase in cells from mice treated with TMA. IL-4 and IL-13 levels were also elevated in the DNCB-treated groups in some situations, but were not significantly different from control values. Neither IL-4 nor IL-13 was elevated in cells from control group mice or mice treated with DINP. Similarly, TMA significantly increased IL-4 and IL-13 mRNA levels in lymph node cells. DNCB treatment increased IL-4 and IL-13 mRNA in some cases, but these differences, while sometimes statistically significant, were well below levels associated with TMA treatment. DINP treatment had no effect on IL-4 or IL-13 mRNA

levels.

Applicant's summary and conclusion

Interpretation of results

not sensitising

Executive summary

Bronchial asthma is mediated, in part, by the immunoregulatory cytokines interleukins 4 and 13 (IL-4 and IL-13). These cytokines stimulate IgE synthesis that in turn is associated with airway hyper-responsiveness. Compounds that stimulate IgE synthesis and elicit bronchial reactivity are generally considered to be respiratory sensitizers. To address this question, topical application (and challenge) of test substances (DINP included) to mice followed by measurements of total serum IgE was performed. In addition, auricular lymph nodes were harvested for measurement of IL-4 and IL-13 proteins and their corresponding messenger RNAs. Because skin absorption of high molecular weight phthalates is limited, liver weight increase, a measure of peroxisomal proliferation, was monitored to assure that internal dosing had been achieved. ELISA and RNase protection assays demonstrated that DINP treatment did not significantly affect IgE, IL-4, or IL-13 levels. Similarly, IL-4 and IL-13 mRNA levels were not elevated. In contrast, all of these were significantly elevated by trimellitic anhydride (TMA), a respiratory sensitizer used as the positive control in this assay. Another control, dinitrochlorobenzene (DNCB), a contact sensitizer, also responded as expected, producing smaller but statistically significant increases in IgE and in mRNA for IL-4 and IL-13 but not in the levels of these cytokines. In summary, treatment with DINP did not result in significant elevations indicating that DINP has little, if any, potential to produce anti-body-mediated respiratory allergy.

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Dissemination Dossier

Repeated oral Endpoint.001

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	Equivalent to guideline study with GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1998							1998-01-29
other:	Aristech Chemical Corporation	1995	TSCA 8(e) Submission 8EHQ-0794-13083. Corroborative information in second species. Dated April 12, 1995.						

Materials and methods

Test type

chronic

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 452 (Chronic Toxicity Studies)	

GLP compliance

yes

Test materials**Test material equivalent to submission substance identity**

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear, colorless liquid - Analytical purity: 100% (first shipment), >99% (second shipment) - Lot/batch No.: QCL 9004-273 (both shipments)

Test animals**Species**

mouse

Strain

B6C3F1

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Portage, Michigan - Age at study initiation: 6 weeks - Weight at study initiation: males 12.0-22.8 g and females 12.5-20.3 g - Housing: single - Diet (e.g. ad libitum): Purina Certified Rodent Chow 5002 - Water (e.g. ad libitum): tap water - Acclimation period: 2 weeks ENVIRONMENTAL CONDITIONS - Temperature (°C): 18-26 - Humidity (%): 55±15 - Air changes (per hr): 10 or more - Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration

oral: feed

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS: For each dietary level, the test material was weighed on an appropriate balance and was placed into a pocket that was made in approximately 200 g of feed contained in a glass beaker. The test material/feed was mixed in a Waring blender for approximately 2 to 3 minutes to ensure an apparent homogeneous mixture. Based on dietary level, multiple premixes were required (approximately 30 g test material /premix). The premixes were added to approximately 5 kg of additional feed, mixed in a Hobart mixer for 15 minutes, then transferred to a Patterson-Kelley twin-shell mixer (fitted with an intensifier bar) that contained the remaining amount of basal feed. The diets were mixed for 1 minute/kg feed. DIET PREPARATION - Rate of preparation of diet (frequency): once a week - Storage temperature of food: refrigerated (2-8°C)

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Results of routine concentration analyses indicated that the dietary concentrations were within an acceptable range (92 to 112%) of target concentrations.

Duration of treatment / exposure

104 weeks for the test groups and 78 weeks for the recovery group (high dose level)

Frequency of treatment

7 days per week

Doses/concentrations

ca. 90.3, 275.6, 741.8, 1560.2 mg/kg bw/d in males and ca. 112.0, 335.6, 910.3, 1,887.6 mg/kg bw/d in females

Basis actual ingested

500, 1500, 4000, 8000 ppm

Basis nominal in diet

No. of animals per sex per dose

70 for the test groups and 55 for recovery group (high dose level)

Control animals

yes, concurrent no treatment

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: twice daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: twice daily. In addition, detailed clinical observations by physical examination were performed once each week. Special attention was paid to mass development. The following information on each grossly visible or palpable mass was recorded: time of onset, location, size (small < 1 cm, large > 1 cm), appearance, progression. BODY WEIGHT: Yes - Time schedule for examinations: prior to treatment, and weekly for Weeks 1-17 and once every 4 weeks thereafter. An additional weight was obtained from all groups on the first day of Week 79. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): - Food consumption for each animal determined: Yes, weekly for Weeks 1-16 and once every 4 weeks thereafter. Food consumption was also determined at Week 78. When obvious spillage or wastage of food was recorded for an animal during the detailed physical examination, the estimate of the food consumed by the animal was excluded from the group mean calculation for that particular interval. HAEMATOLOGY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: absolute reticulocyte count, mean cell hemoglobin, corrected leukocyte count, mean cell hemoglobin concentration, erythrocyte count, mean cell volume, hematocrit, platelet count, hemoglobin, reticulocyte count, leukocyte differential and cellular morphology, myeloid/erythroid ratios (at necropsy), leukocyte count. CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, creatinine, urea nitrogen, total bilirubin, sodium, chloride, calcium, potassium, inorganic phosphorus, glucose, total protein, albumin, globulin. URINALYSIS: Yes - Time schedule for collection of urine: During Weeks 26, 52, 78, and 104 - Metabolism cages used for collection of urine: Yes, urine collection racks - Animals fasted: Yes - Parameters checked: total volume, calcium, chloride, creatinine, creatinine clearance, osmolality, phosphorus, potassium, sodium, appearance/color, specific gravity (calculated),

protein, glucose, ketones, bilirubin, occult blood, microscopic sediment, pH, turbidity, urobilinogen

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes All animals found dead or sacrificed in extremis (using sodium pentobarbital and exsanguination) during the study were subjected to a gross postmortem examination. Fifteen mice/sex/group in the control, and all test groups were sacrificed after 78 weeks of compound administration. Following at least 104 weeks of compound administration, the surviving mice were necropsied. At the interim and terminal sacrifices, the following organs (when present) were weighed: brain with stem, spleen, kidneys (paired weight), liver/gallbladder (drained), testes with epididymides (paired weight), lung, uterus. Five mice/sex/group in all test groups following 78 weeks and five mice/sex/group in all test groups and recovery group following 104 weeks were designated for hepatocellular proliferation studies. Before sacrifice, these animals were labelled with BrdUrd (20 mg/ml) for 72 hrs using ALZET osmotic pump, Model No. 1003D. Tissues from liver (standard tissue samples from left and right median, and right anterior lobes) and duodenum from each animal designated for hepatocellular proliferation studies and were preserved in 10% neutral-buffered formalin. The remainder of the liver was immediately frozen at approximately -70°C for biochemical analysis.

Other examinations

Hepatocellular Proliferation and Biochemical Analyses: The frozen tissue samples from the three liver lobes (right and left lateral, and median) were evaluated for hepatocellular proliferation and analysis of protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, and DNA concentration from animals designated for hepatocellular proliferation studies during Weeks 79 and 105/106 (i.e. after 78 and 104 weeks of treatment, respectively).

Statistics

Statistical analyses of labeling index, palmitoyl-CoA oxidase activity, DNA and protein were performed using analysis of variance techniques for each sacrifice interval. Comparisons of the control group vs. the treated groups were performed using Dunnett's t-Test. Weekly/monthly body weight, body weight change, and food consumption, mean total body weight change (Weeks 1-78, 78-104, and 1-104) and total food consumption (Weeks 1-78, 78-104, and 1-104), clinical pathology data (except hemolysis and cellular morphology gradings and routine urinalysis data), fasted terminal body weights, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups.

Results and discussions

Effect levels

Endpoint Effect level

Sex Basis for effect level / Remarks

NOAEL	ca. 275.6 mg/kg bw/day (actual dose received)	male	liver weight, body weight
NOAEL	ca. 112 mg/kg bw/day (actual dose received)	female	increased liver weight and increased body weight gain

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: For Weeks 1-104, the adjusted mean survival rate was significantly decreased for the males of the high dose group compared to concurrent control value. Clinical observations noted during the course of the study that exhibited a treatment-related increased incidence were signs of poor health and/or antemortem condition (hunched posture - highest incidence in the 8000 ppm dose group animals, hypoactivity - highest incidence in the 8000 ppm dose group males and the 4000 ppm dose group females, few feces - highest incidence in the 8000 ppm dose group females, and urine stains - predominantly in the 8000 ppm dose group males) and swelling in the ventral -abdominal region, in which the notably increased incidence in the males of the 4000, 8000 ppm and recovery group, and in the females of the 8000 ppm and recovery group. **BODY WEIGHT AND WEIGHT GAIN:** Significantly decreased body weight means occurred most frequently in the males and females of the 4000, 8000 ppm and recovery group. In the male mice, significantly lower (than control values) body weight means were noted for the 500 ppm group at Weeks 14, 15, 45, 57, 69, and 81; the 1500 ppm group at Weeks 69 and 93; the 4000 ppm group at Weeks 29-105; the 8000 ppm group at Weeks 29-105; and the recovery group at Weeks 5-7, 10-11, and 15-105. In the female mice, significant decreases in mean body weight were noted for the 500 ppm group at Weeks 8, 21, and 25; the 1500 ppm group at Weeks 8, 25, 37, 49, 61, and 69-101; the 4000 ppm group at Weeks 29, 37, and 45-105; the 8000 ppm group at 16 and 37-105; and the recovery group at Weeks 29 and 37-105. The overall mean total body weight change values for Weeks 1-104 were statistically decreased for the animals of 4000 and 8000 ppm dose groups, and the recovery group males compared to that of the concurrent control group. **FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):** Total mean food consumption values (Weeks 1-78, 78-104, and 1-104) were generally comparable between groups, with the exception of the significantly increased values noted at Weeks 78-104 for the males of the 4000 and 8000 ppm dose groups. For Weeks 1-104, the average daily compound consumption (mg/kg bw/d) for the 500, 1500, 4000, 8000 ppm dietary levels was 90.3, 275.6, 741.8, 1560.2 mg/kg bw/d in the males and 112.0, 335.6, 910.3, 1887.6 mg/kg bw/d in the females, respectively. For Weeks 1-78 in the recovery group, the average daily compound consumption was 1377.4 mg/kg bw/d in the males and 1581.0 mg/kg bw/d in the females. **HAEMATOLOGY:** The lower leukocyte, lymphocyte, and/or segmented neutrophil counts were observed in animals of the 8000 ppm and recovery group (often significantly). **CLINICAL CHEMISTRY:** The higher (frequently significantly) total protein, albumin, and globulin was observed in the males of the 8000 ppm and recovery group. Higher (frequently significantly) aspartate aminotransferase and alanine aminotransferase was observed in the males of the 8000 ppm dose group and in the males and females of the recovery group. **URINALYSIS:** Significant higher urine volume and lower urine osmolality (with significant lower concentrations of sodium, potassium, and chloride) were observed in the animals of 8000 ppm and recovery groups. **ORGAN WEIGHTS:** At the interim sacrifice, mean

absolute kidney weights were significantly decreased in the males of the 1500, 4000 and 8000 ppm dose groups. Absolute and relative liver weight means were increased in the males and females of the 4000 ppm dose group (liver weight relative to body weight was significantly increased in the males of the 4000 ppm dose group) and significantly increased in the males and females of the 8000 ppm dose group. At terminal sacrifice, treatment-related effects were again evident in the kidney (males) and liver (males and females) weights. For kidney in the males of the 1500, 4000, 8000 ppm and recovery group, absolute and relative to brain weight means were significantly decreased; relative to body weight means were also decreased (significantly only in 4000 ppm group). For liver in males of the 4000, 8000 ppm and recovery group, mean absolute and relative weights were significantly increased. For liver in females of 4000, 8000 ppm and recovery group, mean absolute and relative weights were increased, but the differences were not significant compared to the control female values. GROSS PATHOLOGY: At the Week 79 sacrifice, most notable was an increased incidence of the following necropsy findings: liver-mass in the males of the 1500, 4000, 8000 ppm dose groups, and in the females of the 8000 ppm dose group; enlarged liver in the 8000 ppm dose group females; and distended urinary bladder in the 8000 ppm dose group males. At necropsy for study termination, the most frequently observed gross findings were lung-mass (primarily in male mice, all groups); liver-mass, which occurred in greatest numbers in mice of the 4000, 8000 ppm and recovery group; enlarged spleen (predominantly in the females, all groups); and granular pitted/rough kidneys in 8000 ppm dose group females. Distended urinary bladder was noted most frequently in the males of the 4000 and 8000 ppm dose groups. HISTOPATHOLOGY: NON-NEOPLASTIC: At the interim and terminal sacrifice, microscopic evaluation revealed test article-related changes in the liver of mice of both sexes and in the kidneys of female mice. The liver changes in the 8000 ppm group were consisted of increased cytoplasmic eosinophilia, diffuse hepatocellular enlargement, and pigment. In the kidneys of the females of the 8000 ppm group, the incidence of chronic progressive nephropathy was increased. No correlation of increased kidney weights in males was observed. No correlation of decreased relative-to-brain weights for the testis/epididymis was observed in histological changes. HISTOPATHOLOGY: NEOPLASTIC: An increased incidence of hepatocellular neoplasia was present in males and females of the 8000 ppm group in addition to non-neoplastic changes. In mice of the recovery group following the recovery period, the nonneoplastic changes were not present, although the incidence of hepatocellular neoplasia was increased in female mice (37%, 13/35) compared to that of the control (7%, 3/42) and lower dose groups. The total incidence of hepatocellular neoplasia in males of 4000 ppm group (47%, 28/60) was also greater than that observed in the control males (23%, 16/70). Statistical analyses indicated there were no significant increases in the total incidence (all deaths combined) of hepatocellular adenoma in any of the treated male groups versus the control males; and only the incidences of 18/70 and 8/50 in the females of the 4000 and 8000 ppm groups, respectively, were significantly increased compared to the control females. For hepatocellular carcinoma, only the incidences of 17/60 and 20/70 in the males of the 4000 and 8000 ppm groups, respectively, and of 7/60, 18/70, and 13/50 in the females of the 4000, 8000 ppm and recovery groups, respectively, were significantly increased compared to the respective same sex of the controls. In the females of the 1500 ppm group, although the incidences of hepatocellular adenoma or carcinoma were not statistically different compared to the incidences in controls, the incidence (10/60) for hepatocellular adenoma/carcinoma combined was significantly greater than the combined incidence (3/70) in the controls. Corresponding to the statistically significant increases of liver neoplasms in the mid-high and high dose groups, significant positive trends were

indicated for hepatocellular carcinomas and hepatocellular adenoma/carcinoma (combined) in males, and for hepatocellular adenomas and carcinomas, as well as hepatocellular adenoma/carcinoma (combined) in females OTHER FINDINGS: Cell proliferation: After 78 and 104 weeks of DINP administration, there was no evidence of sustained cell proliferation in the livers of male or female mice from the 8000 ppm group compared to livers from the control group. DINP administration did not induce significant increases in the mean labeling index for livers of the 8000 ppm mice compared to controls. Biochemical analyses: Increases in palmitoyl CoA oxidase, a monitor of the level of peroxisome proliferation were observed after 78 and 104 weeks in the livers of mice from 8000 ppm group. Analyses of liver DNA concentration did not detect any biologically relevant differences in mean values between the control and the 8000 ppm group after 78 or 104 weeks of DINP administration. Conversely, liver mean protein concentration was significantly increased in the mice of the 8000 ppm group compared to controls at both analysis intervals.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentbf32.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-37484bc0-4a27-46b5-ae85-8372a557057b%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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Dissemination Dossier

Repeated oral Endpoint.002

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study with GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2000							2000-06-30

Materials and methods

Test type

subchronic

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according	OECD Guideline 408 (Repeated Dose 90-Day Oral	yes additional satellite

to Toxicity in Rodents) groups

Principles of method if other than guideline

According to guideline OECD408 with additional satellite groups for evaluation of hepatocellular proliferation and peroxisome proliferation. 15 animals/sex/dose group were evaluated 3, 20 and 90 days after study initiation.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear colorless liquid - Analytical purity: 99.9% - Lot/batch No.: QCL 9004-273

Test animals

Species

mouse

Strain

B6C3F1

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Raleigh, North Carolina - Age at study initiation: approximately 6 weeks - Weight at study initiation: males 18-24 g and females 15-20 g - Housing: 2 per cage - Diet (e.g. ad libitum): Purina Certified Rodent Chow 5002 - Water (e.g. ad libitum): tap water ENVIRONMENTAL CONDITIONS - Temperature (°C): ca. 22 - Humidity (%): 50±20 - Air changes (per hr): 10 or more - Photoperiod (hrs dark / hrs light):

12/12

Administration / exposure

Route of administration

oral: feed

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS: Each dose level was prepared by placing feed into an appropriately sized glass beaker. A depression was made in the center of the feed and the weighed test material was added to the depression. This test material-feed combination was mixed with approximately 200 g of feed for 2-3 minutes in a Waring blender to form a homogeneous premix. Additional premixes were needed as the amount of test material increased at the higher dose levels. The premix(es) were then added to 5 kg of feed in a Hobart blender and mixed for 10 minutes. The premix from the Hobart blender was added to the required amount of feed for the dose level and mixed in a Patterson-Kelly Twin Shell mixer (2 cu. ft.) fitted with an intensifier bar for 1 min/kg. At no time during the mixing procedure was plastic allowed to come in contact with the test material. DIET PREPARATION - Rate of preparation of diet (frequency): every 4 weeks - Storage temperature of food: 2-6°C

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Routine concentration analyses of each formulation demonstrated values within 10% of target concentration.

Duration of treatment / exposure

13 weeks

Frequency of treatment

7 days per week

Doses/concentrations

341, 904, 2365, 5471 mg/kg bw/day for males and 389, 1041, 2834, 6070 mg/kg bw/day for females

Basis

actual ingested

1500, 4000, 10000, 20000 ppm

Basis nominal in diet

No. of animals per sex per dose

10, except in 10000 and 20000 ppm group where males/females were 11/9 and 9/11, respectively.

Control animals

yes, concurrent no treatment

Details on study design

- Rationale for selecting satellite groups: To evaluate the hepatocellular proliferation and peroxisomal proliferation, additional corresponding groups of 15 mice/sex/group at each dose level plus a positive control (WY 14463) of 15 mice/sex/group were evaluated 3, 30 and 90 days after study initiation.

Positive control

WY 14463 [4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid was administered in 15 males and 15 females at a dosage level of 1000 ppm to evaluate hepatocellular proliferation and peroxisomal proliferation.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: twice daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: twice daily BODY WEIGHT: Yes - Time schedule for examinations: prior to treatment and weekly thereafter FOOD CONSUMPTION: - Food consumption for each animal determined: Yes OPTHALMOSCOPIC EXAMINATION: Yes - Time schedule for examinations: prior to treatment and during week 13 - Dose groups that were examined: all HAEMATOLOGY: Yes - Time schedule for collection of blood: at the end of the treatment - Anaesthetic used for blood collection: Yes (CO2/O2 anesthesia) - Animals fasted: Yes - How many animals: all - Parameters checked: Absolute Reticulocyte Count, Cell Morphology, Corrected Leukocyte Count, Erythrocyte Count, Hematocrit, Hemoglobin, Leukocyte Count, Leukocyte differential, Myeloid/Erythroid Ratio (Bone marrow obtained from the femur), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MLV), Platelet, Reticulocyte Count CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: at the end of the treatment - Animals fasted: Yes - How many animals: all - Parameters checked: Alanine Aminotransferase, Albumin, Globulin, Albumin/Globulin Ratio, Aspartate Aminotransferase, Blood Urea Nitrogen, Calcium, Chloride,

Creatinine, Gamma Glutamyltransferase, Glucose, Inorganic Phosphorus, Potassium, Sodium, Total Bilirubin, Total Protein URINALYSIS: Yes - Time schedule for collection of urine: during 13 week - Metabolism cages used for collection of urine: Yes, collection racks - Animals fasted: Yes - Parameters checked: Appearance, Bilirubin, Glucose, Ketones, Microscopic Examination of Sediment, Occult Blood, pH, Protein, Specific Gravity, Urobilinogen, Urine Calcium, Urine Chloride, Urine Creatinine, Creatinine Clearance, Urine Osmolality, Urine Phosphorus, Urine Potassium, Urine Sodium, Urine Volume

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes All animals were weighed on the day of scheduled necropsy and were sacrificed by exsanguination under sodium pentobarbital anesthesia. The following organs were weighed: brain with stem, spleen, kidneys (paired weight), testes with epididymides (paired weight), liver with gallbladder (drained), uterus, lung. Tissues from a number of organs were preserved in 10% neutral-buffered formalin. In the satellite study, 72 h before sacrifice, animals were labeled with BrdUrd (20 mg/ml; 1 μ l/hr) for 72 hours using ALZET osmotic pumps, Model No 1003D (ALZA Corporation, Palo Alto, CA). Tissues from liver (standard tissue samples from left and right median, and right anterior lobes) and duodenum from each animal were preserved in 10% neutral-buffered formalin and examined microscopically. The remainder of the liver was immediately frozen at approximately -70°C for biochemical analysis.

Other examinations

Hepatocellular Proliferation: Tissue samples from the formalin fixed liver and duodenum of Satellite animals (from animals scheduled for sacrifice at days 4, 31 and 91) in control, 10000 ppm and positive-control groups were processed for evaluation of hepatocellular proliferation. Animals of the 10000 ppm group were evaluated due to decreased survival in the animals of the 20000 ppm group following implantation of osmotic pumps. Biochemical Analysis: Samples from the frozen liver sections of Satellite animals (from animals scheduled for sacrifice at Days 4, 31 and 91) in control, 1500, 4000, 10000 ppm and positive-control groups were analyzed for protein concentration, cyanide-insensitive palmitoyl-CoA oxidation and DNA concentration. Animals of the 10000 ppm group were evaluated due to decreased survival in the animals of the 20000 ppm group following implantation of osmotic pumps.

Statistics

Mean body weight (Weeks 6 and 14), mean body weight change (Weeks 1-13), mean food consumption (Weeks 6 and 13), mean total food consumption (Weeks 1-13), hematology (except cell morphology gradings), serum chemistry, urine chemistry, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups using Levene's test, Dunnet's test and ANOVA. If variances of untransformed data were heterogeneous, a series of transformations was performed in an effort to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were

routinely performed at the 5% two tailed probability level.

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
NOAEL	< 341 mg/kg bw/day (actual dose received)	male	kidney weight
NOAEL	389 mg/kg bw/day (actual dose received)	female	kidney weight
LOAEL	341 mg/kg bw/day (actual dose received)	male	kidney weight
LOAEL	1041 mg/kg bw/day (actual dose received)	female	liver weight

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: No treatment related clinical signs were observed. One female of 10000 ppm group did not survive to termination. **BODY WEIGHT AND WEIGHT GAIN:** Body weights and body weight change were decreased in the males and females of 20000 ppm group with statistical significance at Weeks 6 and 14 in absolute body weight and from initiation to Week 13 in body weight change. **FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):** Total food consumption was significantly increased in the males of the 10000 and 20000 ppm groups, and females in the 10000 ppm group. Weekly food consumption was increased in the males and females of 20000 ppm group at Week 6 and in the females of the 10000 ppm group at Week 13. Mean overall compound consumption (mg/kg bw/d) for weeks 1-13 for 1500, 4000, 10000, 20000 ppm dietary levels in the males was 341, 904, 2365, 5471 mg/kg bw/day, respectively, and in the females was 389, 1041, 2834, 6070 mg/kg bw/day, respectively. **OPHTHALMOSCOPIC EXAMINATION:** No treatment related findings were noted. **HAEMATOLOGY:** No treatment related changes were noted. **CLINICAL CHEMISTRY:** significant increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the males of 20000 ppm group was observed indicative of hepatocellular injury. **URINALYSIS:** Significant decreases in urinary sodium, chloride, and creatinine in the males and females of the 20000 ppm group was observed. Increase in urine volume in the males of the 10000 and 20000 ppm groups, and lower specific gravity in the males of the 20000 ppm group was observed which may reflect a compromise in the ability of the renal tubular epithelial cells to concentrate urine. **ORGAN WEIGHTS:** Significant treatment related absolute and relative organ weight changes were noted in the uterus (decrease), spleen, (decrease), liver/gallbladder (increase), kidney (decrease for the males but increase for the females), and testes/epididymides (decrease) generally in the animals of 20000 ppm group, but extended to the 1500 ppm group for the kidney (decrease relative to brain weight in the males), 4000 ppm group for the liver (increased absolute and relative weight) and 10000 ppm group (increased absolute and relative weight of liver/gallbladder, decreased absolute weight of testes/epididymides, decreased absolute and relative weight of kidney only in males). **GROSS PATHOLOGY:** Necropsy findings included enlarged liver in the males of the 4000 ppm group, and males and females of the 10000 and 20000 ppm groups; granular/pitted/rough kidneys in the

males and females of the 20000 ppm group; and small uterus in the females of the 20000 ppm group. HISTOPATHOLOGY: NON-NEOPLASTIC: Treatment-related histomorphological alterations were noted in the mice of the 10000 and 20000 ppm groups. Hepatocellular enlargement occurred as a moderate to moderately severe diffuse change in the mice of the 20000 ppm group and was accompanied by pigment accumulation in Kupffer cells and bile canaliculi, individual cell degeneration/necrosis, and a low incidence of focal necrosis. Centrilobular to midzonal hepatocellular enlargement was present in the mice of the 10000 ppm group. These histomorphologic liver changes correlated with the increased absolute and relative liver weights in the 10000 and 20000 ppm groups; however, no histomorphologic correlate was noted for the increased liver weights in the males of the 4000 ppm group. Other histomorphologic changes which occurred in mice of the 20000 ppm group were tubular nephrosis in the kidneys, immature/abnormal sperm forms in the epididymides, lymphoid depletion in the spleen and thymus, hypoplasia in the uterus, and absence of Corpora lutea in the ovaries. The changes in the spleen and uterine weights correlated with the microscopic findings noted in these tissues. Treatment-related microscopic alterations were not observed in tissue sections from the mice of the 1500 and 4000 ppm groups.

Remarks on results including tables and figures

Hepatocellular Proliferation and Biochemical analysis: At the first interim sacrifice, the background Labeling Index (an indicator of cell proliferation) in control group was higher and more variable than at later sacrifice times. This was observed in both male and female animals. The Labeling Index (LI) was not increased by the DINP at any of the dose levels examined. In fact, in the first interim sacrifice, the LI was decreased in both male and female mice. The lack of an increase in the LI was observed even though large increases in palmitoyl-CoA oxidase activity were observed. In contrast, large increases in both the LI and palmitoyl-CoA oxidase activity were observed in the positive control group. The LI response in the positive control group peaked at the second sacrifice and decreased by about half at the terminal sacrifice. There was a large increase in peroxisome proliferation as indicated by increases in palmitoyl-CoA oxidase activity without a cell proliferative response as indicated by the Labeling Index in the 10000 ppm animals after 4, 31, and 90 days. Levels of DNA varied randomly among the groups and at the different timepoints. Statistical analysis confirmed the lack of a consistent pattern. Protein analysis also did not indicate a clear pattern with either the 10000 ppm or the positive control group.

Applicant's summary and conclusion

Executive summary

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentc71d.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-98b97d6c-a7c7-489b-8428-4ac77da8019b%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > *Document*

 Dissemination Dossier

 Repeated oral Endpoint.003

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study with GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1998							1998-10-08
publication	Hall, M. et al.	1999	EFFECTS OF DI-ISONONYL PHTHALATE (DINP) ON PEROXISOMAL MARKERS IN THE MARMOSSET - DINP IS NOT A PEROXISOME PROLIFERATOR	J. Toxicological Sci., 24, 237-244					

Materials and methods

Test type
subchronic

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 409 (Repeated Dose 90-Day Oral Toxicity in Non-Rodents)	no
according to	other guideline: Requirements of Ministry of Health and Welfare for Japan, Food and Drug Administration for the USA, Organisation for Economic Co-operation and Development: Testing of Chemicals, and European Economic Communities .	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate (DINP) - Physical state: clear and colourless liquid - Analytical purity: 99.2 % - Lot/batch No.: 0022 and 0012 - Storage condition of test material: cool store (not exceeding 15°C) - Stability under test conditions: at least 48 hours at 21°C and at least 15 days at 4°C

Test animals

Species
monkey

Strain

Marmoset

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Harlan UK - Age at study initiation: 16-25 months - Weight at study initiation: 314-462 g - Housing: single - Diet (e.g. ad libitum): 10 g of malt loaf, 25 g of pelleted primate diet, Mazuri (Special Diets Services Ltd., England) together with 65 g of fresh fruit each morning of each day. - Water (e.g. ad libitum): public supply (Essex and Suffolk Water Company, England), was freely available, except when urine was being collected. - Acclimation period: 3 weeks ENVIRONMENTAL CONDITIONS - Temperature (°C): 24 - Humidity (%): 65 - Air changes (per hr): at least 10 - Photoperiod (hrs dark / hrs light): 10/14

Administration / exposure**Route of administration**

oral: gavage

Vehicle

other: methylcellulose plus tween

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS: The DINP solutions were prepared for administration as a series of graded concentrations in 1% methylcellulose plus 0.5 % tween dosing solutions. The formulations were prepared by adding the appropriate amount of DINP to the vehicle and hand stirring. The mixture was then homogenised further using a high shear mixer for at least one minute. The mixtures were then magnetically stirred during the subsequent aliquoting. All formulations were prepared freshly each week and issued to the animal unit daily. All prepared formulations were stored in glass jars at 4°C and protected from light. VEHICLE - Concentration in vehicle (mg/ml): 20, 100, 500 - Amount of vehicle (if gavage): 5 ml/kg bw/d

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The measurements of the intended concentrations of 20, 100 and 500 mg/ml were 20.2, 97.1 and 473.0 mg/ml, respectively.

Duration of treatment / exposure

at least 13 weeks

Frequency of treatment

once each day, seven days per week

Doses/concentrations

100, 500 and 2500 mg/kg bw/d

Basis actual ingested

No. of animals per sex per dose

4

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: selected based on results from a preliminary study performed at Huntingdon Life Sciences (Report No. KHK055/972500). The dosage of 2500 mg/kg bw/day DINP, using a concentration of 500 mg/ml given at a volume dosage of 5 ml/kg bw was considered to be close to the maximum practical dosage. In the preliminary study, dosages of up to 2500 mg/kg bw/day DINP were not associated with any overt signs of toxicity. - Justification for the test system: The sub-human primate was chosen because of its acceptance as a predictor of toxic change in man and the requirement for a non-rodent species by regulatory agencies. The common marmoset, *Callithrix jacchus*, was used because of the availability of historical control data in the testing laboratory.

Positive control

Clofibrate administered at dosage of 500 mg/kg bw/d similarly as reference substance.

Examinations**Observations and examinations performed and frequency**

CAGE SIDE OBSERVATIONS: Yes - Time schedule: daily - Cage side observations checked: evidence of animal ill-health, such as vomitus, blood or loose faeces. DETAILED CLINICAL

OBSERVATIONS: Yes - Time schedule: daily during the first week of treatment, twice weekly during Weeks 2 to 4 and weekly thereafter. In addition, detailed weekly physical examination was performed on each animal. BODY WEIGHT: Yes - Time schedule for examinations: weekly FOOD CONSUMPTION: - Food consumption for each animal determined and mean daily diet consumption: Yes OPTHALMOSCOPIC EXAMINATION: Yes - Time schedule for examinations: before commencement of treatment and during Week 12 of treatment - Dose groups that were examined: all HAEMATOLOGY: Yes - Time schedule for collection of blood: before commencement of treatment and during Weeks 6 and 13 of treatment (before dosing) - Anaesthetic used for blood collection: No - Animals fasted: Yes, only in samples collected before commencement of treatment and during Week 13 of treatment - How many animals: all - Parameters checked: Packed cell volume (PCV), Haemoglobin concentration (HB), Erythrocyte count (RBC), Total leucocyte count (WBC), Platelet count (PLAT), Mean cell haemoglobin concentration (MCHC), Mean cell haemoglobin (MCH), Mean cell volume (MCV) CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: before commencement of treatment and during Weeks 6 and 13 of treatment (before dosing) - Animals fasted: Yes, only in samples collected before commencement of treatment and during Week 13 of treatment - How many animals: all - Parameters checked: Alkaline phosphatase activity (ALP), Alanine amino-transferase activity (ALT), Aspartate amino-transferase activity (AST), Lactate dehydrogenase isoenzymes (LDH), Gamma-glutamyl transpeptidase activity (GGT), Glucose concentration (GLUC), Total bilirubin concentration (BILT), Total cholesterol concentration (CHOL), Free cholesterol concentration (FCHOL), Total triglyceride concentration (TRIG), Creatinine concentration (CREA), Urea concentration (UREA), Total protein concentration (TP), Electrophoretic protein fractions (albumin, alpha-1 globulin, alpha-2 globulin, beta globulin, gamma globulin), Sodium (Na), potassium (K), Chloride (Cl), calcium (Ca), inorganic phosphorus (Phos). URINALYSIS: Yes - Time schedule for collection of urine: before commencement of treatment and during Weeks 5 and 12 of treatment (Six animals failed to produce a urine sample in Week 12) - Metabolism cages used for collection of urine: No data - Animals fasted: Yes - Parameters checked: appearance, volume, pH, specific gravity, protein, glucose, ketones, bilirubin, blood, sodium, potassium, chloride, sediments. OTHER: Before commencement and during Week 12 of treatment additional blood samples (1.2 ml) were collected into glass tubes containing lithium heparin as anticoagulant for analysis of following parameters: Oestradiol and Testosterone.

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes All animals killed and any found dead were subjected to a detailed necropsy. The following organs were weighed: Adrenals, Brain, Epididymides, Heart, Kidneys, Liver, Lungs, Ovaries, Pituitary, Prostate with urethra sample, Salivary glands, Seminal vesicles, Spleen, Testes, Thymus, Thyroid with parathyroids, Uterus with cervix.

Other examinations

Peroxisome proliferation: immediately after removal and weighing of the liver, samples (minimum 5 g) were taken from all animals killed on completion of the 13 Week treatment

period and frozen by immersion in liquid nitrogen and stored at approximately -75°C until analyzed for protein concentration, Cyanide-insensitive palmitoyl CoA oxidase activity, Cytochrome P450 concentration and Lauric acid hydroxylase activity.

Statistics

The significance of inter-group differences in haematology (excluding the incidence of morphological abnormalities evident on blood smears), blood chemistry and urinalysis (volume, specific gravity, pH and electrolytes only) were assessed by Student's t-test using a pooled error variance. For organ weights and bodyweight gain, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used. Inter-group differences in macroscopic pathology and histopathology were assessed using Fisher's Exact test. For peroxisome proliferation parameters, homogeneity of variance was tested using Levene's test.

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
NOAEL	500 mg/kg bw/day (nominal)	male/female	body weight and weight gain
LOAEL	2500 mg/kg bw/day (nominal)	male/female	body weight and weight gain

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: There were two deaths (one untreated female and one male given clofibrate) during Week 13 of treatment. Both of these deaths were incidental and were not related to treatment. From approximately Week 3 of treatment, ungroomed coat, reddening of the skin around the anus and reddening of the back of the legs was observed for animals receiving DINP at 2500 mg/kg bw/day. Salivation was observed throughout the study for animals receiving clofibrate. This sign became apparent immediately after dosing but was absent 1 to 2 hours later. Emesis was also occasionally observed in these animals during the first two weeks of treatment. **BODY WEIGHT AND WEIGHT GAIN:** Body weight losses or low body weight gain were observed for two males and one female receiving 2500 mg/kg bw/day DINP when compared with the Controls. With the exception of one male, low body weight gain or body weight losses were observed for all animals receiving clofibrate. **FOOD CONSUMPTION:** Food consumption was unaffected for animals receiving DINP or clofibrate. **OPHTHALMOSCOPIC EXAMINATION:** no treatment related findings. **HAEMATOLOGY:** Haematology investigations did not reveal any treatment-related findings for animals receiving DINP. Slightly low packed cell volumes, haemoglobin concentrations, erythrocyte counts and slightly high mean cell haemoglobin and mean cell volumes were observed for animals receiving clofibrate. Platelet counts were also slightly high for these females. **CLINICAL CHEMISTRY:**

There were no blood chemistry changes URINALYSIS: Urinary sodium and chloride levels were slightly low for females receiving clofibrate. There were no treatment-related changes in the urine of animals receiving DINP. ORGAN WEIGHTS: There were no clear treatment-related organ weight changes for animals which received DINP. Slightly high absolute and body weight-relative kidney weights were noted for animals which received clofibrate. Absolute and body weight-relative salivary gland weights were also slightly high for females which received clofibrate. GROSS PATHOLOGY: Macroscopic examination at necropsy revealed a thin build for one male and one female which received 2500 mg/kg bw/day DINP. There were no other findings which were considered to be related to treatment with DINP or clofibrate. HISTOPATHOLOGY: NON-NEOPLASTIC: There were no microscopic findings which were considered to be related to treatment with DINP or clofibrate. OTHER FINDINGS: no changes in testosterone or estradiol concentrations were observed, which were considered to be related to treatment with either DINP or clofibrate.

Remarks on results including tables and figures

Peroxisome proliferation: Clofibrate treatment led to statistically significant increases in microsomal protein and cytochrome P450 concentrations in females, and in palmitoyl CoA oxidase and lauric acid hydroxylase activities of both sexes. The data obtained following oral administration of DINP to marmosets for 13 weeks provide no evidence that the compound does not act as a peroxisome proliferator in primates.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentcb34.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-f16722ea-c9df-418b-90c0-a9636e5a7817%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > *Document*

 Dissemination Dossier

 Repeated oral Endpoint.004

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	key study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	Comparable to guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1998							1998-05-13

Materials and methods

Test type

chronic

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 452 (Chronic Toxicity Studies)	

GLP compliance

yes

Test materials**Test material equivalent to submission substance identity**

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear, colorless liquid - Analytical purity: >99% - Lot/batch No.: QCL 9004-273

Test animals**Species**

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Portage, Michigan - Age at study initiation: 6 weeks - Weight at study initiation: males 96-134 g and females 82-109 g - Housing: single - Diet (e.g. ad libitum): Purina Certified Rodent Chow 5002 - Water (e.g. ad libitum): tap water - Acclimation period: 2 weeks ENVIRONMENTAL CONDITIONS - Temperature (°C): 22 - Humidity (%): 50±20 - Air changes (per hr): 10 or more - Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure**Route of administration**

oral: feed

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS: For each dietary level, the test material was weighed on an appropriate balance and was placed into a pocket that was made in approximately 200 g of feed contained in a glass beaker. The test material/feed was mixed in a Waring blender for approximately 2 to 3 minutes to ensure an apparent homogeneous mixture. Based on dietary level, multiple premixes were required (approximately 30 g test material /premix). The premixes were added to approximately 5 kg of additional feed, mixed in a Hobart mixer for 15 minutes, then transferred to a Patterson-Kelley twin-shell mixer (fitted with an intensifier bar) that contained the remaining amount of basal feed. The diets were mixed for 1 minute/kg feed. **DIET PREPARATION** - Rate of preparation of diet (frequency): once a week - Storage temperature of food: refrigerated (2-8°C)

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Results of routine concentration analyses indicated that all formulations were within 15% of target.

Duration of treatment / exposure

104 weeks for the test groups and 78 weeks for the recovery group (high dose level)

Frequency of treatment

7 days per week

Doses/concentrations

ca. 29.2, 88.3, 358.7, and 733.2 mg/kg bw/d for males and ca. 36.4, 108.6, 442.2, and 885.4 mg/kg bw/d for females

Basis actual ingested

500, 1500, 6000, 12000 ppm

Basis nominal in diet

No. of animals per sex per dose

500 and 1500 ppm dose group: 70 males and 70 females 0, 6000 and 12000 ppm dose group: 85 males and 85 females Recovery (12000 ppm) group: 55 males and 55 females

Control animals

yes, concurrent no treatment

Positive control

[4-Chloro-6-(2,3-xylidino)- 2-pyrimidinylthio] acetic acid was used as positive control for Hepatocellular Proliferation and Biochemical Analyses. Fifteen males were administered the positive control substance for 13 weeks at dietary level of 1000 ppm.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: twice daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: twice daily. In addition, detailed clinical observations by physical examination were performed once each week. Special attention was paid to mass development. The following information on each grossly visible or palpable mass was recorded: time of onset, location, size, appearance, progression. BODY WEIGHT: Yes - Time schedule for examinations: prior to treatment, and weekly for Weeks 1-17 and once every 4 weeks thereafter. An additional weight was obtained from all groups on the first day of Week 79. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): - Food consumption for each animal determined: Yes, weekly for Weeks 1-16 and once every 4 weeks thereafter. Food consumption was also determined at Week 78. When obvious spillage or wastage of food was recorded, the estimate of the food consumed by the animal was excluded from the group mean calculation for that particular interval. HAEMATOLOGY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: absolute reticulocyte count, mean cell hemoglobin, corrected leukocyte count, mean cell hemoglobin concentration, erythrocyte count mean cell volume, hematocrit, platelet count, hemoglobin, reticulocyte count, leukocyte differential and cellular morphology, myeloid/erythroid ratios (at necropsy), leukocyte count. CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, creatinine, urea nitrogen, total bilirubin, sodium, chloride, calcium, potassium, inorganic phosphorus, glucose, total protein, albumin, globulin. URINALYSIS: Yes - Time schedule for collection of urine: During Weeks 26, 52, 78, and 104 - Metabolism cages used for collection of urine: Yes, urine collection racks - Animals fasted: Yes - Parameters checked: total volume, calcium, chloride, creatinine, creatinine clearance, osmolality, phosphorus, potassium, sodium, appearance/color, specific gravity (calculated), protein, glucose, ketones, bilirubin, Urinalysis, occult blood, microscopic sediment, pH, turbidity, urobilinogen

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes All animals found dead or sacrificed in extremis (using sodium pentobarbital and exsanguination) during the study were subjected to a gross postmortem examination. Fifteen mice/sex/group in the control, and all test groups were

sacrificed after 78 weeks of compound administration. Following at least 104 weeks of compound administration, the surviving mice were necropsied. At the interim and terminal sacrifices, the following organs (when present) were weighed: brain with stem, spleen, kidneys (paired weight), liver/gallbladder (drained), testes with epididymides (paired weight), lung, uterus. Five mice/sex/group in all test groups were designated for hepatocellular proliferation studies following 78 weeks and five mice/sex/group in all test groups and recovery group were designated for hepatocellular proliferation studies following 104 weeks. Before sacrifice, these animals were labelled with BrdUrd (20 mg/ml) for 72 hrs using ALZET osmotic pump. Tissues from liver (standard tissue samples from left and right median, and right anterior lobes) and duodenum from each animal designated for hepatocellular proliferation studies and were preserved in 10% neutral-buffered formalin. The remainder of the liver was immediately frozen at approximately -70°C for biochemical analysis.

Other examinations

Hepatocellular Proliferation and Biochemical Analyses: The frozen tissue samples from the three liver lobes (right and left lateral, and median), and the duodenum were evaluated for hepatocellular proliferation and analysis of protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, and DNA concentration from five animals/sex from control, 12000 ppm and recovery group sacrificed during Weeks 1, 2, and 13, and from five animals/sex from control, 6000 and 12000 ppm groups sacrificed after 104 weeks of treatment.

Statistics

Trend analysis of survival was evaluated at the 5.0% one-tailed probability level. Weekly body weights and food consumption and the following intervals for mean body weight change (Weeks 1-53 and 1-105) and total food consumption (Weeks 1-52 and 1-104), clinical pathology data (except hemolysis and cellular morphology gradings and routine urinalysis data), fasted terminal body weights, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups.

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
NOAEL	ca. 88.3 mg/kg bw/day (actual dose received)	male	liver and kidney toxicity, mononuclear cell leukemia
NOAEL	ca. 108.6 mg/kg bw/day (actual dose received)	female	liver and kidney toxicity, mononuclear cell leukemia

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: Survival at Week 104 in the males of the 12000 ppm dose group was significantly lower than the control group. Analysis of the females revealed no difference in survival between control and treated groups. No treatment related clinical signs were observed. **BODY WEIGHT AND WEIGHT GAIN:** The body weight in the animals of the 12000 ppm and recovery groups was significantly lower during weeks 5-105 and 2-105, respectively. **FOOD CONSUMPTION AND COMPOUND INTAKE** (if feeding study): In females of the 12000 ppm and recovery groups, statistically significant lower mean food consumption values were noted most frequently. However, even though these females were fed the same dietary concentration (12000 ppm), significantly lower mean weekly food consumption values were noted at 18/31 measurement intervals for the 12000 ppm group, but only 9/31 intervals for the recovery group during Weeks 1-78. Therefore, the statistically significant intergroup differences in mean weekly and total food consumption were spurious findings. For Weeks 1-104, the average daily compound consumption (mg/kg bw/d) for the 500, 1500, 6000, 12000 ppm dietary levels was 29.2, 88.3, 358.7, and 733.2 mg/kg bw/d in the males and 36.4, 108.6, 442.2, and 885.4 mg/kg bw/d in the females, respectively. For Weeks 1-78 in the recovery group, the average daily consumed dose was 637.3 mg/kg/day in males, and 773.6 mg/kg/day in females. **HAEMATOLOGY:** Significant changes in the hematology data included mild decreases in the erythrocyte mass (erythrocyte count, hemoglobin, and hematocrit) in the 12000 ppm and recovery groups at most intervals, however, the mean values for the recovery group rats were, in general, not significantly different from controls at Week 104. Hematopoietic neoplasia (leukemia) was observed in the peripheral blood smears from several animals of the 12000 ppm and recovery groups. **CLINICAL CHEMISTRY:** Increases (often significant) were observed in the mean values for urea nitrogen, alanine aminotransferase, and aspartate aminotransferase primarily in the 12000 ppm and recovery groups throughout the study. The elevations in these analytes corresponded to the histologic findings noted in the kidneys and livers of the males of the 6000 ppm and recovery group, and animals (both sexes) of the 12000 ppm group. **URINALYSIS:** The mean values for urine volume were significantly increased in the males of the 12000 ppm group at Week 104. The mean creatinine clearance rate was significantly increased in the females of the 12000 ppm group at Week 104. The change was of low magnitude and was not accompanied by a significant change in the mean value for urine volume, urine creatinine, or serum creatinine. Decreases were observed in the mean values for urine calcium, urine potassium, urine chloride, and urine osmolality in some treated groups during the study. The significant changes in the aforementioned urinary chemistry tests are of questionable toxicologic importance as they occurred inconsistently over time and did not correlate with concurrent serum chemistry changes. **ORGAN WEIGHTS:** Liver organ weights (absolute and relative to body) for all interim sacrifices were significantly increased with dose in animals of the 6000 and 12000 ppm groups, compared to the control group. Spleen and kidney weights (absolute and relative to body) were also statistically increased in the 6000 and 12000 ppm groups when compared to the control group of animals sacrificed at Week 79. Absolute and relative weights for testis/epididymis or uterus in the 6000 and 12000 ppm groups were not statistically different compared to controls. In the animals sacrificed after at least 104 weeks of treatment, liver weights (absolute and relative to body or brain) were noted as statistically increased in males and females of the 6000 and 12000 ppm groups. Liver weights in animals of the recovery group were increased when compared to control but not significantly. Absolute and relative spleen and kidney weights in females of the 12000 ppm group were significantly increased. Statistically significant increases of spleen and kidney weights were observed in

females of the 6000 ppm group. There were no statistically significant differences in absolute and relative weights for testis/epididymis or uterus in any of the treated groups compared to same sex in controls. GROSS PATHOLOGY: In males and females of the 6000, 12000 ppm and recovery group, that died or were sacrificed in extremis during the study (unscheduled deaths), the incidence of the following necropsy findings was notably increased: enlarged spleen and granular/pitted/rough appearance of the liver, as well as dark area of the stomach, thoracic cavity fluid, small testis and small seminal vesicle, and uterine mass. At the Week 79 sacrifice, a liver mass was observed in each of two males of the 12000 ppm group; and various gross-pathologies of the liver (enlarged, irregular shape, granular/pitted/rough appearance and raised area) were observed in the females of the 12000 ppm group, when compared to the control group. Small testis and small seminal vesicle were not observed in any males killed from the control, 6000 and 12000 ppm group, and the incidence of uterus mass was 0/15, 2/15, and 1/15 in females of the control, 6000 and 12000 ppm group, respectively. At necropsy for study termination, the following abnormalities were observed to have a dose-related increase in the males and females of the 6000 and 12000 ppm groups: enlarged spleen, enlarged and/or granular/pitted/rough appearance of the liver (seen primarily in the males), dark kidney, dark area of the stomach, various uterine pathologies (cyst, mass, or thickened wall). The incidences for small testis and small seminal vesicle were similar across all groups. HISTOPATHOLOGY: NON-NEOPLASTIC: In the 6000 ppm dose group, there was no histopathological correlate to the increased liver weight. Livers from the rats killed at Week 79 and at study termination were remarkably free of treatment-related histologic findings, with no histologic evidence of hepatocellular enlargement. Treatment-related histopathologic lesions in the kidney at Week 79 and study termination included an increase in the incidence and severity of mineralization of the renal papilla in males, but not females, and increased incidence and severity of tubule cell pigment in both sexes. In the 12000 ppm group, histological findings in the livers of the males and females were diffuse hepatocellular enlargement (first detected at Week 2), increased cytoplasmic eosinophilia (first detected at Week 13) and increased pigment in Kupffer cell/bile canaliculi (first detected at Week 79). Treatment-related histopathologic lesions in the kidney at Week 79 and study termination, were an increase in the incidence and severity of mineralization of the renal papilla in males, but not females, and increased incidence and severity of tubule cell pigment in both sexes. In the recovery group, treatment-related histological effects in the liver also appeared to be reversible in that diffuse hepatocellular enlargement and increased cytoplasmic eosinophilia were not evident, and the incidence of pigment in Kupffer cell/bile canaliculi was comparable to the control group incidence. Kidney enlargement also appeared to be reversible in that mean kidney weights (absolute and relative) in the males and females were comparable to control values. However, mineralization of the renal papilla in males, and renal tubule cell pigment in both sexes, was only partially reversible with the incidence and severity approximating values in 6000 ppm group. HISTOPATHOLOGY: NEOPLASTIC: In the 6000 ppm dose group, the incidence of mononuclear cell leukemia was 49% and 45% in males and females, respectively, compared to 34% and 26% in the control males and females, respectively. In the 12000 ppm group, increased incidence of neoplasms (first detected at Week 79) in liver was observed. Malignant tubule cell carcinoma was detected in kidneys of two males. The incidence of mononuclear cell leukemia was 46% and 46% in males and females, respectively, compared to 34% and 26% in the control males and females, respectively. In the recovery group, there was absence of an appreciable increase in liver neoplasms compared to the control group. Unlike the liver, treatment-related neoplasia in the kidney of males, and hematopoietic tissue of

both sexes, was not reversible. No kidney neoplasms were detected in females, but malignant tubule cell carcinoma was found in four of the males, of which three were killed following the recovery phase. The incidence of mononuclear cell leukemia was 62% and 48% in males and females, respectively, compared to 34% and 26% in the control males and females, respectively. OTHER FINDINGS: Cell proliferation: Significant increases in the labeling index were observed during Week 1 in the livers of male and female rats treated with the test material at all dose levels. The response was not observed at Week 2, 13, or 104. Large increases in the labeling index were observed in the positive control animals at Week 1, but questionable responses were observed at Weeks 2 and 13. No positive control animals were included for the Week 104 sacrifice. Biochemical analyses: Increases in palmitoyl CoA oxidase, a monitor of the level of peroxisome proliferation were observed in rats of the 12000 ppm group at all timepoints. At Week 104, there was also an elevation in palmitoyl-CoA oxidase activity in female rats of the 6000 ppm group. Male rats treated at 6000 ppm did not have elevated enzyme levels. DNA and Protein Analyses: Levels of DNA varied randomly among the groups and at the different timepoints. Statistical analysis confirmed the lack of a consistent pattern. Protein analysis also did not indicate a clear pattern in the 6000, 12000 ppm or positive control groups. Liver weights were significantly elevated in animals of the 12000 ppm group throughout the study, and also in the positive control animals included at 1, 2 and 13 weeks.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument8d9d.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-f71cacea-32d3-427f-a170-4106fcc4b1e1%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > *Document*

 Dissemination Dossier

 Repeated oral Endpoint.006

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1971							
study report		1982							

Materials and methods

Principles of method if other than guideline

Supporting informaiton

Test materials

Test material equivalent to submission substance identity

yes

Results and discussions

Effect levels

Endpoint

Effect
level

Sex

Basis for effect level /
Remarks

other: Results similar to those previously reported.

Applicant's summary and conclusion

Executive summary

ExxonMobil 1971

MRD 70-46 (DINP, CAS 68515-48-0) was administered at dietary levels of 5 – 50 -500 mg/kg/day to 10 rats of each sex during three months. Observations and records were performed daily for mortality and signs of systemic toxicity and weekly for individual body weight, food consumption and behaviour. Clinical laboratory studies were performed on five rats of each sex from the control and each test group.

At month 1 and month 3, haematology assessment was performed and consisted of hematocrit and hemoglobin determinations, erythrocyte count, and total and differential leukocyte count. Clinical biochemistry investigations were carried out at month 1 and month 3 with fasting blood sugar, blood urea nitrogen, total serum protein, total serum bilirubin, serum glutamic-pyruvic transaminase, serum alkaline phosphatase measures and serum electrophoresis. At month 3 serum albumin, serum sodium, serum potassium, serum chloride, carbon dioxide, serum calcium and serum glutamic-oxaloacetic transaminase measures were performed. Urine analysis at month 1 and month 3 consisted of specific gravity, pH, ketone bodies, total protein bilirubin assessment and microscopic examination of the sediment.

Necropsy was performed on the only rat which died during the study. At termination, all the other animals were sacrificed, and necropsies were led for gross observations and histopathological examinations. The following tissues from each animal were preserved in 10% neutral buffer formalin: brain, pituitary, thoracic spinal cord, eye, thyroid, lung, heart, liver, spleen, kidney, adrenal, stomach, pancreas, small and large intestines, mesenteric lymph node, urinary bladder, testis with epididymis, seminal vesicle, ovary, uterus, skin, rib junction, bone marrow, nerve with muscle and unusual lesions. Some tissues were examined microscopically from 5 males and 5 females in the control and 500 mg/kg/d-MRD 70-46 groups: pituitary, thyroid, heart, liver, spleen, kidney, adrenal, stomach, pancreas, small and large intestines, mesenteric lymph node, urinary bladder, testis, seminal vesicle, ovary, bone marrow and unusual lesions. In addition, sections of liver kidneys and unusual lesions were examined from 5 males and 5 females in each of the remaining group.

Body weight gains in male from 50 mg/kg/day were slightly lower than, but not statistically different from those for the control groups. Food consumption was comparable among the control and treated groups. One male died at 50 mg/kg/day. Eye lesions occurred in few animals as well as alopecia in several rats in various test groups and broken nose in two rats during the study. At 500 mg/kg/day, a statistically significant increase of absolute and relative liver weights was observed when compared to control animals in males (16.06 vs. 12.4 g; 3.54 vs. 2.60%) and in females (9.19 vs. 7.32 g; 3.42 vs. 2.64%) with hepatocytic hypertrophy in males (minimal to moderate in 5/5 males) and in females (minimal to slight in 2/5 females). No remarkable

biochemical changes were observed. At 500 mg/kg/day, a statistically significant increase of absolute and relative kidney weights was observed in females (2.2 vs. 1.87 g and 0.818 vs. 0.652%) and males (3.64 vs. 3.33g and 0.8 vs. 0.699%) but was not statistically significant. Neither remarkable biochemical changes nor microscopically compound related changes were observed.

The absolute and relative testis weights were comparable among control and treated groups. No remarkable microscopic changes were observed in ovaries and testes preserved in 10% neutral buffered formalin.

It can be assumed from this 3-month study that the NOAEL is 150 mg/kg/day based on the increase of kidney and liver weights associated with hepatocytic hypertrophy observed at 500 mg/kg/day.

ExxonMobil 1982

Groups of 15 Sprague Dawley rats of each sex were fed diets containing 0-0.3-1.0% MRD 82-41 (DINP, CAS 68515-48-0) which correspond, respectively to 201-690 mg/kg/day in males and to 251-880 mg/kg/day in females.

There was one death at 1% during the final week of the study. Few in-life observations were noted during the study. Mean body weights and food consumption showed normal patterns in all groups until week 12 of the study. At this time, treated animals showed a sharp decrease in body weight means, statistically significant. This might be related to a watering system uncoupling during week 12.

Hematology data (slight decrease of red blood cells, haemoglobin and hematocrit) suggested a mild anemia from 0.3%.

A moderate to severe decrease of the triglycerides levels was observed from 0.3% in both sexes (43 mg/dl at 1% in males vs. 167 mg/dl in control group) with a clear dose related response whereas cholesterol levels were not clinically different from the control group. At 1%, there was a statistically increase of alkaline phosphatase in both sexes (in males 223 UI/l vs. 146 UI/l in controls and in females 159 UI/l vs. 107 UI/l in controls) and a slight increase, statistically significant, of Alanine Aminotransferase (ALT) in males only (47 UI/l vs. 36 UI/l in controls). These biochemical changes were associated with a statistically significant increase of relative liver weights from 0.3% in males and females and increase of absolute liver weights at 1% only in males (23.0 g vs. 19.2 g in controls) and from 0.3% in females (11.2 and 12.7 g at 0.3 and 1% vs. 9.8 g in controls, respectively).

Differences in urine chemistry values were only observed in males. At week 4, there were increases in glucose with a clear dose-related response and in volume, extreme increase in renal epithelial cells and statistically significant decrease of urine osmolarity values. In females from 0.3%, urine protein values were increased. At week 8 in males, urine osmolarity was moderately lower than control and the extreme increase in renal epithelial cells was still observed from 0.3%. Finally, at week 13, in males urine volume and glucose concentration were moderately higher than controls (statistically and clinically different) at 1%, respectively 20 ml vs. 14 ml in controls and 5.7 ml/16H vs. 3.7 ml/16H in controls; urine protein was moderately lower, statistically and clinically different, than controls at 0.3% without dose-response relationship (9.9, and 18.1 mg/16H at 0.3 and 1% vs. 24.9 mg/16H in controls). These urine chemistry changes were associated with a statistically significant increase of relative kidney weight from 0.3% in both sexes and of the absolute kidney weight from 0.3% in females (2.15 and 2.23 g at 0.3 and 1% vs. 1.87 g in controls) and at 1% in males (3.95 g vs. 3.44 g in controls).

Discoloration, scattered tan foci, and dilatation of the renal pelvis were observed in few treated

animals at the necropsy. No histological data are provided. A slight, statistically significant, increase of relative testis weights was noted at 1% associated with a slight increase (not statistically significant) of the absolute testis weights. No histological data are provided. In this 13-week study, no NOEL is identified. The LOEL is 0.3% (201–251 mg/kg/d) based on the increase of liver weight associated with a decrease of the triglycerides levels and the increase of kidney weight associated with urine chemistry changes observed at this dose.

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Dossier > Document

 **Dissemination Dossier**

 **Repeated oral Endpoint.007**

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	OECD guideline study; GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1987							1987-12-11

Materials and methods

Test type

subchronic

Test guideline

Qualifier	Guideline	Deviations
	according to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Palatinol N

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Dr . Karl Thomae GmbH , Biberach a . d . Riss - Housing: single housing in V2A - cages, Type DK III , BECKER & Co., Castrop - Rauxel (floor area about 900 cm²) - Diet (e.g. ad libitum): Kliba -343-diet rat/mouse/hamster, Klingentalmühle AG, CH-4303 Kaiseraugst - Water (e.g. ad libitum): ENVIRONMENTAL CONDITIONS - Temperature (°C): 20 - 24 °C - Humidity (%): 30 - 70 % - Air changes (per hr): fully air-conditioned - Photoperiod (hrs dark / hrs light): 12 h (12 h light from 6.00 - 18.00 h, 12 h dark from 18.00 h - 6.00 h)

Administration / exposure

Route of administration

oral: feed

Duration of treatment / exposure

3 months

Frequency of treatment

continuously in the diet

Doses/concentrations

ca. 150-380, 510-1200, 1500-3200 mg/kg/d (3000, 10000, 30000 ppm in the diet)

Basis

No. of animals per sex per dose

10

Control animals

yes, concurrent no treatment

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
NOAEL	150 — < 380 mg/kg bw/day (nominal)	male/female	
LOAEL	150 — 380 mg/kg bw/day (nominal)	male/female	

Observations

Remarks on results including tables and figures

No mortality occurred. In the highest dose group the body weights were significantly lowered and the water uptake was increased in both sexes. The hair coat was orange-brown colored around the genital region. The clinical-biochemical examinations showed significantly reduced triglycerides and significantly increased activities of alanine amino transferase and of alkaline phosphatase in both sexes. Bilirubin was significantly increased in females and albumin was significantly increased in males. Some male animals showed increased urea and creatinine. Hematology revealed significant reductions in hemoglobin, median cellular volume and hemoglobin content of erythrocytes in both sexes, and significantly reduced hematocrit in females. Polychromasia and anisocytosis were enhanced in female rats. Absolute and relative liver weights, as well as relative kidney weights were significantly increased in both sexes. The relative, but not the absolute testes weight was increased; this alteration was classified as not substance related. The histological examination showed hypertrophy of the liver cells in both sexes and centrilobular adipose degeneration with pyknosis in males. The renal cortical tubular epithelial cells were affected in males. In the 10000 ppm group water consumption was increased in females. Triglycerides were reduced and the activity of alanine amino transferase was increased in both sexes; alkaline phosphatase activity was enhanced in

females. In males hemoglobin content of the erythrocytes was lowered. Absolute and relative liver weights and relative kidney weights were increased in both sexes; absolute kidney weights were increased in males. Hypertrophy of liver cells was observed in males. In the 3000 ppm group water consumption was temporarily increased in females. The triglycerides showed a trend to reduction. Alterations in alimentary adipose infiltration into liver cells were observed at all dose groups in both sexes.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument52f2.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-1911bac3-dc4a-49ba-add6-8c7bd41b9657%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > *Document*

 Dissemination Dossier

 Repeated oral Endpoint.008

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study with GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1991							1991-04-22

Materials and methods

Test type

subacute

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according	other guideline: EPA Toxic Substances Control Act Test Guidelines, 40	

to CFR Part 798

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear, colorless liquid - Analytical purity: 99.9% - Lot/batch No.: QCL 9004-273

Test animals

Species

mouse

Strain

B6C3F1

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Raleigh, North Carolina - Age at study initiation: 6 weeks - Weight at study initiation: males 21-26 g and females 16-24 g - Housing: 2 per cage - Diet (e.g. ad libitum): Purina Certified Rodent Chow 5002 - Water (e.g. ad libitum): tap water - Acclimation period: 2 weeks ENVIRONMENTAL CONDITIONS - Temperature (°C): 21-23 - Humidity (%): 60-63 - Air changes (per hr): 9.4-11.8 - Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration

oral: feed

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS: Each dose level was prepared by adding the proper amount of test material (per level) into a glass beaker filled with feed. Each level was then premixed in a Waring blender with approximately 200 g of feed for approximately 2 to 3 minutes to ensure an apparent homogeneous mixture. Based on the amount of test material required to be added to each diet level, it was necessary to do multiple premixes at those levels. Waring blender premixes were added to feed to total 5 kg (per level) and mixed for 10 minutes in a Hobart mixer. The remaining preweighed bulk feed was placed in a Patterson-Kelley twin-shell mixer fitted with an intensifier bar. The 5-kg premixes (per level) were added to the bulk feed and mixed for 1 minute/kg. DIET PREPARATION - Rate of preparation of diet (frequency): once in 4 week - Storage temperature of food: 2-6 °C

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Concentration analysis for each dose level of the test diet was within 2% of target.

Duration of treatment / exposure

4 weeks

Frequency of treatment

7 days per week

Doses/concentrations

ca. 635, 1378, 2690 and 6518 mg/kg bw/day for males and ca. 781, 1672, 2470 and 6921 mg/kg bw/day for females

Basis actual ingested

3000, 6000, 12500, 25000 ppm

Basis nominal in diet

No. of animals per sex per dose

10

Control animals

yes, concurrent no treatment

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: twice daily for mortality and moribundity
DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: once daily
BODY WEIGHT: Yes - Time schedule for examinations: at study initiation and weekly thereafter
FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): - Food consumption: Yes, throughout the course of the study, feed spillage/waste was observed in all treated groups and the control groups. In order to accurately reflect food consumption values, the spilled feed was collected from beneath each cage and weighed. The spilled/wasted feed weight was recorded and added to the feeder weight at the conclusion of the appropriate week. - Compound intake calculated: Yes
HAEMATOLOGY: Yes - Time schedule for collection of blood: during week 5 - Anaesthetic used for blood collection: No - Animals fasted: Yes - How many animals: all - Parameters checked: leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HGB), platelet count (PLATELET), hematocrit (HCT), leukocyte differential corrected leukocyte count (COR WBC), cell morphology. Bone marrow smears were prepared at the time of necropsy for the evaluation of myeloid/erythroid ratio (M/E).
CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: during week 5 - Animals fasted: Yes - How many animals: all - Parameters checked: sodium, potassium, chloride, total protein (T PROT), total bilirubin (T BILI), blood urea nitrogen (BUN), creatinine (CREAT), glucose, albumin, calcium, inorganic phosphorus (IN PHOS), aspartate aminotransferase (AST), alanine aminotransferase (ALT), globulin

Sacrifice and pathology

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
For each terminally killed animal, the following organs were weighed: liver with gallbladder, kidneys and testes with epididymides. Organ-to-terminal-body-weight ratios were calculated.

Statistics

Weekly absolute body weights, body weight change (Weeks 1-4), weekly food consumption, total food consumption (Weeks 1-4), clinical pathology data (except cell morphology gradings), fasted terminal body weights, and organ weight data were evaluated by analysis of variance (ANOVA) using Dunnett's procedure for comparing treated means to the appropriate control means.

Results and discussions

Effect levels

Endpoint Effect level

Sex Basis for effect level / Remarks

NOAEL	ca. 781 mg/kg bw/day (actual dose received)	female	hepatocytomegaly, liver/gallbladder weight
LOAEL	ca. 1525 mg/kg bw/day (actual dose received)	female	hepatocytomegaly, liver/gallbladder weight
LOAEL	ca. 635 mg/kg bw/day (actual dose received)	male	hepatocytomegaly, liver/gallbladder weight

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: No mortality and clinical signs were observed. **BODY WEIGHT AND WEIGHT GAIN:** The mean body weight values of the males and females of the 25000 ppm dose group at weeks 3 and 4 were significantly lower than controls. Mean body weight values of females of all other dose groups were significantly elevated for at least one interval compared to the mean female control value at sporadic intervals, but the differences were slight. The mean total body weight changes (weeks 1-4) were also negative in the males and females of the 25000 ppm dose group, and significantly lower than corresponding controls for both sexes. Although the 12500 ppm dose group female total body weight change value was significantly elevated, this difference from control was not considered to be treatment related. **FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):** The mean weekly food consumption (weeks 2, 3, and 4) and total food consumption (weeks 1-4) values were significantly decreased for the females of the 25000 ppm dose group, but not for the males of same group. Weekly and overall (weeks 1-4) mean food efficiency values were negative in both sexes of the 25000 ppm dose group, with the exception of a positive value for the females at week 1. Mean overall compound consumption (mg/kg bw/d) for weeks 1-4 for 3000, 6000, 12500 and 25000 ppm dietary levels in the males were 635, 1378, 2690 and 6518 mg/kg bw/day, respectively, and in the females were 781, 1672, 2470 and 6921 mg/kg bw/day, respectively. **HAEMATOLOGY:** no treatment related changes were observed. **CLINICAL CHEMISTRY:** Relative to corresponding controls, animals of the 25000 ppm dose group had elevated serum alanine aminotransferase activity (both sexes) and blood urea nitrogen levels (males only), although the statistics were based on values from four or fewer animals in the 25000 ppm dose group. **ORGAN WEIGHTS:** Significant and dose-related decreases were observed in the mean absolute and relative kidney and testes/epididymides weights in dose groups 6000-25000 ppm males, with the exception of the increased relative kidney weight in the 25000 ppm group. Significantly increased liver/gallbladder weight parameters (absolute and relative) were observed in all groups of compound-treated animals with the exception of 3000 ppm dose group females. **GROSS PATHOLOGY:** Gross observations were noted in the livers of males of dose groups 6000 and 25000 ppm, and females of dose groups 12500 and 25000 ppm, and were characterized as enlarged, dark, and/or having pale areas. **HISTOPATHOLOGY: NON-NEOPLASTIC:** Hepatocellular enlargement was present in livers of females of the 6000-25000 ppm dose groups and males of the 3000-25000 ppm dose groups with a clear dose response relative to severity. Focal coagulative necrosis and/or separate chronic inflammatory foci occurred in livers of males of the 25000 ppm dose group, and at a lower incidence in females of the 12500 and 25000 ppm dose groups. Tubular nephrosis was observed in kidneys of all males and females of the 25000 ppm dose group, and males of the 12500 ppm dose group. Lesions of the reticuloendothelial

system were noted in males and females of the 25000 ppm dose group, and included atrophy of the spleen and lymphoid depletion in the thymus. Lesions of the epididymis were observed in all males of the 25000 ppm dose group and were characterized as increased cellular debris. In all females of the 25000 ppm dose group, lesions of the reproductive tract included a virtual absence of corpora lutea in the ovaries and atrophic uteri.

Remarks on results including tables and figures

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4cdc-1b7a-e044-00144fd73934/showdocument99bc.html?treeUuid=DISS-828e03e1-4cdc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4cdc-1b7a-e044-00144fd73934&uuid=AGGR-8fe9935c-0be5-4704-bf44-c9fb133854c8%2FDISS-828e03e1-4cdc-1b7a-e044-00144fd73934>

Dossier > Document

 Dissemination Dossier

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Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Study result type	experimental result	Study period	24 May - 02 June 1982
Reliability	2 (reliable with restrictions)		
Rationale for reliability	A well constructed and documented study and although not conducted to any guidelines, the principles and methods followed those of regulated studies and some aspects of the study were conducted according to the principles of Good Laboratory Practice.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1982							1982-12-03

Materials and methods

Test type

other: one week pre-chronic dietary

Limit test

no

Test guideline

Qualifier

Guideline

Deviations

no guideline available

Principles of method if other than guideline

A one week prechronic feeding study in 24 male rats was performed to compare the response of two test compounds to an untreated control group over selected biological endpoints.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): MRD-82-40 and MRD-82-41 - Molecular formula (if other than submission substance): not given - Molecular weight (if other than submission substance): not given - Substance type: technical material - Physical state: clear liquid - Analytical purity: assumed to be 100% - Impurities (identity and concentrations): N/A - Purity test date: not given - Lot/batch No.: not given - Expiration date of the lot/batch: not given - Stability under test conditions: not given - Storage condition of test material: room temperature

Test animals

Species

rat

Strain

other: Fischer 344

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Breeding Laboratories, Inc., Kingston, N.Y. USA. - Age at study initiation: Approx. 4 - 6 weeks - Weight at study initiation: 153.0 to 172.7 grams -

Fasting period before study: N/A - Housing: Individual cages; suspended stainless steel; 7 x 10 x 7 inches - Diet: Purina Certified Chow 5002 (mash) - ad libitum - Water: automatic water system - ad libitum - Acclimation period: 14 days ENVIRONMENTAL CONDITIONS - Temperature (°C): between 20 to 24°C - Humidity (%): between 40 and 70% RH - Air changes (per hr): information not given - Photoperiod (hrs dark / hrs light): 12 hours light, 12 hours dark by automatic timer IN-LIFE DATES: From: May 11 1982 To: June 2 1982

Administration / exposure

Route of administration

oral: feed

Vehicle

other: Purina Certified Rat Chow 5002 (mash)

Details on oral exposure

DIET PREPARATION - Rate of preparation of diet (frequency): once only - Mixing appropriate amounts with (Type of food): 2% of the pure undiluted test material (assumed to be 100% pure) mixed w/w with the diet - Purina Certified Rat Chow (5002 mash) - Storage temperature of food: room temperature VEHICLE - Justification for use and choice of vehicle (if other than water): The chemical is absorbed by the oral route - Concentration in vehicle: 2% mixed on a grams weight percent basis (grams test material/100 grams of feed) - Amount of vehicle (if gavage): N/A - Lot/batch no. (if required): Batch No. of diet not given - Purity: N/A

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Analysis of test materials: A 10 ml archival sample was collected of each compound by the Bio/dynamics West Feed Mixing Group. These samples were transferred to the Compound preparation Department of the East Laboratory. Compound Preparation Summary: The compounds were received via Federal Express on May 3 1982 (MRD-82-40, MRD-82-41). On May 6, 1982, approximately 125 grams of each were transferred to Exxon Industrial Hygiene Analytical Laboratory for stability and analytical determinations. On May 21 1982, 160 grams of each compound were transferred to Bio/dynamics West, to be mixed with feed in the Diet preparation Departments. When the feed was mixed it was brought back to the East Lab, where concentration verification samples were taken. These samples, collected on May 26 1982, the first day of dosing, were then transferred on May 28 1982, to the Exxon Industrial Hygiene analytical Laboratory. At the completion of the study, June 2 1982, another set of concentration samples were taken and delivered to Exxon Laboratories for Analysis. Finally, on June 9 1982,

one 10ml sample of both compounds (which were collected as retention samples by Bio/West) were transferred to Exxon Analytical as per the Study Sponsor's instructions. Analysis of test material in the feed: Purity and Stability were determined by the Sponsor. Homogeneity were sampled as described below and Analytical determinations were also performed by the Sponsor. Similarly concentration verification was sampled as described below and Analytical determinations were performed by the Sponsor. Sampling of diets: - Homogeneity samples: The homogeneity samples were taken by the Bio/dynamics West mixing personnel as follows: One approx. 10 gram sample was collected from each of three different points from each mix prior to discharging the diets from the blender. The approximate blender sampling points were as follows: 1. top left, 2. top right, and 3. bottom centre. These homogeneity samples were taken from both test groups. Analysis of the individual samples were performed in duplicate by the Sponsor and the means of the analytical values among the three points were compared to confirm homogeneity. Concentration verification samples: The concentration verification samples were taken by the East Laboratory Compound preparation Department as follows: One approximately 10 gram sample was collected from a randomly-selected point from each test and control feed bucket prior to administering the feed to the animals. Analysis of the individual samples was performed in duplicate by the Sponsor. Any blend exhibiting a verification from the target concentration of +/- 10% was re-mixed and re-analysed to verify concentration.

Duration of treatment / exposure

7 days

Frequency of treatment

ad libitum

Doses/concentrations

2% (w/w) in diet

Basis nominal in diet

No. of animals per sex per dose

8 Males

Control animals

yes, plain diet

Details on study design

- Dose selection rationale: - Rationale for animal assignment (if not random): N/A (randomly

allocated by computer) - Rationale for selecting satellite groups: N/A - Post-exposure recovery period in satellite groups: N/A - Section schedule rationale (if not random): N/A

Positive control

Not Applicable

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: once in the morning (before 10:30 am) and once in the afternoon (at approx. 3:00 pm) DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: once a day for the 7 days BODY WEIGHT: Yes - Time schedule for examinations: DAY -1, DAY 0 (initiation) and DAY 7. (see Table 1) FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Food consumption measured after one week - Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: No - 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: No WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): Not applicable OPHTHALMOSCOPIC EXAMINATION: Not applicable HAEMATOLOGY: Yes - Time schedule for collection of blood: DAY 7 - Anaesthetic used for blood collection: Yes (methoxyflurane) - Animals fasted: YES - How many animals: ALL 3 groups (8/group) CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: DAY 6 - Animals fasted: No - How many animals: ALL 3 groups (8/group) URINALYSIS: Not applicable NEUROBEHAVIOURAL EXAMINATION: Not applicable

Sacrifice and pathology

GROSS PATHOLOGY: Yes All animals were subjected to necropsy examination and the following tissues and organs were preserved in 10% neutral buffered formalin from each rat: Liver Kidneys Testes (with epididymides) Unusual lesions HISTOPATHOLOGY: Yes The following tissues were prepared for histological examination from each rat: Liver Kidneys Testes (with epididymides) Unusual lesions.

Statistics

The following parameters were analyzed statistically: mean body weight mean food consumption mean organ weights organ/body weights ratios clinical laboratory findings ANOVA: performed on the mean bodyweights and on the mean food consumptions. Duncan's Multiple Range Test for Variables: performed on the body weight means for each weigh period and on the food consumption data. The following analysis was performed on the mean organ weights, organ/body weight ratios and clinical laboratory findings. Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a

multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Duncan's test was used to determine which means were significantly different from the control. If a non parametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summary rank test (Dunn) was used to determine which treatments differed from control.

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
no NOAEL identified only one dose concentration per test material		male	

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

yes

Food consumption and compound intake (if feeding study)

yes

Food efficiency

no data

Water consumption and compound intake (if drinking water study)

not examined

Ophthalmoscopic examination

not examined

Haematology

no effects

Clinical chemistry

yes

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

no effects

Gross pathology

no effects

Histopathology: non-neoplastic

no effects

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY: All animals survived until study termination date. There were no significant toxicological observations recorded during the study. Slight red nasal discharge was recorded for two animals in Group I (controls) and one animal in Group II. One animal in Group III developed slight alopecia on both forepaws which later became scabbed. Two animals from Group II developed a slight ocular discharge on Day 6 (probably related to the orbital bleeding in the morning of Day 6). Also due to eye bleeding, one animal in Group I exhibited extreme eye damage, and one animal in Group II exhibited slight eye damage. **BODY WEIGHT AND WEIGHT GAIN:** Group I showed some weight gain from pre-test to termination. Group II and Group III gained weight from pre-test to initiation. Statistical analysis

of the means revealed no differences among the group means. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Group I consumed the most feed, followed by Group III. Group II consumed the least amount of feed. Statistical analysis of the means showed a significant difference among the groups. HAEMATOLOGY: There were no statistical or clinical differences noted in any of the haematology parameters. CLINICAL CHEMISTRY: Non-fasted (day 6) 1). Glucose: The means of Groups II & III did not differ statistically or clinically from the controls. 2). Cholesterol: The means of Groups II and III were moderately suppressed as compared to the control group (statistically and clinically significant). 3). Triglycerides: The means of Group II and III were severely suppressed as compared to the control group (statistically and clinically significant). Fasted (day 7) 1). Uric acid: No statistical or clinical differences noted in the means. 2). Glucose: The means of Group II and III were slightly lower than the control group. These findings are statistically significant and probably clinically significant. 3). Urea Nitrogen: No statistical or clinical differences noted in the means. 4). Glutamic Oxalacetic transaminase: No statistical or clinical differences noted in the means. 5). Glutamic Pyruvic Transaminase: No statistical or clinical differences noted in the means. 6). Triglycerides: The means of Groups II and III are slightly lower than the control group (Group III is statistically significant). Differences are probably clinically significant. 7). Alkaline Phosphatase: The mean of Group II is slightly lower than control group; statistically and clinically significant. The mean of Group III is not statistically or clinically different. 8). Total Protein: The mean of Group III is slightly lower than the control group (statistically significant). Clinical significance is questionable. The mean of Group II is not statistically or clinically different. 9). Cholesterol: The mean of Group I is slightly higher than the control group (statistically and clinically significant). The mean of Group III is not statistically different from the control group. 10). Albumin: The mean of Group II is slightly higher than the control Group. Although this finding is statistically significant, its clinical significance is questionable. The mean of Group III is not statistically or clinically different from the control group. 11). Calcium: The mean of Group II is slightly higher than the control group. Although this finding is statistically significant, it is not clinically significant. The mean of Group III is slightly lower than the control group. Although this finding is statistically significant it is not clinically significant. 12). Chloride: Chlorine showed no clinical or statistical difference. ORGAN WEIGHTS: There were no statistical differences among the means for body weight and testes/epididymis weight. The means did differ statistically for both liver and kidney weights. Kidney to body weight ratio means did differ statistically, whereas the testes/epididymis to body weight ratios for Groups II and III were higher in relation to the controls (Group I). The kidney to body weight and liver to body weight ratios were higher for Groups II and III. GROSS PATHOLOGY: Cervical lymph nodes were reddened in 4 animals in Group I, and in 2 animals in Group II. The lobes and surfaces of the lungs were mottled tan, pink and red for 1 animal in Group I and for 1 animal in Group II. The liver was slightly congested on all lobes and surfaces in 1 animal in Group II and 3 animals in Group III. HISTOPATHOLOGY: NON-NEOPLASTIC: No microscopic changes were present in any of the tissues examined which were related to the oral feeding of 2% MRD-82-40 or 2% MRD-82-41 in the diet when each treated group was compared to the control group.

Remarks on results including tables and figures

Table 1 Body weights (grams)

Group I: Control Basal Lab Diet

Animal Number	Day -1 (sorting body weights)	Day 0 (pre-test body weights)	Day 7 fasted weights (terminal body weights)
043	153.0	158.1	150.6
ICA003	157.0	157.8	160.7
ICA067	159.0	162.8	165.6
ICA039	161.0	167.5	171.3
ICA019	162.0	165.0	164.3
ICA035	164.0	167.7	170.6
ICA051	166.0	170.4	170.5
ICA007	168.0	171.9	173.6
MEAN	161.3	165.1	165.9
standard deviation.	1.7	1.9	2.7

Group II: 2% (w/w) MRD-82-40

Animal Number	Day -1 (sorting body weights)	Day 0 (pre-test body weights)	Day 7 fasted weights (terminal body weights)
ICA072	151.0	153.0	150.6
ICA037	159.0	162.9	154.3
ICA064	159.0	162.9	156.8
ICA002	161.0	162.7	162.5
ICA063	162.0	165.5	154.3
ICA057	164.0	166.6	162.3
ICA012	165.0	168.6	162.4
ICA052	168.0	172.7	170.9
MEAN	161.1	164.4	159.3
standard deviation.	1.8	2.0	2.3

Group III: 2% (w/w) MRD-82-41

Animal Number	Day -1 (sorting body weights)	Day 0 (pre-test body weights)	Day 7 fasted weights (terminal body weights)
ICA078	153.0	154.6	146.2
ICA018	156.0	159.2	151.8
ICA048	159.0	162.0	157.2
ICA053	160.0	164.0	159.1
ICA027	162.0	168.4	163.5
ICA056	163.0	170.6	160.4
ICA049	165.0	167.4	161.8
ICA023	169.0	171.8	169.8

MEAN	160.9	164.8	158.7
standard deviation.	1.8	2.1	2.6

Applicant's summary and conclusion

Conclusions

There were no significant in-life gross toxic effects from either of the test materials when administered to rats for 7 days. No clinical or statistical differences were noted in any of the haematology parameters. However, mean weight and organ to body weight ratios were increased for both liver and kidneys in rats fed on diet treated with MRD-82-40 and with MRD-82-41, and the testes / epididymis to body weight ratio was significant for rats fed on diet treated with MRD-82-41 compared to the controls.

Executive summary

A one week pre-chronic feeding study in rats was performed at Bio/dynamics Laboratory, East Millstone NJ, USA on behalf of Exxon Corporation research and Environmental Health Division, East Millstone, NJ, USA. The aim was to compare the response of two test compounds to an untreated control group over selected biological endpoints. Twenty four male Fischer 344 rats were placed into three equal groups, one control (basal diet) and two test groups (MRD-82-40 and MRD-82 -41). The test material was administered on a 2% weight-to-weight basis of the basal laboratory diet. Body weights were recorded pre-dose, at initiation and at termination and clinical observations performed daily. Hematological parameters were measured on blood samples from fasted animals at termination and clinical chemistry parameters were measured from fasted and non-fasted animals. Food consumption was measured from initiation to termination. There were no mortalities and all animals were euthanized at termination, subjected to post mortem examination and selected organs examined histopathologically. There were no significant in-life gross toxic effects from test material administration.

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 Dissemination Dossier

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Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study with GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1991							1991-08-12

Materials and methods

Test type

subchronic

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according	other guideline: EPA Toxic Substances Control Act test guidelines, 40	no

to CFR Part 798

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear colorless liquid - Analytical purity: 99.9% - Lot/batch No.: QCL 9004-273 - Supplier: Aristech Chemical Corp. - Storage condition of test material: room temperature

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Raleigh, North Carolina - Age at study initiation: approximately 6 weeks - Weight at study initiation: males 110-131 g and females 90-108 g - Housing: 2 per cage - Diet (e.g. ad libitum): Purina Certified Rodent Chow 5002 - Water (e.g. ad libitum): tap water ENVIRONMENTAL CONDITIONS - Temperature (°C): ca. 22 - Humidity (%): 50±20 - Air changes (per hr): 10 or more - Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration

oral: feed

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS: Each level was prepared by placing feed into an appropriately sized glass beaker. A depression was made in the center of the feed and the weighed test material was added to the depression. This test material-feed combination was mixed with approximately 200 g of feed for 2-3 minutes in a Waring blender to form a homogeneous premix. Additional premixes were needed as the amount of test material increased at the higher dose levels. The premix(es) were then added to 5 kg of feed in a Hobart blender and mixed for 10 minutes. The premix from the Hobart blender was added to the required amount of feed for the dose level and mixed in a Patterson-Kelly Twin Shell mixer (2 cu. ft.) fitted with an intensifier bar for 1 min/kg. At no time during the mixing procedure was plastic allowed to come in contact with the test material. DIET PREPARATION - Rate of preparation of diet (frequency): every 4 weeks - Storage temperature of food: 2-6°C

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The percent of target concentration was low (less than 10% of target) and varied by more than 10% from its duplicate sample for one prestudy homogeneity sample taken from the middle of the mix for 2500 ppm dose group. A remix of the same test diet was performed and analysis of homogeneity samples produced results within 10% of the target concentration and with acceptable variance between duplicate samples. The percent of target concentration was high (greater than 10% of target) at the Day 35 stability analysis of the test diet mix of the 20000 ppm dose group for Weeks 1-3 and low at the Day 35 stability analysis of the test diet mix of the 2500 ppm dose group for Weeks 8-11. All other analyses were within $\pm 10\%$ of the target concentration, with acceptable variance between duplicate samples.

Duration of treatment / exposure

13 weeks

Frequency of treatment

7 days per week

Doses/concentrations

ca. 175.8, 354.6, 719.6, 1544.7 mg/kg bw/day for males and ca. 218.9, 438.0, 823.8, 1687.1 mg/kg bw/day for females

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes All animals were weighed on the day of scheduled necropsy and were sacrificed by exsanguination under sodium pentobarbital anesthesia. The following organs were weighed: brain with stem, spleen, kidneys, testes with epididymides, liver, uterus, lung

Statistics

Mean body weight (Weeks 1, 5, 9, and 14), mean body weight change (Weeks 1-13), mean food consumption (Weeks 1, 4, 8, and 13), mean total food consumption (Weeks 1-13), hematology (except cell morphology gradings), serum chemistry, urine chemistry, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups.

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
LOAEL	ca. 176 mg/kg bw/day (actual dose received)	male	kidney and liver weight
LOAEL	ca. 219 mg/kg bw/day (actual dose received)	female	kidney weight

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: No mortality was observed. An increased incidence of urine stains occurred in female rats receiving 10000 and 20000 ppm. **BODY WEIGHT AND WEIGHT GAIN:** Low body weight gain was observed in high-dose animals of both sexes. Relative to corresponding control values, the depression in body weight gain was more pronounced in high-dose females than in males. **FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):** Food intake was slightly decreased in high-dose females, but was comparable between control and high dose-males. Weekly compound consumption in the females of all dose groups tended to be slightly greater than values in corresponding male dosage groups. Mean overall compound consumption (mg/kg bw/d) for weeks 1-13 in the males for 2500, 5000, 10000, 20000 ppm dietary levels was 176, 355, 720, 1545 mg/kg bw/day, respectively, and in the females was 219, 438, 824, 1687 mg/kg bw/day, respectively. **OPHTHALMOSCOPIC EXAMINATION:** There were no ophthalmoscopic findings related to compound treatment. **HAEMATOLOGY:** Mean erythrocyte count, mean hematocrit, and mean hemoglobin were significantly lower in the males and females of the 10000 and 20000 ppm dose groups, and in the males of the 5000 ppm dose group when compared to the respective control values. There were no other significant differences in the hematology data. **CLINICAL CHEMISTRY:** increased serum albumin levels and/or decreased serum globulin levels were observed in males and females from the 10000 and 20000 ppm groups. Serum globulin levels were also decreased in females receiving 5000 ppm. Blood urea nitrogen levels were markedly elevated in males from the 10000 and 20000 ppm groups and slightly elevated in females from the 20000 ppm group. **URINALYSIS:** Evaluation of urine chemistry data revealed significantly increased mean urine sodium and potassium excretion values in the females of the 10000 ppm dose group when compared to respective control values. There were no other significant differences in urine chemistry data. Evaluation of urinalysis data revealed a slight increase in urine specific gravity in the males of the 20000 ppm dose group, accompanied by increases in urine protein, leukocytes, and amorphous material. Increases in incidence and grade for

leukocytes and amorphous material were also noted in the males of the 10000 ppm dose group. Urinalyses in the females were generally comparable between control and treated groups. ORGAN WEIGHTS: Mean liver weight parameters were significantly increased in a dose-dependent manner in all groups, with the exception of low-dose females. Mean kidney weight parameters were significantly increased in all treated groups. Uterine weight parameters in high-dose females were markedly lower than the corresponding values in control females. GROSS PATHOLOGY: Enlarged and dark livers were observed in two males of the 20000 ppm dose group. Dark areas of the glandular stomach were observed in three males of the 20000 ppm dose group, one female of the 2500 ppm dose group, and three females of the 20000 ppm dose group. HISTOPATHOLOGY: NON-NEOPLASTIC: Microscopic evaluation revealed hepatocellular enlargement in the high dose group males (periportal) and females (centrilobular). Treatment-related renal findings were present in the males of the 5000-20000 ppm dose group and consisted of granular casts and a higher severity of regenerative/basophilic tubules. Vesiculation and an increased severity of acute inflammation were present in the stratified squamous epithelium of the nonglandular stomach in selected animals from the 10000 and 20000 ppm dose groups.

Remarks on results including tables and figures

Applicant's summary and conclusion

Executive summary

http://apps.echa.europa.eu/registered_data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumente950.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934+%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-f2c6b1cf-afac-4d15-8126-7f3a3499daf4%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934

Dossier > *Document*

 Dissemination Dossier

 Repeated oral Endpoint.011

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Acceptable, well-documented study, GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1986							1986-01-13
publication	Lington AW, Bird MG, Plutnick RT, Stubblefield WA, and Scala RA.	1997	Chronic toxicity and carcinogenic evaluation of diisononylphthalate in rats.	Fundamental and Applied Toxicology (36):79-89					

Materials and methods

Test type

combined repeated dose and carcinogenicity

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): MRD-83-260 - Substance type: clear, colorless liquid - Analytical purity: assumed 100%

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Breeding Laboratories. - Age at study initiation: 6 weeks - Weight at study initiation: males (106-143 g), females (88-110 g) - Fasting period before study: N/A - Housing: individually - Diet (e.g. ad libitum): ad libitum - Water (e.g. ad libitum):

ad libitum - Acclimation period: 19 days ENVIRONMENTAL CONDITIONS - Temperature (°C): 20-22 - Humidity (%): 68-76 - Air changes (per hr): N/A - Photoperiod (hrs dark / hrs light): 12/12 IN-LIFE DATES: From: 1983-03-21 To: 1985-03-17

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): Purina rodent chow - Storage temperature of food: room temperature

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

Diet

Frequency of treatment

Daily

Doses/concentrations

0, 0.03, 0.30, 0.60 %
Basis nominal in diet

No. of animals per sex per dose

110 per sex per dose

Control animals

yes, concurrent no treatment

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: weekly BODY WEIGHT: Yes - Time schedule for examinations: weekly 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 HAEMATOLOGY: Yes CLINICAL CHEMISTRY: Yes URINALYSIS: Yes

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes

Results and discussions

Effect levels

Endpoint	Effect level	Sex Basis for effect level / Remarks
LOAEL	0.3 % in diet	Increased liver and kidney weights increased incidence of non-neoplastic changes) increased liver and kidney weights increased incidence of non-neoplastic changes

Observations

Remarks on results including tables and figures

Applicant's summary and conclusion

Executive summary

Groups of 220 (110/sex) Fischer 344 rats, (6 weeks of age at initiation of dosing) were administered dietary concentration of 0 - 0.03 - 0.3 - 0.6% (w/w) DINP (MRD 83-260, CAS 68515-48-0, assumed to be 100% pure for purposes of dosing for a period up to 2 years. The mean daily intakes of DINP over 2 years were 15, 152 and 307 mg/kg/day for male rats corresponding to dose levels of 0.03 - 0.3 and 0.6%, respectively and 18, 184 and 375 mg/kg/day for female rats, respectively. Preselected subgroups of 10 rats/sex/dose level were scheduled for interim sacrifice after 6, 12 and 18 months and remaining rats were sacrificed at 24 months.

The animals were observed twice daily for viability. Clinical examinations (nature, onset and

duration of signs, palpable tissue masses), body weight and food consumption were recorded weekly. Hematological, clinical-chemical and urinalysis parameters were determined in rats sacrificed at the 6, 12 and 18-month intervals and in 20 rats/sex/dose level at study termination. Hematological (RBC count, Hct, Hb, total and differential WBC count, MCV, MCH, MCHC, platelet, reticulocyte counts), clinico-chemical parameters (total protein, albumin, globulin, BUN, cholesterol, triglycerides, creatinine, glucose, alkaline phosphatase, AST, ALT, total bilirubin, calcium, sodium, potassium, chloride, serum osmolality) and urinalysis (pH, specific gravity, ketones, bilirubin, occult blood, volume, glucose, proteins, creatinine, sodium, potassium, osmolality, renal epithelial count) were determined in rats sacrificed at the 6, 12 and 18-month intervals and in 20 rats/sex/dose level at study termination. It should be noted that gamma glutamyl transpeptidase and ornithine decarboxylase was not measured.

Complete gross necropsy was conducted in all animals sacrificed at 6, 12, 18 months and all survivors at study termination, and also on moribund sacrificed rats in which organ weights were not determined. Selected organs (adrenals, brain, heart, kidney, liver, ovaries, spleen, testes and thyroid/parathyroids) were weighed and tissues were preserved in 10% neutral buffered formalin and processed (paraffin technique) for light microscopic examination.

The morphological examination of the liver was conducted on livers of two rats/sex/group by electron microscopy for evidence of peroxisome proliferation induction at study termination. Liver specimens from the right lobe were excised, minced and rapidly fixed by immersion with Karnofsky fixative and then prepared for microscopy evaluation.

Histopathological examinations were conducted on all gross lesions, all tissue masses, epididymides, kidneys, liver, lungs, ovaries, pituitary, spleen, testes and thyroid in rats sacrificed at the 6, 12 and 18-month intervals; all gross lesions, tissues masses kidney, liver from rats of the low and mid-dose group sacrificed at study termination; and on adrenals, aorta (abdominal), brain, epididymides, esophagus, eyes with optic nerve, femoris muscle with sciatic nerve, harderian glands, heart, kidneys, large intestine (colon, cecum), liver, lungs, mammary glands, mesenteric lymph nodes, nasal turbinates/cavity, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, seminal vesicles, skin, small intestines (duodenum, jejunum, ileum), spinal cord (cervical, midthoracic, lumbar), spleen, sternum with marrow, stomach, testes, thymus, thyroids/parathyroids, trachea, urinary bladder, uterus, vagina, all gross lesions and all tissue masses from all control and high-dose rats.

Results

Survival was greater than 60% in all groups at study termination. A slight decrease in overall survival was indicated in the mid- and high-dose groups of either sex compared to controls, depending on the statistical method: No significant difference was obtained using the Weibull technique which is sensitive to model parameters; the Cox and generalized Kruskal-Wallis tests indicated a significant difference in survival in mid- and high-dose female rats.

Clinical in-life observations: the incidence of clinical in-life observations was relatively low throughout the 24-month test period. With the possible exception of a low incidence of urinary staining noted for the treated females, mainly during the first year of study, there were no in-life findings that appeared indicative of treatment related toxicity (palpable masses which were mainly subcutaneous, ocular changes were the most common findings observed at a similar incidence in treated and control rats).

Food consumption: slight and sporadic but in many cases statistically significant changes in weekly food consumption values occurred in both sexes during the study; overall consistent changes consisted of a significant reduction of food consumption in the high-dose males during

the last 12 months of the study and of a slight increase of food consumption in the mid and high-dose females during the first 12 months of the study. Body weight changes: when compared with controls, the high-dose males exhibited a statistically significant, dose-related decrease in body weight beginning at 12 months of treatment and persisting until termination (4 to 7% reduction in body weight compared to the control group). There were not statistically significant changes of bw in females which exhibited small, but in some cases statistically significant differences, compared to controls: treated females weighed slightly more than controls until the last 6 months of the study when they displayed slight decreases in bw.

Organ weights: both males and females from the mid and high-dose groups exhibited a statistically significant, dose-related increase in relative kidney and liver weights throughout most of the treatment period; the absolute liver and kidney weights demonstrated a similar trend. At termination in the high-dose level absolute liver and kidney weights were increased, respectively in males by 20% and 8% (liver and kidney weights were 13.94 and 3.19 g vs. 11.63 and 2.95 g) and in females by 27.3 and 9% (liver and kidney weights were 10.25 and 2.30 g vs. 8.27 and 2.10 g). Those changes in organ weights were correlated to histopathological findings at the 18-month sacrifice.

At study termination, the mid- and high-dose males exhibited a statistically significant, but not dose-related, increase in absolute and relative spleen weights and the high-dose females a statistically significant increase in relative spleen weight. Relative, but not absolute adrenal weights were slightly, but significantly increased in both sexes. No treatment-related changes were observed in the absolute or relative organ weights for ovaries, brain, heart, or thyroid/parathyroid.

At study termination, a statistically significant increase in relative testis weights was observed at the high dose associated with a slight, not statistically significant, increase of 13% in absolute testis weight (6.22 g vs. 5.48 g in the control group).

Hematology: in general, the mean hematologic values were within normal limits for both sexes at 6, 12 and 18 months (few slight but statistically significant differences were seen in males for Hb, MCH and MCHC values and in females for MCV, MCHC and platelet values, all of which were within biological limits and thus not considered as treatment related). At study termination, some possible treatment related effects on hematologic values were observed. In the mid and high-dose males, there was a dose dependent increase, possibly treatment related although not statistically significant compared to controls, of white blood cell count (WBC). There were two animals at the 0.3% level that had extremely elevated WBC's and the occurrence of abnormally high WBC's increased with dose. The mid-dose females also exhibited a statistically significant increase in mean WBC count as compared to controls. The red blood cell count, hemoglobin and hematocrit mean values were slightly lower for mid- and high-dose males compared to control values and only statistically significant at the high-dose level. The high-dose males and females also exhibited an increased frequency of occurrence of nucleated RBC, polychromatophilic red cells, and reticulocytes when compared with control animals.

Serum chemistry: no remarkable findings at 6, 12 and 18 months. Some parameters were statistically increased (BUN, A/G ratio, creatinine) but were judged unlikely to be of biological significance due to a lack of dose response and thus considered as not treatment related. On the other hand, parameters usually associated with liver function (Alkaline phosphatase, AST, ALT) were weakly, but generally at statistical significance, increased in the mid and high-dose males at all study intervals; however, at study termination, considerable variations were seen in individual values of liver enzymes suggesting that these changes may be not related to treatment. In

addition, the serum chemistry profile in females did not reveal significant changes in liver function parameters.

Urine chemistry: statistically significant changes in some of the urinary parameters were seen in males throughout the study, especially in high-dose males which exhibited increased urine volumes at all time intervals; potassium and glucose levels were increased in mid and high-dose males at the 6, 12 and 18-month intervals but not at study termination. An increase in excretion of renal epithelial cells was also noted from 0.3% in males at 6 months but not at later treatment periods.

Gross effects at necropsy: no grossly observable and treatment-related abnormalities were apparent at any of the interim sacrifices. At study termination, however, mid and high-dose males and high-dose females exhibited an increased incidence of splenic enlargement when compared with control animals.

Histopathological findings: Ultrastructural examination of liver specimens from representative rats of each sex from the four groups did not reveal any treatment-related peroxisome proliferation. Lesions were observed in the liver and kidney of high-dose rats (minimal to slight centrilobular to midzonal hepatocellular enlargement in high-dose rats; minimal increase in tubular cell pigment was noted in the renal tubular epithelium in high-dose male). Pertaining to liver findings, an increased incidence of spongiosis hepatitis was noted in males given 0.3 and 0.6% DINP (respectively 51 and 62 treated animals vs. 24 controls), and of hepatocellular enlargement in both sexes at the high dose (9 in males and 11 in females vs. 1 in controls of both sexes). Focal necrosis was increased in both sexes from 0.3% DINP, but was only significant in males of the high-dose group (26 in treated animals vs. 10 in controls). Hepatopathy associated with leukaemia was also increased in mid and high-dose groups, but only significantly from 0.3% in males (34 and 33 in treated animals vs. 22 in controls) and at 0.6% in females (33 in treated animals vs. 16 controls). In addition at 24 months, lesions in the liver consisting of slight centrilobular to midzonal hepatocellular enlargement in a small number of rats were comparable to the findings at the 18-month sacrifice. A histopathology peer review and pathology working group review (Experimental Pathology Laboratories, 1999) were conducted on selected lesions of the liver observed in the two chronic/carcinogenicity studies with Fischer 344 rats with DINP and confirmed the previous conclusions concerning liver histopathological findings. In kidneys, a minimal increase in tubular cell pigment was noted in the tubular epithelium of high-dose male rats, sacrificed after 6, 12 and 18 -month treatment. At 24 months, histopathology examinations revealed an increased severity of tubular cell pigmentation in animals with advanced leukaemia (see Table 4.27).

Concerning kidney findings in male rats, a retrospective evaluation of archived tissue was conducted by immunohistochemical techniques (Caldwell et al., 1999) to measure the accumulation of α 2u-globulin in male rat kidneys following DINP treatment. Using archived tissue obtained at 12-month interim sacrifice, a dose-dependant increase of α 2u-globulin was identified by immunohistochemical staining techniques (IHC) in male kidneys only. An increase in cell proliferation was confirmed by immunohistochemical detection of proliferating cell nuclear antigen (PCNA) and located especially in the areas of α 2u-globulin accumulation. Hematoxyllin and eosin-stained sections revealed tubular epithelial hypertrophy and regeneration consistent with the immunohistopathological findings. These findings were related to the α 2u-globulin mechanism of male rat specific nephropathy which is not regarded as relevant to humans.

Conclusion

Based on slight decreased survival data in females, liver findings in males (increased incidence of spongiosis hepatitis and increased serum levels of usual liver parameters), increase of absolute and relative liver and kidney weights in both sexes and other findings (e.g. increase of relative and absolute spleen weights in males) from 0.3% a NOAEL for chronic hepatic and renal effects of 0.03% might be considered which corresponds approximately to 15 and 18 mg/kg/day for males and females, respectively.

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Dossier > Document

 **Dissemination Dossier**

 **Repeated dermal Endpoint.001**

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	1969
Reliability	2 (reliable with restrictions)		
Rationale for reliability	An old study conducted before GLP or standard guidelines were introduced. However, the study was undertaken at a recognised laboratory, and there are sufficient data reported to provide a supplementary assessment for a second route of exposure.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1969							1969-08-01

Materials and methods

Test type

subacute

Limit test

no

Test guideline

Qualifier
no guideline available

Guideline

Deviations
no data

Principles of method if other than guideline

A standard design for repeat dermal application toxicity study

GLP compliance

no

Test materials

Test material equivalent to submission substance identity
yes

Details on test material

A clear, oily liquid with a faint, unpleasant odour. Received on 10 January 1969 and assumed to be 100% active.

Test animals

Species
rabbit

Strain

New Zealand White

Sex

male/female

Administration / exposure

Type of coverage
occlusive

Vehicle

unchanged (no vehicle)

Analytical verification of doses or concentrations

no

Duration of treatment / exposure

24 hours per day, five days per week for six weeks

Frequency of treatment

Once a day, five days a week

Doses/concentrations

Basis nominal per unit body weight

No. of animals per sex per dose

Four male at 0.5 ml/Kg and four males at 2.5 ml/Kg

Control animals

yes, sham-exposed

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
NOAEL systemic	ca. 500 mg/kg bw/day (nominal)	no data	Mild dermal irritation, but no systemic effects on kidney or liver

Observations

Remarks on results including tables and figures

There was no evidence of systemic toxicity from repeated dermal exposures to MRD-69-4 as based on general appearance and behavior, clinical laboratory studies, and gross and microscopic visceral pathology.

Compound effect was confined to gross and microscopic alterations of the skin. Grossly, MRD-

69-4 at the 0.5 ml/kg/day level produced generally mild dermal irritation which was slightly more severe than irritation produced by mineral oil at 2.5 ml/kg/day; at the 2.5 ml/kg/day level, slight or moderate (abraded skin only) erythema and slight desquamation were noted through the fifth week of application.

Histologically, skin sections showed slight acanthosis, slight hyperkeratosis, and slight dermatitis of comparable severity in control and compound-treated animals. Sections of kidney, liver, and skin from control and test animals were comparable histologically; lesions encountered were consistent with incidental disease.

Applicant's summary and conclusion

Conclusions

There was no evidence of systemic toxicity from repeated daily dermal exposure to MRD-69-4 for six weeks. Mild irritation was observed at the application sites of animals treated with MRD-69-4, although there was no histopathological difference in the severity of the skin changes between test and control animals.

Executive summary

In a six week dermal toxicity study, groups of four New Zealand White rabbits received 24-hour daily applications of MRD-69-4 at 0.5 or 2.5 ml/Kg, five times a week, to the closely clipped intact (two animals per group) or abraded (two animals per group) abdominal skin, wrapped with gauze adhesive tape and fitted with neck collars. Control animals (three males and one female) received mineral oil at 2.5 ml/Kg. Clinical signs were recorded daily, haematology and urinalysis undertaken initially and terminally, and histopathology was performed on the liver, kidneys and skin. There was no evidence of systemic toxicity. Mild irritation was observed at the application sites of animals treated with MRD-69-4, although there was no difference in the severity of histopathological skin changes apparent between test and control animals. The systemic NOEL was considered to be 2.5 ml/kg/day (estimated to be 2500 mg/kg/day) and the local NOAEL was considered to be 0.5 mg/kg/day (estimated to be 500 mg/kg/day).

The dermal toxicity study was considered to be a suitable supporting study for classification.

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 Dissemination Dossier

 Repeated inhal Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	The study is rated a "2" because appropriate testing methods were used; however, the study does not follow and accepted guideline or indicate compliance with GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1981							

Materials and methods

Test type

subchronic

Limit test

no

Principles of method if other than guideline

As described previously, Pegg (1979) GM Research Reports

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

no

Test animals**Species**

rat

Strain

Sprague-Dawley

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS - Age at study initiation: adult - Weight at study initiation: 125g - Housing: individually in polycarbonate wire top cages - Diet: ad libitum - Water: ad libitum - Acclimation period: 7 days

Administration / exposure**Route of administration**

inhalation: aerosol

Type of inhalation exposure

whole body

Vehicle

unchanged (no vehicle)

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION Data not available
TEST ATMOSPHERE Repeated exposures to nonradioactive DIDP were conducted through whole body exposure in a 30L cylindrical glass jar. Animals were restrained in wire mesh cylinders and supported on a suspended screen floor. The chamber atmosphere was assayed for DIDP content on each exposure day. Cascade impactor samples for determination of aerosol particle size taken every other day during the exposure period.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

5 consecutive days, 2 days recovery and another 5 days exposure (10 total exposures)

Frequency of treatment

6 hours/ day

Doses/concentrations

500 mg/m³

Basis nominal conc.

MMAD / GSD

MMAD = 0.98 μ m

No. of animals per sex per dose

8 rats/dose 6 control rats

Control animals

yes, concurrent no treatment

Details on study design

Body weights of the animals were taken immediately before and after each 6 hour exposure period. After exposure, animals were returned to the vivarium. Control animals were exposed simultaneously in an identical chamber flushed with filtered room air. Rats were observed daily for body weight gain, appearance and gross behavior. At the end of the exposure regimen

animals were held for observation for 3 weeks. Animals were sacrificed at the end of the observation period and tissue samples taken for histopathology.

Results and discussions

Effect levels

Endpoint	Effect level	Sex	Basis for effect level / Remarks
NOAEC	500 mg/m ³ air	male	No systemic toxicity

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

yes

Food consumption

no data

Food efficiency

no data

Water consumption

no data

Ophthalmoscopic examination

no data

Haematology

no data

Clinical chemistry

no data

Urinalysis

no data

Neurobehaviour

no data

Organ weights

no data

Gross pathology

no data

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

no data

Details on results

The nominal chamber atmosphere concentration for the repeated exposure toxicity studies was 500 mg/m³. Daily, mean analytical concentrations ranged from 468 to 571 mg/m³. The mean analytical concentration for the ten exposure periods was 505 mg/m³. The mass median aerodynamic diameter of the DIDP aerosol was 0.98 µm. Animals exposed repeatedly to 500 mg/m³ DIDP exhibited no marked outward signs of toxicity during exposures. The rate of body weight gain was not different between control and exposed animals. Occasional changes in focal pulmonary histology were observed. Animals sacrificed 3 weeks following termination of exposure exhibited moderate increases in the width of alveolar septa with slight interstitial mixed inflammatory reactions. Alveolar macrophages and type II pneumocytes were increased in number. Peribronchial lymphoid tissue appeared slightly more prominent in exposed animals. Liver, kidney and spleen from DIDP treated animals exhibited no obvious histologic alteration except for slight hepatic fatty metamorphosis.

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

DIDP has been used as read across as it is a structure related material to DINP. The exposure regimen did not result in lethality when animals were observed for up to 3 weeks following treatment. Pulmonary irritation, however was apparent.

Executive summary

In a 2-week study (General Motors Research Laboratories, 1981) designed to evaluate the fate of DIDP (see Section 5.1), toxicity was also assessed. DIDP was administered to 8 male rats (6 for control) by inhalation (aerosol) at analytical concentration of $505 \pm 7 \text{ mg/m}^3$ (MMAD: $0.98 \mu\text{m}$) 6 hours a day, 5 times per week. Rats were observed daily for body weight gain, appearance and gross behavior. Animals were sacrificed at the end of the observation period (3 weeks) and tissue samples taken for histopathology. There were no marked outward signs of toxicity during exposure. The rate of body weight gain was not different between control and exposed animals. Effects in the lungs were: moderate increase in the width of alveolar septa with slight interstitial mixed inflammatory reactions, alveolar macrophages and type II pneumocytes were increased in number, peribronchial lymphoid tissue appeared slightly more prominent. In liver, spleen and kidneys, no obvious histologic alterations were noted except for a slight hepatic fatty metamorphosis. No systemic toxicity, but local irritant effects were observed at the concentration tested, thus a NOAEL of 0.5 mg/l (500 mg/m^3) can be assumed.

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 **Dissemination Dossier**

 **Genetic in vitro Endpoint.001**

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	comparable to guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1986							1986-12-10

Materials and methods

Type of genotoxicity

gene mutation

Type of study

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

Principles of method if other than guideline

Method: Ames, B.N. et al.: Mutation Research 31, 347-364

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

no

Details on test material

Palatinol N

Method

Species/strain

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
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Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation	with and without
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Metabolic activation system

Test concentrations

20, 100, 500, 2500, 5000 µg/plate

Controls

Negative controls

Solvent / vehicle controls	yes DMSO
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True negative controls

Positive controls	yes
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Positive control substance	other: 2-aminoanthracene, N-methyl-N'-nitro-N-nitroso-guanidine, 4-nitro-o-phenylenediamine, 9-aminoacridine chloride monohydrate
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Remarks

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	
Vehicle controls valid	yes
Negative controls valid	
Positive controls valid	yes

Remarks on results including tables and figures

standard plate test with and without metabolic activation (Aroclor induced rat liver S-9); no bacteriotoxicity was observed

Mutagenicity: An increase in the number of his revertants was not observed either without S-9 mix or after the addition of a metabolizing system. According to the results of the present study, the test substance Palatinol N is not mutagenic in the Ames test under the experimental conditions chosen here.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934.showdocument61a5.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-2abf2beb-8ac4-45f5-bca4-9ac72ff0cb34%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 **Dissemination Dossier**

 **Genetic in vitro Endpoint.002**

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	10.01.1996-13.02. 1996
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered to be equivalent to international test guidelines for a chromosome aberration study		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1996							1996-03-08
publication	McKee RH, Przygoda RT, Chirdon MA, Engelhardt G, Stanley M.	2000	Di(isononyl) phthalate (DINP) and di(isodecyl) phthalate (DIDP) are not mutagenic.	J Appl Toxicol. 2000 Nov-Dec;20(6):491-7.					

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate - Substance type: Technical material - Physical state: Colourless liquid -Stability under test conditions: Room temperature

Method

Target gene

Not applicable

Species/strain

Species/strain	Chinese hamster Ovary (CHO)
Details on mammalian cell lines (if applicable)	WBL clone, supplied by Merck Research Laboratories, West Point , Pennsylvania, USA. Received 5 June 1992
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	Rat

Test concentrations

0 (vehicle control), 5, 10, 20, 40, 80 and 160 micrograms/ml. The treatment groups and test concentrations are tabulated in the 'overall remarks and attachments' window below.

Vehicle

- Vehicle(s)/solvent(s) used: Acetone - Justification for choice of solvent/vehicle: Solubility

Controls

Negative controls	no
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	7,12-dimethylbenzanthracene and, N-Methyl-N-Nitro-N-Nitrosoguanidine
Remarks	None

Details on test system and conditions

METHOD OF APPLICATION: in medium; in agar (plate incorporation); preincubation; in suspension; as impregnation on paper disk DURATION - Preincubation period: None - Exposure duration: 3 or 20 hours - Expression time (cells in growth medium): 20 or 44 hours - Fixation time (start of exposure up to fixation or harvest of cells): 20 or 44 hours SPINDLE INHIBITOR (cytogenetic assays): Yes (Colcemid) STAIN (for cytogenetic assays): Yes (5% Giemsa) NUMBER OF REPLICATIONS: 2 NUMBER OF CELLS EVALUATED: 200 per per treatment group DETERMINATION OF CYTOTOXICITY: - Method: mitotic index; number of mitotic cells per 1000 total cell count OTHER EXAMINATIONS: - Determination of polyploidy: Included in aberration totals. If marked increases had been noted the frequency would have been calculated based on 500 metaphases in the particular flask - Determination of endoreplication: Not included in aberration totals. If marked increase had been noted the frequency would have been calculated based on 500 metaphases in the particular flask.

Evaluation criteria

See 'overall remarks and attachments' window below

Statistics

See 'overall remarks and attachments' window below

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	yes
Vehicle controls valid	yes
Negative controls valid	not applicable
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS - Effects of pH: No data - Effects of osmolality: No data - Evaporation from medium: No data (unlikely as substance has low vapor pressure and a Log P o/w relatively high) - Water solubility: Doses were significantly higher than the estimated water solubility of DINP (2 µg/ml). - Precipitation: precipitation was thought to have occurred on some occasions at 40, 80 or 160 µg/ml - Other confounding effects: No data
RANGE-FINDING/SCREENING STUDIES: YES A toxicity pre-test was performed to determine appropriate doses for the main study. COMPARISON WITH HISTORICAL CONTROL DATA: No quantitative data reported.

Remarks on results including tables and figures

A tabulated summary of results is presented in the 'overall remarks and attachments' window below.

No statistically significant differences were observed between the test and vehicle control groups in the percentage of aberrant cells following treatment with the test substance at 40, 80 or 160 µg/l, either with or without metabolic activation, for the initial or repeat assays.

Although there was a statistically significant trend of the series without metabolic activation for the 20-hour harvest in the repeat assay, the response at the high dose (160 µg/ml) was not extreme and is within the normal range of the vehicle control. It was not supported by an observable dose response, and was not supported by other results at other harvests. Examination of the replicates showed that one flask at 160 µg/ml, had 6 aberrant cells out of 100 while the other flask at the same dose had only 2 aberrant cells out of 100. It appeared that the one flask (6/100) was anomalous and not representative of the other data. Based on this data analysis, there was no statistically significant relationship between dose and the number of aberrant cells.

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

The test substance did not induce chromosomal aberrations in chinese hamster ovary cells

Executive summary

Chinese hamster ovary (CHO) cells were exposed to di-isononyl phthalate (DINP) in acetone at concentrations of 5,10, 20, 40, 80 or 100 µg/ml media, for 3 hours (with and without metabolic activation) in an initial assay, or 3 hours (with metabolic activation) and 20 hours (without metabolic activation) in a repeat assay, and harvested after 20 or 44 hours incubation, respectively. DINP did not induce chromosomal aberrations in the cultured CHO cells at any of the evaluted dose levels (40, 80 and 160 µg/ml) with and without metabolic activation.

The study was considered acceptable for classification, and satisfied the requirements for mammalian cell chromosomal aberration studies.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumente6e3.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-28b3f821-4c48-46e5-ae26-40a21d6c16ca%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 **Genetic in vitro Endpoint.003**

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	4.1.1996 to 15.1.1996
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered to be equivalent to international test guidelines for a bacterial gene mutation test.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1996							1996-03-08
publication	McKee RH, Przygoda RT, Chirdon MA, Engelhardt G, Stanley M.	2000	Di(isononyl) phthalate (DINP) and diisodecyl phthalate (DIDP) are not mutagenic.	J Appl Toxicol. 2000 Nov-Dec;20(6):491-7.					

Materials and methods

Type of genotoxicity

gene mutation

Type of study

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

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	no

Principles of method if other than guideline

There is no explicit statement in the report that the study was undertaken in accordance with a specific guideline, although there is a reference cited for OECD 471, 1983.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate - Substance type: Technical material - Physical state: Colourless liquid - Stability under test conditions: No data - Storage condition of test material: Room temperature

Method

Target gene

Histidine frameshift and base pair substitution.

Species/strain

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Details on mammalian cell lines (if applicable)	Supplied by Dr. B. N. Ames, and characterised 1 - 3 February 1995 in accordance with published procedures of Maron and Ames, 1983.

Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	Rat S9 mix
Species/strain	S. typhimurium TA 1538
Details on mammalian cell lines (if applicable)	Supplied by Dr. B. N. Ames, and characterised 1 - 3 February 1995 in accordance with published procedures of Maron and Ames, 1983.
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	Rat S9 mix

Test concentrations

Initial assay: 0.5, 5, 50, 500 and 5000 micrograms/plate Repeat assay: 250, 500, 1000, 2500 and 5000 micrograms/plate

Vehicle

- Vehicle(s)/solvent(s) used: acetone (test substance) and DMSO (positive control substances) - Justification for choice of solvent/vehicle: Solubility

Controls

Negative controls	no
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	9-aminoacridine Plus: 2-Aminoanthracene, N-Methyl-N-Nitro-N-Nitrosoguanidine, 2-Nitrofluorene
Remarks	Four positive control substances were used

Details on test system and conditions

METHOD OF APPLICATION: in medium; in agar (plate incorporation) DURATION - Exposure duration: 2 days NUMBER OF REPLICATIONS: Three DETERMINATION OF CYTOTOXICITY - Method: notable reduction in background lawn or > than 50% reduction in the number of revertant colonies compared to the vehicle control.

Evaluation criteria

An individual dose was considered positive if the mean revertant count on the test plates was equal to or greater than three times the mean number of spontaneous revertants on the vehicle control plates. A positive result for the assay was defined as a dose-related increase in the mean number of revertant colonies over at least three concentrations of test substance including at least one positive dose

Statistics

The mean revertant colony count and standard deviation were determined for each dose point (Sendcor and Cochran, 1989)

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	not determined
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes
Species/strain	S. typhimurium TA 1538
Metabolic activation	with and without
Test system	strain/cell type:
Genotoxicity	negative
Cytotoxicity	not determined
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS - Effects of pH: No data - Effects of osmolality: No data - Evaporation from medium: No data - Water solubility: No data - Precipitation: Beading of the test substance observed on the plates at doses equal to or greater than 250 micrograms/plate in all tester strains , with and without metabolic activation - Other confounding effects: RANGE-FINDING/SCREENING STUDIES: Yes To determine the appropriate doses

for the main assay. Toxicity was not observed at any dose tested with or without metabolic activation. 5000 micrograms/plate was selected as the high dose for the main mutagenicity study.

Remarks on results including tables and figures

DINP did not induce significant increases in mean revertant colonies (equal to or greater than three times the acetone controls) in tester strains TA 98, TA 100, TA 1535, TA1537 or TA 1538 at any dose level tested with or without metabolic activation in either the initial or repeat assays.

Toxicity was not observed in either the initial or repeat assay. Beading of the test substance was observed in both the initial and repeat assays in all strains of bacteria at ≥ 250 micrograms/plate.

The positive control substances produced at least a three fold increase in revertant colonies when compared with the vehicle control in their respective strains.

The test assay data summaries for the initial and repeat tests are reported in the attached 'background information' window below.

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

DINP was not mutagenic in any strain of *Salmonella typhimurium* tested, which included exposure to concentrations above the solubility of the test substance (250 micrograms/plate and higher)

Executive summary

This study was conducted to evaluate the mutagenic potential of di-isononyl phthalate (DINP) in five selected strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, TA 1537 and TA 1538). Test concentrations were 0, 5, 50, 500 and 5000 $\mu\text{g}/\text{plate}$ (initial assay) or 250, 500, 1000, 2500 and 5000 $\mu\text{g}/\text{plate}$ (repeat, confirmatory assay) in acetone with or without S9 mix. Concurrent positive and vehicle controls were employed for all strains. DINP was not mutagenic in any strain of *Salmonella typhimurium*.

The study was considered acceptable for classification, and satisfied the guidelines for bacterial gene mutation studies.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentaec4.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-365998a5-ff1a-4c7c-bbaf-3803469789cf%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Genetic in vitro Endpoint.004

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for
reliability Acceptable, well documented study.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Barber ED, Cifone M, Rundell J, Przygoda R, Astill BD, Moran E, Mulholland A, Robinson E, Schneider B.	2000	Results of the L5178Y mouse lymphoma assay and the Balb/3T3 cell in vitro transformation assay for eight phthalate esters.	J Appl Toxicol. 2000 Jan- Feb;20(1):6 9-80.					

Materials and methods

Type of genotoxicity

other: L5178 mouse lymphoma mammalian cell mutation assay and the Balb/3T3 cell transformation assay

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	

Principles of method if other than guideline

L5178Y Mouse Lymphoma Assay: This mammalian cell mutagenicity assay is used to determine whether a chemical is capable of inducing a change in cultured mammalian cells (mouse lymphoma L5178Y cells) measured as acquired resistance to trifluorothymidine (the result of forward mutation at the thymidine kinase locus). In brief, cells are treated with the test chemical and then placed into suspension cultures with selective medium for replication and fixation of induced mutations. Cells are then plated for colony growth, and after several days, colony numbers and colony size are recorded. The number of mutant colonies is a measure of the ability of the test chemical to induce a genetic change at the thymidine kinase locus in these transformed cells. The mouse lymphoma L5178Y/TK +/- assay can detect both point mutations and chromosomal alterations. **Balb/3T3 Cell In vitro transformation assay:** The BALB/c 3T3 cell transformation assay is designed to allow the expression of transformed foci (IARC/NCI/EPA Working Group, 1985). In the standard assay, the cells are seeded at 1×10^4 cells/60-mm dish in at least 12 replicates and exposed to a test chemical in the culture medium for 72 h from 24 h after inoculation. On termination of exposure, the treatment medium is replaced with normal medium and the cultures are maintained for a further 4 to 6 weeks receiving medium change twice a week. In these conditions, the cells grow to sub-confluence in a week and attain confluence in 8 days. During this growing phase, carcinogen-treated cells can divide several times and some DNA damage caused by the carcinogen can be fixed as mutations. After confluence the normal cells move to a stationary phase but the transformed cells continue multiplying to form foci.

GLP compliance

no data

Test materials**Test material equivalent to submission substance identity**

no

Details on test material

- Name of test material (as cited in study report): DINP

Method

Target gene

L5178Y Mouse Lymphoma Assay - forward mutation at the thymidine kinase locus Balb/3T3
Transformation Assay - foci formation

Species/strain

Species/strain	mouse lymphoma L5178Y cells
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Details on mammalian cell lines (if applicable)	
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Additional strain characteristics	
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Metabolic activation	with and without
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Metabolic activation system	S9 rat liver fraction
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Species/strain	mammalian cell line, other: Balb/3T3
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Details on mammalian cell lines (if applicable)	
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Additional strain characteristics	
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Metabolic activation	not applicable
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Metabolic activation system	
-----------------------------	--

Test concentrations

L5178Y Mouse Lymphoma Assay - 1.5, 3, 4, 5, 6, 8 ul/ml media Balb/3T3 Transformation
Assay - 0.125, 0.398, 1.250, 2.50, 3.750 ul/ml media

Vehicle

unchanged

Results and discussions

Test results

Species/strain	mouse lymphoma L5178Y cells
----------------	-----------------------------

Metabolic activation	not applicable
----------------------	----------------

Test system	
Genotoxicity	negative
Cytotoxicity	yes
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes
Species/strain	mammalian cell line, other: Balb/3T3
Metabolic activation	
Test system	
Genotoxicity	negative
Cytotoxicity	
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Applicant's summary and conclusion

Interpretation of results

negative

Executive summary

Eight phthalate esters, with alcohol chain lengths of 1-11 carbon atoms and with various degrees of branching, were tested in vitro in the L5178Y mouse lymphoma mammalian cell mutation assay and in the Balb/3T3 cell transformation assay. The tests were performed as part of a voluntary testing agreement between the Chemical Manufacturers Association's Phthalate Esters Panel and the United States Environmental Protection Agency (US EPA). The esters tested were: dimethyl phthalate (DMP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), di-n-hexyl, n-octyl, n-decyl phthalate (610P), di-isononyl phthalate (DINP), di-heptyl, nonyl, undecyl phthalate (711P), di-isodecyl phthalate (DIDP) and di-undecyl phthalate (DUP). Both DMP and DBP were found to produce significant increases in the mutant frequency in the mouse lymphoma assay in the presence but not in the absence of an Aroclor-induced rat liver activation system (S-9). Ester 610P gave equivocal results in the mouse lymphoma assay in the presence and absence of rat liver S-9. There was no indication of mutagenic potential for any of the other test materials in the mouse lymphoma assay, and none of the test materials increased transformation frequency in the Balb/3T3 cell transformation assay. Aldehyde metabolites of the de-esterified alcohols are postulated to play a role in the positive results for DMP and DBP.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument63be.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-ac3d9c12-f0de-4c74-a9cb-13f7422d5975%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Genetic in vitro Endpoint.005

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	OECD guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1995-04-13

Materials and methods

Type of genotoxicity

gene mutation

Type of study

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

Test guideline

Qualifier	Guideline	Deviations
according	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	

substance phenylendiamine, 9-aminoacridine chloride monohydrate
Remarks

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	
Vehicle controls valid	yes
Negative controls valid	
Positive controls valid	yes

Remarks on results including tables and figures

Toxicity : No bacteriotoxic effect was observed. Mutagenicity: An increase in the number of his revertants was not observed both in the standard plate test and in the preincubation test either without S-9 mix or after the addition of a metabolizing system. Assessment: According to the results of the present study, the test substance Diisononylphthalat IGS 21002 is not mutagenic in the Ames test under the experimental conditions chosen here.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument5ac4.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-f912f691-923f-480e-b083-09ddcafd52%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Genetic in vivo Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	4 (not assignable)
Rationale for reliability	Secondary literature

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	European Commission	2003	1,2-benzenedicarboxylic acid, di-C8-10- branched alkyl esters, C9-rich and di-“isononyl” phthalate (DINP)	Risk Assessment Report Vol. 35 (EUR 20784 EN)					

Materials and methods

Test materials

Test material equivalent to submission substance identity

no

Results and discussions

Test results

Sex

Genotoxicity negative

Toxicity no data

Vehicle controls valid no data

Negative controls valid no data

Positive controls valid no data

Applicant's summary and conclusion

Executive summary

In an in vivo cytogenetic assay, DINP (R-1218, CAS 28553-12-0) was administered orally to 3 groups of Fisher 344 rat at doses of 5-1.7-0.5 ml/kg/d, diluted in corn oil, during 5 days (Microbiological Associates, 1981d). Samples of femoral bone marrow were analysed for chromosomal aberrations after the treatment period. Concurrent olive oil and triethylenemelamine dosed animals served as negative and positive controls, respectively. In a preliminary range finding study, no toxic effects were detected at levels up to 10 ml/kg DINP. There were no changes in the mitotic index at any dose tested. No changes were observed (only one animal had chromosomal abnormality at dose of 1.7 mg/kg/d (chromatid breaks) and the test was considered to be negative. However, cytogenetic analysis has been performed on only 50 cells/animal and mitotic index calculated by observation of 100 cells only, such values are low and can weaken the significance of the test.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument4c32.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-9071d190-3b02-4950-a23f-e5a17bb2cd1d%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Carcinogenicity Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study with GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1998							1998-01-29

Materials and methods

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OTS 798.3300 (Carcinogenicity)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear, colorless liquid - Analytical purity: 100% (first shipment), >99% (second shipment) - Lot/batch No.: QCL 9004-273 (both shipments)

Test animals

Species

mouse

Strain

B6C3F1

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Portage, Michigan - Age at study initiation: 6 weeks - Weight at study initiation: males 12.0-22.8 g and females 12.5-20.3 g - Housing: single - Diet (e.g. ad libitum): Purina Certified Rodent Chow 5002 - Water (e.g. ad libitum): tap water - Acclimation period: 2 weeks ENVIRONMENTAL CONDITIONS - Temperature (°C): 18-26 - Humidity (%): 55±15 - Air changes (per hr): 10 or more - Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration

oral: feed

Details on exposure

PREPARATION OF DOSING SOLUTIONS: For each dietary level, the test material was weighed on an appropriate balance and was placed into a pocket that was made in approximately

200 g of feed contained in a glass beaker. The test material/feed was mixed in a Waring blender for approximately 2 to 3 minutes to ensure an apparent homogeneous mixture. Based on dietary level, multiple premixes were required (approximately 30 g test material /premix). The premixes were added to approximately 5 kg of additional feed, mixed in a Hobart mixer for 15 minutes, then transferred to a Patterson-Kelley twin-shell mixer (fitted with an intensifier bar) that contained the remaining amount of basal feed. The diets were mixed for 1 minute/kg feed. DIET PREPARATION - Rate of preparation of diet (frequency): once a week - Storage temperature of food: refrigerated (2-8°C)

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Results of routine concentration analyses indicated that the dietary concentrations were within an acceptable range (92 to 112%) of target concentrations.

Duration of treatment / exposure

104 weeks for the test groups and 78 weeks for the recovery group (high dose level)

Frequency of treatment

7 days per week

Post exposure period

26 weeks

Doses / concentrations

ca. 90.3, 275.6, 741.8, 1560.2 mg/kg bw/d in males and ca. 112.0, 335.6, 910.3, 1,887.6 mg/kg bw/d in females

Basis actual ingested

500, 1500, 4000, 8000 ppm

Basis nominal in diet

No. of animals per sex per dose

70 for the test groups and 55 for recovery group (high dose level)

Control animals

yes, concurrent no treatment

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: twice daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: twice daily. In addition, detailed clinical observations by physical examination were performed once each week. Special attention was paid to mass development. The following information on each grossly visible or palpable mass was recorded: time of onset, location, size (small < 1 cm, large > 1 cm), appearance, progression. BODY WEIGHT: Yes - Time schedule for examinations: prior to treatment, and weekly for Weeks 1-17 and once every 4 weeks thereafter. An additional weight was obtained from all groups on the first day of Week 79. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): - Food consumption for each animal determined: Yes, weekly for Weeks 1-16 and once every 4 weeks thereafter. Food consumption was also determined at Week 78. When obvious spillage or wastage of food was recorded for an animal during the detailed physical examination, the estimate of the food consumed by the animal was excluded from the group mean calculation for that particular interval. HAEMATOLOGY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: absolute reticulocyte count, mean cell hemoglobin, corrected leukocyte count, mean cell hemoglobin concentration, erythrocyte count, mean cell volume, hematocrit, platelet count, hemoglobin, reticulocyte count, leukocyte differential and cellular morphology, myeloid/erythroid ratios (at necropsy), leukocyte count. CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, creatinine, urea nitrogen, total bilirubin, sodium, chloride, calcium, potassium, inorganic phosphorus, glucose, total protein, albumin, globulin. URINALYSIS: Yes - Time schedule for collection of urine: During Weeks 26, 52, 78, and 104 - Metabolism cages used for collection of urine: Yes, urine collection racks - Animals fasted: Yes - Parameters checked: total volume, calcium, chloride, creatinine, creatinine clearance, osmolality, phosphorus, potassium, sodium, appearance/color, specific gravity (calculated), protein, glucose, ketones, bilirubin, occult blood, microscopic sediment, pH, turbidity, urobilinogen

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes All animals found dead or sacrificed in extremis (using sodium pentobarbital and exsanguination) during the study were subjected to a gross postmortem examination. Fifteen mice/sex/group in the control, and all test groups were sacrificed after 78 weeks of compound administration. Following at least 104 weeks of compound administration, the surviving mice were necropsied. At the interim and terminal sacrifices, the following organs (when present) were weighed: brain with stem, spleen, kidneys (paired weight), liver/gallbladder (drained), testes with epididymides (paired weight), lung,

uterus. Five mice/sex/group in all test groups following 78 weeks and five mice/sex/group in all test groups and recovery group following 104 weeks were designated for hepatocellular proliferation studies. Before sacrifice, these animals were labelled with BrdUrd (20 mg/ml) for 72 hrs using ALZET osmotic pump, Model No. 1003D. Tissues from liver (standard tissue samples from left and right median, and right anterior lobes) and duodenum from each animal designated for hepatocellular proliferation studies and were preserved in 10% neutral-buffered formalin. The remainder of the liver was immediately frozen at approximately -70°C for biochemical analysis.

Other examinations

Hepatocellular Proliferation and Biochemical Analyses: The frozen tissue samples from the three liver lobes (right and left lateral, and median) were evaluated for hepatocellular proliferation and analysis of protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, and DNA concentration from animals designated for hepatocellular proliferation studies during Weeks 79 and 105/106 (i.e. after 78 and 104 weeks of treatment, respectively).

Statistics

Statistical analyses of labeling index, palmitoyl-CoA oxidase activity, DNA and protein were performed using analysis of variance techniques for each sacrifice interval. Comparisons of the control group vs. the treated groups were performed using Dunnett's t-Test. Weekly/monthly body weight, body weight change, and food consumption, mean total body weight change (Weeks 1-78, 78-104, and 1-104) and total food consumption (Weeks 1-78, 78-104, and 1-104), clinical pathology data (except hemolysis and cellular morphology gradings and routine urinalysis data), fasted terminal body weights, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups.

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Sex	Basis for effect level / Remarks
NOAEL	carcinogenicity	ca. 275.6 mg/kg bw/day (actual dose received)	male	hepatocellular adenomas and carcinomas (nominal dose: 1500ppm)
NOAEL	carcinogenicity	ca. 112 mg/kg bw/day (actual dose received)	female	hepatocellular adenomas and carcinomas (nominal dose: 500ppm)

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: For Weeks 1-104, the adjusted mean survival rate was significantly decreased for the males of the high dose group compared to concurrent control

value. Clinical observations noted during the course of the study in which there was a treatment-related increased incidence were signs of poor health and/or antemortem condition (hunched posture - highest incidence in the 8000 ppm dose group animals, hypoactivity - highest incidence in the 8000 ppm dose group males and the 4000 ppm dose group females, few feces - highest incidence in the 8000 ppm dose group females, and urine stains - predominantly in the 8000 ppm dose group males) and swelling in the ventral -abdominal region, in which the notably increased incidence in the males of the 4000, 8000 ppm and recovery group, and in the females of the 8000 ppm and recovery group. **BODY WEIGHT AND WEIGHT GAIN:** Significantly decreased body weight means occurred most frequently in the males and females of the 4000, 8000 ppm and recovery group. In the male mice, significantly lower (than control values) body weight means were noted for the 500 ppm group at Weeks 14, 15, 45, 57, 69, and 81; the 1500 ppm group at Weeks 69 and 93; the 4000 ppm group at Weeks 29-105; the 8000 ppm group at Weeks 29-105; and the recovery group at Weeks 5-7, 10-11, and 15-105. In the female mice, significant decreases in mean body weight were noted for the 500 ppm group at Weeks 8, 21, and 25; the 1500 ppm group at Weeks 8, 25, 37, 49, 61, and 69-101; the 4000 ppm group at Weeks 29, 37, and 45-105; the 8000 ppm group at 16 and 37-105; and the recovery group at Weeks 29 and 37-105. The overall mean total body weight change values for Weeks 1-104 were statistically decreased for the animals of 4000 and 8000 ppm dose groups, and the recovery group males compared to that of the concurrent control group. **FOOD CONSUMPTION AND COMPOUND INTAKE** (if feeding study): Total mean food consumption values (Weeks 1-78, 78-104, and 1-104) were generally comparable between groups, with the exception of the significantly increased values noted at Weeks 78-104 for the males of the 4000 and 8000 ppm dose groups. For Weeks 1-104, the average daily compound consumption (mg/kg bw/d) for the 500, 1500, 4000, 8000 ppm dietary levels was 90.3, 275.6, 741.8, 1560.2 mg/kg bw/d in the males and 112.0, 335.6, 910.3, 1887.6 mg/kg bw/d in the females, respectively. For Weeks 1-78 in the recovery group, the average daily compound consumption was 1377.4 mg/kg bw/d in the males and 1581.0 mg/kg bw/d in the females. **HAEMATOLOGY:** The lower leukocyte, lymphocyte, and/or segmented neutrophil counts were observed in animals of the 8000 ppm and recovery group (often significantly). **CLINICAL CHEMISTRY:** The higher (frequently significantly) total protein, albumin, and globulin was observed in the males of the 8000 ppm and recovery group. Higher (frequently significantly) aspartate aminotransferase and alanine aminotransferase was observed in the males of the 8000 ppm dose group and in the males and females of the recovery group. **URINALYSIS:** Significant higher urine volume and lower urine osmolality (with significant lower concentrations of sodium, potassium, and chloride) were observed in the animals of 8000 ppm and recovery groups. **ORGAN WEIGHTS:** At the interim sacrifice, mean absolute kidney weights were significantly decreased in the males of the 1500, 4000 and 8000 ppm dose groups. Absolute and relative liver weight means were increased in the males and females of the 4000 ppm dose group (liver weight relative to body weight was significantly increased in the males of the 4000 ppm dose group) and significantly increased in the males and females of the 8000 ppm dose group. At terminal sacrifice, treatment-related effects were again evident in the kidney (males) and liver (males and females) weights. For kidney in the males of the 1500, 4000, 8000 ppm and recovery group, absolute and relative to brain weight means were significantly decreased; relative to body weight means were also decreased (significantly only in 4000 ppm group). For liver in males of the 4000, 8000 ppm and recovery group, mean absolute and relative weights were significantly increased. For liver in females of 4000, 8000 ppm and recovery group, mean absolute and relative weights were increased, but the differences were not

significant compared to the control female values. GROSS PATHOLOGY: At the Week 79 sacrifice, most notable was an increased incidence of the following necropsy findings: liver-mass in the males of the 1500, 4000, 8000 ppm dose groups, and in the females of the 8000 ppm dose group; enlarged liver in the 8000 ppm dose group females; and distended urinary bladder in the 8000 ppm dose group males. At necropsy for study termination, the most frequently observed gross findings were lung-mass (primarily in male mice, all groups); liver-mass, which occurred in greatest numbers in mice of the 4000, 8000 ppm and recovery group; enlarged spleen (predominantly in the females, all groups); and granular pitted/rough kidneys in 8000 ppm dose group females. Distended urinary bladder was noted most frequently in the males of the 4000 and 8000 ppm dose groups. HISTOPATHOLOGY: NON-NEOPLASTIC: At the interim and terminal sacrifice, microscopic evaluation revealed test article-related changes in the liver of mice of both sexes and in the kidneys of female mice. The liver changes in the 8000 ppm group were consisted of increased cytoplasmic eosinophilia, diffuse hepatocellular enlargement, and pigment. In the kidneys of the females of the 8000 ppm group, the incidence of chronic progressive nephropathy was increased. No correlation of increased kidney weights in males was observed. HISTOPATHOLOGY: NEOPLASTIC: An increased incidence of hepatocellular neoplasia was present in males and females of the 8000 ppm group in addition to non-neoplastic changes. In mice of the recovery group following the recovery period, the nonneoplastic changes were not present, although the incidence of hepatocellular neoplasia was increased in female mice (37%, 13/35) compared to that of the control (7%, 3/42) and lower dose groups. The total incidence of hepatocellular neoplasia in males of 4000 ppm group (47%, 28/60) was also greater than that observed in the control males (23%, 16/70). Statistical analyses indicated there were no significant increases in the total incidence (all deaths combined) of hepatocellular adenoma in any of the treated male groups versus the control males; and only the incidences of 18/70 and 8/50 in the females of the 4000 and 8000 ppm groups, respectively, were significantly increased compared to the control females. For hepatocellular carcinoma, only the incidences of 17/60 and 20/70 in the males of the 4000 and 8000 ppm groups, respectively, and of 7/60, 18/70, and 13/50 in the females of the 4000, 8000 ppm and recovery groups, respectively, were significantly increased compared to the respective same sex of the controls. In the females of the 1500 ppm group, although the incidences of hepatocellular adenoma or carcinoma were not statistically different compared to the incidences in controls, the incidence (10/60) for hepatocellular adenoma/carcinoma combined was significantly greater than the combined incidence (3/70) in the controls. Corresponding to the statistically significant increases of liver neoplasms in the mid-high and high dose groups, significant positive trends were indicated for hepatocellular carcinomas and hepatocellular adenoma/carcinoma (combined) in males, and for hepatocellular adenomas and carcinomas, as well as hepatocellular adenoma/carcinoma (combined) in females. OTHER FINDINGS: Cell proliferation: After 78 and 104 weeks of DINP administration, there was no evidence of sustained cell proliferation in the livers of male or female mice from the 8000 ppm group compared to livers from the control group. DINP administration did not induce significant increases in the mean labeling index for livers of the 8000 ppm mice compared to controls. Biochemical analyses: Increases in palmitoyl CoA oxidase, a monitor of the level of peroxisome proliferation were observed after 78 and 104 weeks in the livers of mice from 8000 ppm group. Analyses of liver DNA concentration did not detect any biologically relevant differences in mean values between the control and the 8000 ppm group after 78 or 104 weeks of DINP administration. Conversely, liver mean protein concentration was significantly increased in the mice of the 8000 ppm group compared to controls at both analysis intervals.

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

Essentially, this study identified the same target organ (liver) as in the rat studies, however, at a higher NOAEL and LOAEL. The mouse is less sensitive than the rat. Therefore, for risk characterization, the resulting NOAEL of the rat studies i.e. 88 mg/kg bw/day is used.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument081e.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-cf78e136-4c87-471a-bfa6-947a30fa8999%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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[Administrative Data](#) [Data source](#) [Materials and methods](#)

[Results and discussions](#)

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability	Acceptable, well documented study report which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1999							1999-08-24

Materials and methods

Limit test

no

Principles of method if other than guideline

A Histopathology Peer Review and a Pathology Working Group (PWG) Review were conducted in accordance with EPA Pesticide Regulation (PR) Notice 94-5, August 24, 1994 which sets forth a procedure to be followed for submission of pathology re-reads to the Agency. The peer review and PWG review were conducted of selected lesions of the liver and spleen observed in two chronic studies with a commercial plasticizer, Di(isononyl)phthalate (DINP), in F344 rats. Specifically, the PWG review evaluated the significance of spongiosis hepatitis, foci of cellular

alteration, and primary hepatocellular neoplasms in the liver, and the significance of mononuclear cell leukemia. The purpose of this review is to have a panel of experts examine the selected lesions in the liver and spleen, provide a consensus diagnosis for each lesion examined, and to discuss the toxicologic and biologic significance of the findings. The first of the two studies was conducted for the Exxon Corporation (Exxon Study Number 326075; MRD-83-260) at Covance Laboratories as Study Number HLA 145-605. The second study was conducted for the Aristech Chemical Corporation at Covance Laboratories (Study Number Covance 2598-104).

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear, colorless liquid - Analytical purity: 99%

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Administration / exposure

Route of administration

oral: feed

Analytical verification of doses or concentrations

yes

Duration of treatment / exposure

105 weeks for HLA Study (No. 145-605); 104 weeks for Covance Study (No. 2598-104) including 78 weeks in recovery group.

Frequency of treatment

7 days per week

Post exposure period

26 weeks in the recovery group of the Covance Study.

Doses / concentrations

300, 3000 and 6000 ppm for HLA Study

Basis nominal in diet

ca. 29.2, 88.3, 358.7, and 733.2 mg/kg bw/d for males and ca. 36.4, 108.6, 442.2, and 885.4 mg/kg bw/d for females for HLA Study

Basis actual ingested

500, 1500, 6000, 12000 ppm for Covance Study

Basis nominal in diet

15, 152, and 307 mg/kg/day for males and 18 , 184, and 375 mg/kg/day for females for Covance Study

Basis actual ingested

No. of animals per sex per dose

110 for HLA Study, 70-85 for Covance Study

Control animals

yes, concurrent no treatment

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Sex	Basis for effect level / Remarks
NOAEL	carcinogenicity	1500 ppm (nominal)	male	hepatocellular carcinoma and mononuclear cell leukemia
NOAEL	carcinogenicity	3000 ppm	female	hepatocellular carcinoma and mononuclear

(nominal)

cell leukemia

Observations

Details on results

HISTOPATHOLOGY: NEOPLASTIC: The results of the Histopathology Peer Review and PWG Review of the liver and spleen generally confirmed the data originally reported by the Study Pathologist. A few differences were noted between the pathology protocols for the two studies concerning the preparation of tissue sections and the tissues which were examined from individual groups. A slight increase in the incidence of mononuclear cell leukemia was observed in male rats in groups given 3000 ppm and greater of DINP and in female rats in groups given 6000 ppm and greater of DINP in the diet as compared to lower dose groups and the controls. The incidence of mononuclear cell leukemia in groups given 1500 ppm and less of DINP and in female rats given 3000 ppm and less of DINP in the diet was similar to the incidence observed in the control groups. In the studies being reviewed, the incidence of mononuclear cell leukemia in most groups is similar to historical values (see free text). Mononuclear cell leukemia increases in incidence with increasing age in F344 rats. In the HLA study, the incidence of mononuclear cell leukemia at the 79-week interim sacrifice and terminal sacrifice ranged from 36.2-58.9% in male rats and from 23.3-50% in female rats. In the Covance study, the incidence of mononuclear cell leukemia at the 79-week interim sacrifice and terminal sacrifice ranged from 33.8-49.2% in male rats and from 18-48% in female rats. Spongiosis hepatitis was increased in incidence in the liver of male rats given 3000 ppm and greater of DINP in the diet as compared to lower dose groups and the controls. The incidence of spongiosis hepatitis in male groups given 1500 ppm and less of DINP in the diet was similar to the incidence observed in the control groups. The incidence of spongiosis hepatitis was similar in all control and treated groups of female rats. Spongiosis hepatitis, often diagnosed as cystic degeneration, is a degenerative change involving the perisinusoidal cells of the liver. Spongiosis hepatitis is a change in the liver that is observed spontaneously with increasing age in F344 rats. Spongiosis hepatitis may be found as an independent lesion in the liver parenchyma or within foci of cellular alteration or hepatocellular neoplasms. In these studies, the Study Pathologist diagnosed spongiosis hepatitis when it occurred regardless of its relation to other hepatic changes which were also present. This makes the interpretation of the significance of spongiosis hepatitis as a possible treatment-related effect difficult (In the Guides for Toxicologic Pathology, the Society of Toxicologic Pathologists recommends that spongiosis hepatitis, when present within foci or tumors, should not be diagnosed separately). In the HLA study, the incidence of foci of cellular alteration and hepatocellular neoplasms was similar in control and treated male and female rats given up to 6000 ppm DINP in the diet. In the Covance study, a slight increase in the incidence of hepatocellular neoplasms was observed in male rats given 6000 and 12000 ppm DINP in the diet and female rats given 12000 ppm DINP in the diet for 104 weeks. In Group given 12000 ppm DINP in the diet for 78 weeks then given only control diet for the remainder of the study, the incidence of hepatocellular neoplasms was similar to control values. The incidence of foci of cellular alteration in the liver in the Covance study was decreased in all treated groups relative to the controls. The slight increase in the incidence of hepatocellular neoplasms at extremely high dietary levels of DINP may be a secondary effect to the known peroxisome proliferation produced by the compound.

Remarks on results including tables and figures

Historical control values (incidence of mononuclear cell leukemia) published by the National Toxicology Program* are summarized below:

Male Rats	Female Rats	
No. Examined	(1936)	(1983)
Range	10-72%	6-31%
Mean	33.6%	20.2%

* Haseman, JK, Eustis, SL, and Arnold, J. (1990). Tumor incidences in Fischer 344 rats: NTP historical data. In: Pathology of the Fischer Rat (Eds.) Boorman, GA, Eustis, SL, Elwell, MR, Montgomery, CA, and Mackenzie, WF, Academic Press, Inc., San Diego, California, pp. 555-564.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument3058.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-8be9a15a-af5c-4b0c-8faf-c3cd65f023b7%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > *Document*

 Dissemination Dossier

 Carcinogenicity Endpoint.003

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	key study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	1 (reliable without restriction)
Rationale for reliability	Guideline study with GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1998							1998-05-13

Materials and methods

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OTS 798.3300 (Carcinogenicity)	no

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear, colorless liquid - Analytical purity: >99% - Lot/batch No.: QCL 9004-273

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Portage, Michigan - Age at study initiation: 6 weeks - Weight at study initiation: males 96-134 g and females 82-109 g - Housing: single - Diet (e.g. ad libitum): Purina Certified Rodent Chow 5002 - Water (e.g. ad libitum): tap water - Acclimation period: 2 weeks ENVIRONMENTAL CONDITIONS - Temperature (°C): 22 - Humidity (%): 50±20 - Air changes (per hr): 10 or more - Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration

oral: feed

Details on exposure

PREPARATION OF DOSING SOLUTIONS: For each dietary level, the test material was weighed on an appropriate balance and was placed into a pocket that was made in approximately 200 g of feed contained in a glass beaker. The test material/feed was mixed in a Waring blender

for approximately 2 to 3 minutes to ensure an apparent homogeneous mixture. Based on dietary level, multiple premixes were required (approximately 30 g test material /premix). The premixes were added to approximately 5 kg of additional feed, mixed in a Hobart mixer for 15 minutes, then transferred to a Patterson-Kelley twin-shell mixer (fitted with an intensifier bar) that contained the remaining amount of basal feed. The diets were mixed for 1 minute/kg feed. DIET PREPARATION - Rate of preparation of diet (frequency): once a week - Storage temperature of food: refrigerated (2-8°C)

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Results of routine concentration analyses indicated that all formulations were within 15% of target.

Duration of treatment / exposure

104 weeks for the test groups and 78 weeks for the recovery group (high dose level)

Frequency of treatment

7 days per week

Post exposure period

26 weeks

Doses / concentrations

ca. 29.2, 88.3, 358.7, and 733.2 mg/kg bw/d for males and ca. 36.4, 108.6, 442.2, and 885.4 mg/kg bw/d for females

Basis actual ingested

500, 1500, 6000, 12000 ppm

Basis nominal in diet

No. of animals per sex per dose

500 and 1500 ppm dose group: 70 males and 70 females 0, 6000 and 12000 ppm dose group: 85 males and 85 females Recovery (12000 ppm) group: 55 males and 55 females

Control animals

yes, concurrent no treatment

Positive control

[4-Chloro-6-(2,3-xylidino)- 2-pyrimidinylthio] acetic acid was used as positive control for Hepatocellular Proliferation and Biochemical Analyses. Fifteen males were administered the positive control substance for 13 weeks at dietary level of 1000 ppm.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: twice daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: twice daily. In addition, detailed clinical observations by physical examination were performed once each week. Special attention was paid to mass development. The following information on each grossly visible or palpable mass was recorded: time of onset, location, size, appearance, progression. BODY WEIGHT: Yes - Time schedule for examinations: prior to treatment, and weekly for Weeks 1-17 and once every 4 weeks thereafter. An additional weight was obtained from all groups on the first day of Week 79. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): - Food consumption for each animal determined: Yes, weekly for Weeks 1-16 and once every 4 weeks thereafter. Food consumption was also determined at Week 78. When obvious spillage or wastage of food was recorded, the estimate of the food consumed by the animal was excluded from the group mean calculation for that particular interval. HAEMATOLOGY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: absolute reticulocyte count, mean cell hemoglobin, corrected leukocyte count, mean cell hemoglobin concentration, erythrocyte count mean cell volume, hematocrit, platelet count, hemoglobin, reticulocyte count, leukocyte differential and cellular morphology, myeloid/erythroid ratios (at necropsy), leukocyte count. CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: During Weeks 26, 52, 78, and 104 - Animals fasted: Yes - How many animals: 10 animals/sex/group - Parameters checked: aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, creatinine, urea nitrogen, total bilirubin, sodium, chloride, calcium, potassium, inorganic phosphorus, glucose, total protein, albumin, globulin. URINALYSIS: Yes - Time schedule for collection of urine: During Weeks 26, 52, 78, and 104 - Metabolism cages used for collection of urine: Yes, urine collection racks - Animals fasted: Yes - Parameters checked: total volume, calcium, chloride, creatinine, creatinine clearance, osmolality, phosphorus, potassium, sodium, appearance/color, specific gravity (calculated), protein, glucose, ketones, bilirubin, Urinalysis, occult blood, microscopic sediment, pH, turbidity, urobilinogen

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes All animals found dead or sacrificed in

extremis (using sodium pentobarbital and exsanguination) during the study were subjected to a gross postmortem examination. Fifteen mice/sex/group in the control, and all test groups were sacrificed after 78 weeks of compound administration. Following at least 104 weeks of compound administration, the surviving mice were necropsied. At the interim and terminal sacrifices, the following organs (when present) were weighed: brain with stem, spleen, kidneys (paired weight), liver/gallbladder (drained), testes with epididymides (paired weight), lung, uterus. Five mice/sex/group in all test groups were designated for hepatocellular proliferation studies following 78 weeks and five mice/sex/group in all test groups and recovery group were designated for hepatocellular proliferation studies following 104 weeks. Before sacrifice, these animals were labelled with BrdUrd (20 mg/ml) for 72 hrs using ALZET osmotic pump. Tissues from liver (standard tissue samples from left and right median, and right anterior lobes) and duodenum from each animal designated for hepatocellular proliferation studies and were preserved in 10% neutral-buffered formalin. The remainder of the liver was immediately frozen at approximately -70°C for biochemical analysis.

Other examinations

Hepatocellular Proliferation and Biochemical Analyses: The frozen tissue samples from the three liver lobes (right and left lateral, and median), and the duodenum were evaluated for hepatocellular proliferation and analysis of protein concentration, cyanide-insensitive palmitoyl-CoA oxidation, and DNA concentration from five animals/sex from control, 12000 ppm and recovery group sacrificed during Weeks 1, 2, and 13, and from five animals/sex from control, 6000 and 12000 ppm groups sacrificed after 104 weeks of treatment.

Statistics

Trend analysis of survival was evaluated at the 5.0% one-tailed probability level. Weekly body weights and food consumption and the following intervals for mean body weight change (Weeks 1-53 and 1-105) and total food consumption (Weeks 1-52 and 1-104), clinical pathology data (except hemolysis and cellular morphology gradings and routine urinalysis data), fasted terminal body weights, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups.

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Sex	Basis for effect level / Remarks
NOAEL	toxicity	88.3 mg/kg bw/day (actual dose received)	male	liver and kidney toxicity
NOAEL	toxicity	108.6 mg/kg bw/day (actual dose received)	female	liver and kidney toxicity
NOAEL	carcinogenicity	88.3 mg/kg bw/day (actual dose received)	male	mononuclear cell leukemia (nominal dose: 1500ppm)

NOAEL carcinogenicity 108.6 mg/kg bw/day (actual female mononuclear cell leukemia dose received) (nominal dose: 6000ppm)

Observations

Details on results

CLINICAL SIGNS AND MORTALITY: Survival at Week 104 in the males of the 12000 ppm dose group was significantly lower than the control group. Analysis of the females revealed no differences in survival between control and treated groups. No treatment related clinical signs were observed. **BODY WEIGHT AND WEIGHT GAIN:** The body weights of the animals from the 12000 ppm and recovery groups was significantly lower during weeks 5-105 and 2-105, respectively. **FOOD CONSUMPTION AND COMPOUND INTAKE** (if feeding study): In females of the 12000 ppm and recovery groups, statistically significant lower mean food consumption values were noted most frequently. However, even though these females were fed the same dietary concentration (12000 ppm), significantly lower mean weekly food consumption values were noted at 18/31 measurement intervals for the 12000 ppm group, but only 9/31 intervals for the recovery group during Weeks 1-78. Therefore, the statistically significant intergroup differences in mean weekly and total food consumption were spurious findings. For Weeks 1-104, the average daily compound consumption (mg/kg bw/d) for the 500, 1500, 6000, 12000 ppm dietary levels was 29.2, 88.3, 358.7, and 733.2 mg/kg bw/d in the males and 36.4, 108.6, 442.2, and 885.4 mg/kg bw/d in the females, respectively. For Weeks 1-78 in the recovery group, the average daily consumed dose was 637.3 mg/kg/day in males, and 773.6 mg/kg/day in females. **HAEMATOLOGY:** Significant changes in the hematology data included mild decreases in the erythrocyte mass (erythrocyte count, hemoglobin, and hematocrit) in the 12000 ppm and recovery group at most intervals, however, the mean values for the recovery group rats were, in general, not significantly different from controls at Week 104. Hematopoietic neoplasia (leukemia) was observed in the peripheral blood smears from several animals of the 12000 ppm and recovery group. **CLINICAL CHEMISTRY:** Increases (often significant) were observed in the mean values for urea nitrogen, alanine aminotransferase, and aspartate aminotransferase primarily in the 12000 ppm and recovery group throughout the study. The elevations in these analytes corresponded to the histologic findings noted in the kidneys and livers of the males of the 6000 ppm and recovery group, and animals (both sexes) of the 12000 ppm group. **URINALYSIS:** The mean values for urine volume were significantly increased in the males of the 12000 ppm group at Week 104. The mean creatinine clearance rate was significantly increased in the females of the 12000 ppm group at Week 104. The change was of low magnitude and was not accompanied by a significant change in the mean value for urine volume, urine creatinine, or serum creatinine. Decreases were observed in the mean values for urine calcium, urine potassium, urine chloride, and urine osmolality in some treated groups during the study. The significant changes in the aforementioned urinary chemistry tests are of questionable toxicologic importance as they occurred inconsistently over time and did not correlate with concurrent serum chemistry changes. **ORGAN WEIGHTS:** Liver organ weights (absolute and relative to body) for all interim sacrifices were significantly increased with dose in animals of the 6000 and 12000 ppm groups, compared to the control group. Spleen and kidney weights (absolute and relative to body) were also statistically increased in the 6000 and 12000 ppm groups when compared to the control group of animals sacrificed at Week 79. Absolute and

relative weights for testis/epididymis or uterus in the 6000 and 12000 ppm groups were not statistically different compared to controls. In the animals sacrificed after at least 104 weeks of treatment, liver weights (absolute and relative to body or brain) were noted as statistically increased in males and females of the 6000 and 12000 ppm groups. Liver weights in animals of the recovery group were increased when compared to control but not significantly. Absolute and relative spleen and kidney weights in females of the 12000 ppm group were significantly increased. Statistically significant increases of spleen and kidney weights were observed in females of the 6000 ppm group. There were no statistically significant differences in absolute and relative weights for testis/epididymis or uterus in any of the treated groups compared to same sex in controls. GROSS PATHOLOGY: In males and females of the 6000, 12000 ppm and recovery group, that died or were sacrificed in extremis during the study (unscheduled deaths), the incidence of the following necropsy findings was notably increased: enlarged spleen and granular/pitted/rough appearance of the liver, as well as dark area of the stomach, thoracic cavity fluid, small testis and small seminal vesicle, and uterine mass. At the Week 79 sacrifice, a liver mass was observed in each of two males of the 12000 ppm group; and various gross-pathologies of the liver (enlarged, irregular shape, granular/pitted/rough appearance and raised area) were observed in the females of the 12000 ppm group, when compared to the control group. Small testis and small seminal vesicle were not observed in any males killed from the control, 6000 and 12000 ppm group, and the incidence of uterus mass was 0/15, 2/15, and 1/15 in females of the control, 6000 and 12000 ppm group, respectively. At necropsy for study termination, the following abnormalities were observed to have a dose-related increase in the males and females of the 6000 and 12000 ppm groups: enlarged spleen, enlarged and/or granular/pitted/rough appearance of the liver (seen primarily in the males), dark kidney, dark area of the stomach, various uterine pathologies (cyst, mass, or thickened wall). The incidences for small testis and small seminal vesicle were similar across all groups. HISTOPATHOLOGY: NON-NEOPLASTIC: In the 6000 ppm dose group, there was no histopathological correlate to the increased liver weight. Livers from the rats killed at Week 79 and at study termination were remarkably free of treatment-related histologic findings, with no histologic evidence of hepatocellular enlargement. Treatment-related histopathologic lesions in the kidney at Week 79 and study termination were an increase in the incidence and severity of mineralization of the renal papilla in males, but not females, and increased incidence and severity of tubule cell pigment in both sexes. In the 12000 ppm group, histological findings in the livers of the males and females were diffuse hepatocellular enlargement (first detected at Week 2), increased cytoplasmic eosinophilia (first detected at Week 13) and increased pigment in Kupffer cell/bile canaliculi (first detected at Week 79). Treatment-related histopathologic lesions in the kidney at Week 79 and study termination, were an increase in the incidence and severity of mineralization of the renal papilla in males, but not females, and increased incidence and severity of tubule cell pigment in both sexes. In the recovery group, treatment-related histological effects in the liver also appeared to be reversible in that diffuse hepatocellular enlargement and increased cytoplasmic eosinophilia were not evident, and the incidence of pigment in Kupffer cell/bile canaliculi was comparable to the control group incidence. Kidney enlargement also appeared to be reversible in that mean kidney weights (absolute and relative) in the males and females were comparable to control values. However, mineralization of the renal papilla in males, and renal tubule cell pigment in both sexes, was only partially reversible with the incidence and severity approximating values in 6000 ppm group. HISTOPATHOLOGY: NEOPLASTIC: In the 6000 ppm dose group, the incidence of mononuclear cell leukemia was 49% and 45% in males and

females, respectively, compared to 34% and 26% in the control males and females, respectively. In the 12000 ppm group, increased incidence of neoplasms (first detected at Week 79) in liver was observed. Malignant tubule cell carcinoma was detected in kidneys of two males. The incidence of mononuclear cell leukemia was 46% and 46% in males and females, respectively, compared to 34% and 26% in the control males and females, respectively. In the recovery group, there was absence of an appreciable increase in liver neoplasms compared to the control group. Unlike the liver, treatment-related neoplasia in the kidney of males, and hematopoietic tissue of both sexes, was not reversible. Although no kidney neoplasms were detected in females, malignant tubule cell carcinoma was found in four of the males, of which three were killed following the recovery phase. The incidence of mononuclear cell leukemia was 62% and 48% in males and females, respectively, compared to 34% and 26% in the control males and females, respectively. OTHER FINDINGS: Cell proliferation: Significant increases in the labeling index were observed during Week 1 in the livers of male and female rats treated with the test material at all dose levels. The response was not observed at Week 2, 13, or 104. Large increases in the labeling index were observed in the positive control animals at Week 1, but questionable responses were observed at Weeks 2 and 13. No positive control animals were included for the Week 104 sacrifice. Biochemical analyses: Increases in palmitoyl CoA oxidase, a monitor of the level of peroxisome proliferation were observed in rats of the 12000 ppm group at all timepoints. At Week 104, there was also an elevation in palmitoyl-CoA oxidase activity in female rats of the 6000 ppm group. Male rats treated at 6000 ppm did not have elevated enzyme levels. DNA and Protein Analyses: Levels of DNA varied randomly among the groups and at the different timepoints. Statistical analysis confirmed the lack of a consistent pattern. Protein analysis also did not indicate a clear pattern in the 6000, 12000 ppm or positive control groups. Liver weights were significantly elevated in animals of the 12000 ppm group throughout the study, and also in the positive control animals included at 1, 2 and 13 weeks.

Remarks on results including tables and figures

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentfaf8.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-82f956a0-aa3a-4b8e-a626-b3c7aa93f6a9%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > Document

 **Dissemination Dossier**

 **Carcinogenicity Endpoint.004**

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability	The test parameters documented do not totally comply with the specific testing guideline and the work has no claim that it was conducted according to GLP, but the study is well conducted and scientifically acceptable.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Lington AW, Bird MG, Plutnick RT, Stubblefield WA, and Scala RA.	1997	Chronic toxicity and carcinogenic evaluation of diisononylphthalate in rats.	Fundamental and Applied Toxicology (36):79-89					
publication	Caldwell DJ, Eldridge SR, Lington AW,	1999	Retrospective evaluation of alpha 2u-globulin accumulation in male rat kidneys	Toxicol Sci. 1999 Sep;51(1):153-60.					

McKee RH. following high doses of diisononyl phthalate.

study report 1986

1986-01-13

Materials and methods

Limit test

no

Test guideline

Qualifier

no guideline followed

Guideline

Deviations

not applicable

Principles of method if other than guideline

Groups of male & female rats were fed diisononyl phthalate (DINP) at dietary levels of 0 (control), 0.03, 0.3 and 0.6 wt% for period up to two years. Interim sacrifices of 10 rats/sex /dose were at 6, 12 and 18 months with the surviving rats sacrificed at 24 months. There were three primary objectives; i). to assess the chronic toxicity and oncogenicity of DINP at a dose close to the maximum tolerated dietary dose of 0.6 wt%; ii). to determine whether DINP could produce hepatic peroxisome proliferation under chronic exposure conditions, and iii). to define a no-observed-effect level (NOEL) for DINP under chronic exposure conditions.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Jayflex DINP - Substance type: technical material - Physical state: not reported - Analytical purity: > 99% DINP (by HPLC analysis) - Impurities (identity and concentrations): Isononyl alcohol (concentration not given) - Composition of test material, percentage of components: not reported - Purity test date: not

reported - Lot/batch No.: not reported - Expiration date of the lot/batch: not reported - Stability under test conditions: not reported - Storage condition of test material: not reported

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Breeding Laboratory (Stoneridge, NY, USA.) - Age at study initiation: 6 weeks - Weight at study initiation: (Mean wts) Males 109 ± 5 grams; Females 89 ± 3 - Fasting period before study: N/A - Housing: Individually housed (except during the first week of acclimation) in suspended stainless steel with indirect bedding and water - Diet (e.g. ad libitum): ad libitum presented weekly - Water (e.g. ad libitum): ad libitum - Acclimation period: not reported ENVIRONMENTAL CONDITIONS - Temperature ($^{\circ}\text{C}$): not reported - Humidity (%): not reported - Air changes (per hr): not reported - Photoperiod (hrs dark / hrs light): not reported IN-LIFE DATES: not reported

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): Purina Certified Rodent Chow 5002 on a fixed percentage (w/w) basis and mixed thoroughly to ensure homogeneity - Storage temperature of food: not reported VEHICLE -N/A

Analytical verification of doses or concentrations

yes

Duration of treatment / exposure

up to 24 months with constant exposure

Frequency of treatment

continuous (freely available diet containing the test material)

Post exposure period

N/A

Doses / concentrations

0.03 wt%

Basis nominal in diet equivalent 15 mg/kg/day for males; 18 mg/kg/day for females

0.3 wt%

Basis nominal in diet equivalent to 152 mg/kg/day for males; 184 mg/kg/day for females.

0.6 wt%

Basis nominal in diet equivalent to 307 mg/kg/day for males; 375 mg/kg/day for females.

No. of animals per sex per dose

110/sex/dose level

Control animals

yes, plain diet

Details on study design

- Dose selection rationale: Based on previous studies which showed that a level of DINP above 0.6 % might jeopardize survival due to kidney lesions. The mid dose (0.3%) was selected to produce lesser effects on the liver and kidney. The low dose of 0.03% was selected as an anticipated no- observed-effect level based on 13-week data and pharmacokinetic considerations in this strain of rats. - Rationale for animal assignment (if not random): Random assignment - Rationale for selecting satellite groups: N/A - Post-exposure recovery period in satellite groups:

N/A

Positive control

Not applicable

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: once daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: weekly BODY WEIGHT: Yes - Time schedule for examinations: weekly on the same day each week 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: No data OPHTHALMOSCOPIC EXAMINATION: No HAEMATOLOGY: Yes - Time schedule for collection of blood: 6, 12 and 18 months and at termination (24 months) - Anaesthetic used for blood collection: Yes (identity) / No / No data - Animals fasted: Yes (overnight, 16 hrs) - How many animals: All animals at interim sacrifice and 20 rats/sex/group at termination No haematology data reported for interim results but results at 24 months reported CLINICAL CHEMISTRY: Yes - Time schedule for collection of blood: 6, 12 and 18 months and at termination (24 months) - Animals fasted: Yes (overnight, 16 hrs) - How many animals: All animals at interim sacrifice and 20 rats/sex/group at termination URINALYSIS: Yes - Time schedule for collection of urine: about two weeks prior to interim: 6, 12 and 18 months and at termination (24 months) - Metabolism cages used for collection of urine: No data - Animals fasted: No NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: Yes (see tables 2 and 3)

Other examinations

Morphological examination of liver: Livers of two rats/sex/dose group including controls were examined by electron microscopy for evidence of hepatic peroxisome induction at study termination.

Statistics

Food consumption, body weights, organ weights, relative organ weights, serum chemistry, haematology, and urine chemistry parameters were evaluated. Bartlett's test of homogeneity of variances. If the variances were equal, the testing for significance was done using a parametric

method, a standard one-way ANOVA. If significant differences among the means were indicated, Dunnett's test was used to determine which treatment groups differed from controls. If the groups did not have equivalent variances, the test of equality of means was performed using the Kruskal- Wallis test. If the means were different, Dunn's summed rank test was used to determine which treatment groups differ significantly from control. The test for equal variance was conducted at the 1% level of significance; all other tests at the 5 and 1% levels. The survival analysis used two methods: i). the Weibull estimate of median survival, and ii). the Kaplan-Meier method with a comparison between groups using both the generalized Kruskal-Wallis test and Cox's test. To assess tumour incidence, three methods were used: i). life table analyses ii). incidental tumour analysis and iii). unadjusted analysis for linear dose response. The latter consisting of a pairwise comparison of each dose group with the control by Fisher exact test and a test for linear trend.

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Sex	Basis for effect level / Remarks
NOEL	other: all biological endpoints	0.03 % wt (equivalent to approximately 17 mg DINP /kg/day)	male/female	overall effects

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

yes

Food consumption and compound intake (if feeding study)

yes

Food efficiency

no data

Water consumption and compound intake (if drinking water study)

not examined

Ophthalmoscopic examination

not examined

Haematology

yes

Clinical chemistry

yes

Urinalysis

yes

Neurobehaviour

not examined

Organ weights

yes

Gross pathology

yes

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

yes

Details on results

CLINICAL SIGNS AND MORTALITY The incidence of clinical in-life observations was relatively low throughout the 24-month test period. The most frequently noted observation included alopecia and scabs on the skin which appeared unrelated to treatment. Survival was greater than 60% in all groups (75-80% in controls) at study termination. A slight decrease in overall survival was noted in the mid and high-dose groups of both sexes compared to controls,

but whether or not this decrease was statistically significant depended on the statistical method employed; i.e. no difference was observed using the Weibull technique but the Cox and generalized Kruskal-Wallis tests indicated a significant difference in survival in mid- and high-dose female rats; (see Table 1).

BODY WEIGHT AND WEIGHT GAIN When compared with controls, the high-dose males had a statistically significant, dose-related decrease in body weight at 12 months of treatment and continued to display this decrease at 18 and 24 months of treatment. The mean body weights for the high-dose group males were approximately 4 to 7 % lower than corresponding controls. There were no statistically significant changes in females. In general, the treated females weighed slightly more than the control animals until approximately the last 6 months of the study, when they displayed a slight decrease in body weight.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study) There were slight decreases in food consumption at the mid and high levels. The mean daily intakes of DINP over 2 years for male rats were 15, 152 and 307 mg/kg/day corresponding to dose levels of 0.03, 0.3 and 0.6 wt % respectively. For female rats, the mean daily intakes were 18, 184, and 375 mg/kg/day, respectively.

FOOD EFFICIENCY No data available

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study) Not applicable

OPHTHALMOSCOPIC EXAMINATION Not applicable

HAEMATOLOGY In general, the mean hematological values were within normal limits for both sexes treated with DINP for 6, 12 and 18 months. However, at study termination, some possible treatment-related effects on haematologic values were observed. The red blood cell (RBC) count and haemoglobin and haematocrit mean values were slightly lower for the mid and high-dose males and females compared to control values but only the high dose males showed a statistically significant decrease in these parameters compared with controls. Both sexes in the high dose group also exhibited an increased frequency of occurrence of nucleated RBC, polychromatophilic red cells, and reticulocytes when compared with control animals.

CLINICAL CHEMISTRY At 6, 12 and 18 months there were no remarkable findings. Some parameters were statistically increased (BUN, A/G ratio, creatinine) but were judged unlikely to be of biological significance due to a lack of dose response. Some of the liver function parameters were weakly increased in the males fed on the mid and high levels of DINP at all time intervals in the study (viz: alkaline phosphatase, SGOT and SGPT). In general, these responses were statistically significant and may be related to treatment. No statistically significant effects were observed on total bilirubin. The results of the female serum chemistry profile revealed no statistically significant treatment-related effects on liver function parameters.

URINALYSIS The qualitative urine chemistry parameters were nonremarkable at all time intervals for both sexes. The quantitative urine chemistry parameters were unaffected in females at all intervals but the males showed slight but statistically significant changes in some of the parameters throughout the study. High dose males exhibited increased urine volumes when compared to the controls at all time intervals. In the mid or high dose males, potassium and glucose showed a statistically significant increase at 6, 12, and 18 months, but not at 24 months. A statistically significant increase in excretion of renal epithelial cells was noted in high-dose males at 6 months but not at later treatment periods.

NEUROBEHAVIOUR Not applicable

ORGAN WEIGHTS Both males and females of the mid- and high- dose groups had a statistically significant, dose-related increase in relative kidney and liver weights throughout most of the treatment period; the absolute liver and kidney weights demonstrated a similar trend. At study termination, the mid- and high- dose males and high dose females exhibited a statistically significant increase in absolute and relative spleen weight compared to controls. Absolute and relative adrenal weights were slightly but significantly increased in high-dose

female rats after 6 and 12 months of treatment. In addition, at study termination, relative but not absolute adrenal weights were slightly but significantly increased in high-dose rats of both sexes. There were no observed treatment related changes in the absolute or relative organ weights for ovaries, testes, brain, heart, or thyroid/parathyroid. GROSS PATHOLOGY No grossly observable and treatment-related abnormalities were apparent at each of the interim sacrifices. At study termination, however, the mid-and high-dose males and high dose females exhibited an increased incidence of splenic enlargement when compared with control animals.

HISTOPATHOLOGY: NON-NEOPLASTIC No treatment-related effects on tissues were observed in rats sacrificed after 6 and 12 months of treatment. At the 18 month sacrifice non-neoplastic lesions were observed in the liver and kidney of treated rats. Minimal to slight centrilobular to midzonal hepatocellular enlargement was present in 9/10 male and 10/10 female rats treated with 0.6% DINP; this effect was absent in controls. In the kidneys, a minimal increase in tubular cell pigment was noted in the tubular epithelium of high-dose rats. After treatment for 24 months, tubular cell pigmentation in the kidney was increased in severity in animals with advanced mononuclear cell leukemia MNCL (see MNCL below in Neoplastic). At terminal sacrifice, a variety of spontaneous disease lesions and incidental findings of the expected type and incidence occurred in control and treated rats without relationship to treatment with the exception of liver lesions. These lesions include regenerative nodules, focal necrosis, spongiosis hepatis and hepatopathy associated with leukemia. Slight centrilobular to midzonal hepatocellular enlargement was present in a small number of rats and was comparable to findings at the 18 month sacrifice. DINP also failed to produce any clear treatment-related effects on the kidneys. Kidney sections revealed chronic progressive nephropathy in most of the rats which was not increased in severity by treatment. Similarly, DINP failed to produce any treatment-related effects on the testes; (see Table 2). HISTOPATHOLOGY: NEOPLASTIC No treatment-related effects on tissues were observed in rats sacrificed after 6, 12 and 18 months of treatment. At 24 months, the most common causes of the unscheduled deaths and/or morbidity were mononuclear cell leukemia (MNCL) and to a lesser extent, pituitary neoplasia. A slight increase in MNCL was noted in the mid- and high-dose rats in both sexes. Table 3 summarizes selected neoplastic lesions (MNCL, liver, kidney and testes) in rats. MNCL occurred at statistically increased incidence in the mid- and high-dose groups of both sexes when compared with the control groups and with a significantly increasing trend over time. In many of the rats, the MNCL was very early and limited to an increase in mononuclear cells in the hepatic sinusoids. The MNCL was associated with a variety of hepatic alterations (see above in non-neoplastic). Renal neoplasms were present in three mid-dose and two high-dose male rats which consisted of two tumour types: transitional cell carcinomas and tubular cell carcinomas; each of which was not statistically significant when compared to control animals. In addition, there was no evidence of any treatment-related preneoplastic renal lesions. HISTORICAL CONTROL DATA (if applicable) Not Applicable OTHER FINDINGS Morphological examination of Liver: Ultrastructural examination of liver specimens from representative rats of each sex from four groups did not reveal any treatment-related peroxisomal proliferation.

Remarks on results including tables and figures

DINP was not considered a liver carcinogen in Fischer 344 rats at a dietary level up to 0.6% DINP. Morphologic evaluations

at 18 or 24 months indicated that hepatic peroxisome proliferation was not induced in the chronic study. At study termination, DINP produced statistically significant increases in mononuclear cell leukemia (MNCL) in the the mid- and high-dose groups of Fischer 344 rats. MNCL in this strain of rats is associated with secondary effects on organ weights (splenic enlargement), haematological parameters (decreases in haemoglobin and haematocrit), clinical chemistry parameters (liver function indices such as ALT, AST, bilirubin etc.), as well as liver lesions such as necrosis (Stromberget *al.*, 1983). A review of the available literature suggests that the increase of MNCL in Fischer 344 rats is a common finding associated with aging in this strain and that the increased incidence in rats treated with DINP is not regarded as relevant to humans.

Table 1:

Survival for Fischer 344 Rats in a Chronic DINP Study at Terminal Sacrifice

DINP dose groups (wt %)	No. in group	No. of unscheduled deaths	No. of survivors at terminal sacrifice	Percentage survivors
Males				
0	81	20	61	75
0.03	80	25	55	69
0.3	80	30	50	62
0.6	80	29	51	64
Females				
0	81	16	65	80
0.03	81	24	57	70
0.3	80	31	49	61 ^a
0.6	80	26	54	68 ^b

^aStatistically significant from control group at 1% level based on Kruskal-Wallis test and Cox's test, but not

statistically significant by the Weibull technique

^bStatistically significant from control group at 5% level based on Kruskal-Wallis test and Cox's test

Table 2

Table 2:

Incidence of Non neoplastic Liver Lesions in Rats fed DINP for 2 years

	Dietary concentration of DINP (wt %)							
	0	0.03	0.3	0.6	0	0.03	0.3	0.6
Sex	Males				Females			
Number examined	81	80	80	80	81	81	80	80
Liver								
Focal necrosis	10	9	16	26	13	11	19	21
Spongiosis hepatis	24	24	51	62	4	1	3	4
Sinusoid ectasia	16	16	24	33	9	4	6	10
Hepatopathy associated with leukemia	22	17	34	33	16	18	24	33
Hepatocellular enlargement	1	1	1	9	1	0	0	11

Foci of vacuolated hepatocytes	15	14	1	0	17	16	8	5
Diffuse fatty changes	12	4	4	0	10	9	3	3
Cholangiectasis	52	25	16	20	10	6	3	5
Regenerative nodules	3	3	6	6	5	7	10	9

Table 3:

Incidence of Neoplastic Liver Lesions in Rats fed DINP for 2 years

	Dietary concentration of DINP (wt %)							
	0	0.03	0.3	0.6	0	0.03	0.3	0.6
Sex	Males				Females			
Number examined	81	80	80	80	81	81	80	80
Liver								
Neoplastic nodules (1)	3	1	1	1	0	2	0	1
Hepatocellular cancer (2)	0	0	0	3	1	0	0	1
Combined (1 and 2)	3	1	1	4	1	2	0	2
Basophilic foci	53	62	48	42	72	64	64	54
Eosinophilic foci	58	50	45	51	59	45	42	32
Kidney								
Transitional cell carcinoma	0	0	3	0	0	0	0	0
Transitional cell adenoma	0	0	0	0	0	0	0	0
Tubular cell carcinoma	0	1	0	2	0	0	0	0
Tubular cell adenoma	0	0		0	0	0	0	0
Hyperplasia of transitional cell epithelium	21	23	31	25	32	39	34	32
Testes: Bilateral interstitial cell tumours	71	64	70	72	-	-	-	-
Hematopoietic system: Mononuclear cell leukemia	33	28	48 ^a	51 ^{a,c}	22	20	30 ^c	43 ^{a,b,c}

^aStatistically significant from control group at 5% level using the Fisher exact test

^bStatistically significant from control group at 5% level using the incidental tumour test

^cStatistically significant from control group at 5% level using the life table test

Applicant's summary and conclusion

Relevance of carcinogenic effects / potential

The study indicates that DINP has a low potential for carcinogenicity in Fischer 344 rats. There was no clear evidence of any carcinogenic effects except for a marginal increase in the incidence of mononuclear cell leukemia (MNCL) in mid- and high-dose rats. The prevailing data indicate

that the histogenic tumour type (MNCL) in Fischer 344 rats does not occur in humans. Moreover, this leukemia in this strain of rats appears to arise from the spleen, but this does not appear to be the case for humans where leukemia originates from affects on the bone marrow. Therefore, this slight increase of MNCL in the mid- and high-dose rats is not considered a relevant risk for humans.

Conclusions

This study supports previous studies that DINP has a low potential for chronic toxicity and carcinogenicity in Fischer 344 rats; DINP was not a liver carcinogen in this strain of rats at a dietary level up to 0.6%. Morphological evaluations also indicated that hepatic peroxisome proliferation was not induced in this chronic study. Organ weights of liver and kidney were increased at the mid- and high-doses but there was no clear evidence of any carcinogenic effects except for a marginal increase in the incidence of mononuclear cell leukemia (MNCL) in the mid- and high dose rats which is not considered a relevant risk for humans.

Executive summary

Groups of 110 'Fischer 344' rats per sex were fed diisononyl phthalate (DINP) at dietary levels of 0, 0.03, 0.3 and 0.6 wt% for periods up to 2 years. Interim sacrifices of 10 pre-designated rats/sex/dose were at 6, 12, and 18 months with surviving animals sacrificed at 24 months. At study termination, survival was in excess of 60% for every group.

At the mid or high dose, there was a slight decrease in food consumption and body weights, a slight increase in mortality, and a dose related increase in relative organ weights of liver and kidney. There were also some slight effects on urinalysis, and on haematologic and clinical chemistry parameters.

There was no observed peroxisome induction in livers of treated rats compared with the controls and no clear treatment-related nonneoplastic or neoplastic lesions were found. However, mononuclear cell leukemia (MNCL) and changes known to be associated with an increased incidence of MNCL were seen in the mid-dose and high-dose groups. Published literature suggests that MNCL is a common finding in aging rats of the strain used in this study and that this increased incidence in rats treated with DINP is not relevant to man.

A clear no-observed-effect level (NOEL) was demonstrated for all biological end points at a dietary level of 0.03 wt% or approximately 17mg/kg/day of DINP.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument00b1.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-698a6e1b-268d-47a8-b6a8-9619f413d391%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > Document

 Dissemination Dossier

 Reproduction Endpoint.001

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	supporting study		
Study result type	experimental result	Study period	25.06.1993 to 12.11.1993
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered to be equivalent to international test guidelines for a one generation study. The study was intended to be a screening study to select doses for a two generation reproductive toxicity test.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1996							1996-03-08

Materials and methods

Test type

one-generation study

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 415 (One-Generation Reproduction Toxicity Study)	no data

Principles of method if other than guideline

There is no explicit statement in the report that the study was undertaken in accordance with a specific guideline, although there are references to the US EPA 'Test guidelines for reproduction and fertility effects' and the EU Annex V 'Two generation reproduction Toxicity test.'

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate - Substance type: Technical material - Physical state: Colourless liquid - Stability under test conditions: No data - Storage condition of test material: Room temperature Analyses of the test material for stability, identity, strength, purity and composition were undertaken prior to dose initiation (in an unknown GLP compliant manner) and after dose initiation (in a GLP compliant manner). The documentation is maintained by the Sponsor and the Testing Laboratory, respectively.

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Breeding Laboratories, Inc. - Age at study initiation: 6-7 wks - Weight at study initiation: Males: 182-222 g; Females: 137-178 g; - Fasting period before study: None - Housing: individual, except during the mating and postpartum period - Diet (e.g. ad libitum): ad libitum - Water (e.g. ad libitum): ad libitum - Acclimation period: 8 days
ENVIRONMENTAL CONDITIONS - Temperature (°C): 68-76 °C - Humidity (%): 40-70% RH
- Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): 12 hours dark/12 hours light
IN-LIFE DATES: From: 25 June 1993 To: 12 November 1993

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): Purina /PMI certified rodent chow (5002 meal) - Storage temperature of food: Room temperature

Details on mating procedure

- M/F ratio per cage: 1/1 - Length of cohabitation: Up to three weeks - Proof of pregnancy: Vaginal plug and/or sperm in vaginal rinse, referred to as Day 0 of pregnancy - After ... days of unsuccessful pairing replacement of first male by another male with proven fertility. No data - Further matings after two unsuccessful attempts: No data - After successful mating each pregnant female was caged (how): Suspended stainless steel and wire mesh cages, and from GD 20 cages fitted with a stainless steel litter pan

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The stability and homogeneity of the test material in feed chow was demonstrated by the testing laboratory after dose initiation. Homogeneity was confirmed at the highest and lowest levels expected during the study. Triplicate samples were collected from the top, middle and bottom of each preparation (nine samples in total). The concentration was the mean of all nine samples. The coefficient of variance ranged from 1.63% to 4.74% . Stability was confirmed for 14 days by measuring the concentrations of selected samples from each of the homogeneity preparations

after room temperature and refrigerated storage. The concentrations of test material in the diets were checked at least once a month and found to be within 10% of the nominal concentrations on all occasions.

Duration of treatment / exposure

Ten weeks before mating and through the mating period, continuing for males until sacrifice following delivery of their last litter sired and females until they were sacrificed following weaning of the F1 animals on PPD 21.

Frequency of treatment

Continuous for parents (males and females)

Details on study schedule

Study terminated at weaning of the F1 animals (PPD21)

Doses / concentrations

0.5, 1.0 and 1.5 % in diet

Basis nominal conc.

No. of animals per sex per dose

30

Control animals

yes, plain diet

Further details on study design

- Dose selection rationale: The high dose of 1.5% was expected to be equivalent to at least 1000 mg/kg/day, the EU and OECD limit dose. The low dosage was expected not to cause any adverse effect on the parents. The middle dose was expected to produce a mild bodyweight reduction. - Rationale for animal assignment (if not random): The parents were selected from the pool, by exclusion of animals with poor health, outlying bodyweight or abnormalities, and assigned to treatment groups using a computer generated randomized procedure to most nearly equalise initial mean bodyweight.

Positive control

No

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes - Time schedule: At least once daily DETAILED

CLINICAL OBSERVATIONS: Yes - Time schedule: Weekly BODY WEIGHT: Yes - Time schedule for examinations: Males weekly; females weekly until confirmation of mating, then on gestation days 0, 7, 14, and 21 and post partum days 0, 4, 7, 10, 14, and 21 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

Estrous cyclicity (Parental animals)

No data

Sperm parameters (Parental animals)

No data Parameters examined in the male parental generation: Testis weight, epididymis weight

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 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 where indicated 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 as soon as possible after the last F1 litter was sired. - Maternal animals: All surviving animals after the last litter of each generation was weaned. GROSS NECROPSY - Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera. The uteri of all females used for mating were examined grossly for evidence of implantations. HISTOPATHOLOGY / ORGAN WEIGHTS The tissues weighed for Parent animals used for mating were: kidneys, testes, epididymides, liver, prostate, seminal vesicles and ovaries. No histopathological examinations were performed.

Postmortem examinations (Offspring)

SACRIFICE - The F1 offspring were sacrificed at 21 days of age. - These animals were subjected to post mortem examinations, with retention of tissues. **GROSS NECROPSY** - Gross necropsy consisted of internal examinations of all F1 pups that died. **HISTOPATHOLOGY / ORGAN WEIGHTS** There were no organ weights recorded or histopathological examination of tissues.

Statistics

1) Continuous data were tested. Bartlett's test for equivalence followed by a standard one way analysis of variance. If the ANOVA was significant Dunnett's test was performed. A linear regression to test for a dose response was also performed and tested for lack of fit. All tests were reported at the 5% and 1% level of significance. If the variance was not significant then a Krustal-Wallis test was performed. 2) Pup weight was analyzed by a standard nested analysis of covariance (Snedecor and Cochran, 1989) with pups nested within dams and dams nested within doses and litter size (both sexes combined) as the covariate. If differences in groups were identified, the Least Significant Difference technique was used to determine which groups differed from the control group (Snedecor and Cochran, 1989). Male and female pups were tested separately. All tests were reported at the 5% and 1% level of significance. 3) Parental reproductive and offspring survival incidences data were analyzed as follows. First a standard chi-square analysis (Snedecor and Cochran, 1989). Next, each treatment group was compared to the control group using a 2X2 Fischer Exact test (Bradley, 1968). Thirdly, Armitage's test for linearity trend in the dosage groups was performed (Snedecor and Cochran, 1989). All tests were reported with a 5% or 1% level of significance.

Reproductive indices

Male mating index Male fertility index Female fertility index Female fecundity index Gestation index

Offspring viability indices

Live birth index Survival index on Days 1, 4, 7, 14 and 21 Lactation index

Results and discussions

Effect levels

Endpoint	Generation	Sex	Effect level	Basis for effect level / Remarks
LOAEL	P	male/female	< 0.5 % diet	
LOAEL	F1	male/female	< 0.5 % diet	

Observations: parental animals

Clinical signs (parental animals)

no effects

Body weight and food consumption (parental animals)

yes

Test substance intake (parental animals)

yes

Reproductive function: estrous cycle (parental animals)

no data

Reproductive function: sperm measures (parental animals)

no data

Reproductive performance (parental animals)

no effects

Organ weights (parental animals)

yes

Gross pathology (parental animals)

no effects

Histopathology (parental animals)

no data

Details on results (parental animals)

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS) Statistically significant lower mean bodyweights were observed in mid and high dose parental animals compared with controls. These lower bodyweights were apparent as early as Weeks 1 and 2 and were observed until scheduled study termination. Similarly, statistically similar lower mean food consumption was observed in the same treatment groups. TEST SUBSTANCE INTAKE (PARENTAL ANIMALS) Concentration in diet (%) Actual dose in mg/kg/day Premating males Premating females 0.5% 591-301 624-364 1.0% 1157-622 1169-734 1.5% 1676-966 1694-1114

Concentration in diet (%) Actual dose in mg/kg/day Gestation females Postpartum females 0.5% 377-404 490-923 1.0% 741-796 1034-1731 1.5% 1087-1186 1274-2246

REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS) There was no statistically significant difference in male mating, male fertility, female fertility, female fecundity or female gestation indices between treated and control animals. Mean days of gestation of treated and control animals were essentially equivalent. However, the mean litter size (12.5) and mean live offspring (11.9) of the high dose animals were lower than controls (14.1 and 13.9 respectively), and the historic control range of this laboratory (16.8-12.6 and 16.1-12.2 respectively).

ORGAN WEIGHTS (PARENTAL ANIMALS) There were statistically significant increases in the mean absolute and/or mean relative liver and kidney weights of both male and female animals at all dose levels, compared with controls. Generally these increases occurred in a dose-related fashion. Differences in reproductive organ weights compared with controls were apparent but not believed to be biologically important.

Observations: offspring

Viability (offspring)

no effects

Clinical signs (offspring)

no effects

Body weight (offspring)

yes

Sexual maturation (offspring)

no data

Organ weights (offspring)

no data

Gross pathology (offspring)

no effects

Histopathology (offspring)

no data

Details on results (offspring)

BODY WEIGHT (OFFSPRING) Dose-related decreases in mean offspring bodyweight were recorded during the postnatal period(PND0-21). There were statistically significant lower mean bodyweights in the high dose males(10.2-46.0%) and females (11.3-46.9%) mid dose females (7.9-26.9) at all weighing intervals and in mean offspring bodyweight of the mid dose males on PND 0, 1,7,14,and 21 (5.7-26.5%) compared with controls.

Remarks on results including tables and figures

Parental animals

There were no clinical signs in the parental animals which were judged to be directly related to treatment with DINP. The majority of animals in all groups had no adverse clinical signs during the premating, mating, postmating, gestation and/or postpartum periods.

Statistically significant lower mean body weights, as well as suppression in body weight gain, were observed primarily in the mid and high-dose parental animals compared with controls. These lower body weights were apparent as early as week 1 or 2, and were observed during the majority of weighing intervals until scheduled study termination. The greatest decrease from controls (up to 23.3%) was observed during the postpartum period. Similarly, statistically significant lower mean food consumption was observed primarily in the mid and high-dose animals compared with controls. These findings were important in their apparent relationship to findings in the offspring and relative organ weights.

There were statistically significant increases in the mean absolute and/or mean relative liver and kidney weights of both male and female animals at all dose levels tested (0.5, 1.0 and 1.5%) compared with controls. Generally, these increases occurred in a dose-related fashion.

Pertaining to male organ toxicity, there was a statistically significant increase in the mean absolute and relative right testis weight, left testis and right epididymis weights and the mean relative left epididymis and seminal vesicle weights in the high-dose males compared with controls. In females, there was a statistically significant decrease in the mean absolute and relative right ovarian and mean absolute left ovarian weights of the high-dose females compared with controls. Histopathological examination was not conducted to confirm if any structural changes occurred.

It was not determined if any structural changes occurred in reproductive organs at any dose level: microscopic evaluation was not performed on any organs in both sexes. Thus significance of organ weight changes could not be assessed because of the limitations of the study. However, there were no statistically significant differences in male mating, male fertility, female fertility, female fecundity, or female gestation indices between treated and control animals. Mean days of gestation of the treated and control groups were essentially equivalent. However, the mean litter size (12.5) and mean live offspring (11.9) of the high-dose animals were lower than controls (14.1 and 13.9, respectively), and the historical control range of this laboratory (16.8-12.6 and 16.1 and 12.2, respectively). There were no statistically significant differences in the mean sex

ratio of the treated offspring compared with controls.

There were no gross post-mortem findings in the parental animals judged to be related to treatment with DINP. The majority of animals in all groups were free of observable abnormalities at post-mortem examination. There were no significant post-mortem findings in the two animals which succumbed prior to scheduled study termination.

Offspring

The mean live birth index (95.2%), Day 4 survival index (85.6%), Day 14 survival index : (92.7%) and lactation index (87.2%) of the high-dose offspring were statistically significantly, decreased compared with controls (live birth: 98.2%, Day 4: 93.1%, Day 14: 98.5% and lactation index: 93.9%). The historical control range from this laboratory was the following: live birth: 99.1-95.2%, Day 4: 99.5-89.0%, Day 14: 100-93.7% and lactation index: 100-86.9%.

There were no treatment-related clinical findings observed in the offspring of any group. The majority of offspring in all groups were free of observable abnormalities from PND 0-21.

Dose-related decreases in mean offspring body weight were observed during the postnatal period (PND 0-21). There were statistically significant lower mean body weights in the high-dose males (10.2-46.0%) and females (11.3-46.9%), mid-dose females (7.9-26.9%) at all weighing intervals and in mean offspring body weight of the mid-dose males on PND 0, 1, 7, 14 and 21 (5.7-26.5%) compared with controls.

There were also statistically significant lower mean body weights in the low-dose males on PND 0, 1, 14 and 21 (6.9-11.2%) and low-dose females (7.5- 10.1%) at all weighing intervals.

Offspring bodyweights (g) of male (M) and female (F) pups during the postnatal period (PND 0-21)

Group	M PND 0	M PND 1	M PND 4	M PND 7	M PND 14	M PND 21
0%	6.98	7.34	9.80	16.02	33.77	54.34
0.5%	6.49**	6.83**	9.18	14.52	30.00**	48.94**
1.0%	6.42**	6.92*	9.12	14.00*	26.23**	39.93**
1.5%	6.27**	6.58**	8.19*	11.04**	20.18**	29.32**
Historical control	6.35-7.02	6.68-7.46	8.53-11.43	13.64-18.74	28.81-36.73	44.89-60.77
Group	F PND 0	F PND 1	F PND 4	F PND 7	F PND 14	F PND 21
0%	6.68	7.05	9.58	15.60	32.72	52.19
0.5%	6.15**	6.52**	8.81*	14.07*	29.40**	47.77**
1.0%	6.05**	6.49**	8.56*	13.24**	25.04**	38.13**
1.5%	5.91**	6.25**	7.84**	10.71**	19.31**	27.71**

Historical control	5.96-6.74	6.30-7.16	8.32-11.05	13.33-17.69	27.22-35.74	42.39-61.19
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*Mean statistically different from control mean (equal to or less than 0.5)

* Mean statistically different from control mean (equal to or less than 0.01)

The majority of animals were free of observable abnormalities at the scheduled terminal sacrifice on PND 21. Similarly, the majority of animals which died prior to scheduled termination (PND 0-20) was free of observable abnormalities, or was too autolyzed to be examined at the necropsy.

Applicant's summary and conclusion

Conclusions

Evidence of toxicity was observed in parents treated at 1.0% or 1.5% DINP, but there was no effect on reproductive indices. Significant suppression of bodyweight gain was observed in the offspring at 0.5%, 1.5% and 1.5% DINP.

Executive summary

In a reproductive toxicity study, groups of 30 male and 30 female rats (Sprague Dawley CD) were exposed to Di-isononylphthalate in the diet at constant concentrations of 0 (control), 0.5%, 1.0% or 1.5%. Parent males and females received treated diet *adlibitum* for ten weeks before mating and during the mating period, and females during gestation and post partum until weaning of the F1 offspring on post partum day 21.

The dosages in mg/kg/day related to concentration in diet were:

Concentration in diet	Actual dose in mg/kg/day	
	Premating male	Premating females
0.5%	591-301	624-363
1.0%	1157-622	1169-734
1.5%	1676-966	1694-1114
Concentration in diet	Actual dose in mg/kg/day	
	Gestation females	Postpartum females
0.5%	377-404	490-923
1.0%	741-796	1034-1731
1.5%	1087-1186	1274-2246

Effects of treatment in parental animals were observed primarily at dose levels of 1.0% and 1.5% and comprised lower bodyweight gain, lower food consumption and increased liver and kidney weights compared with controls. There were no apparent effects on reproductive indices.

Effects of treatment in the offspring, observed in dams treated at 0.5%, 1.0% or 1.5% DINP, were reduced bodyweight on PND 0 and significant suppression of bodyweight gain during the

postnatal period.

A NOAEL was not established for systemic toxicity. No effect was observed on fertility parameters but a decrease of live birth and survival indices occurred at 1.5% which led to a NOAEL of 1%.

The data from this study was used to select lower dosages for a subsequent two generation reproductive toxicity study.

The toxicity study was considered acceptable for classification and essentially satisfied the guideline requirements for one generation reproductive toxicity studies.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument1a6f.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-9251df2b-6a91-4609-b525-81be05827d7b%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > *Document*

 Dissemination Dossier

 Reproduction Endpoint.002

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	1998
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered to be equivalent to international test guidelines for a two-generation rat reproduction study.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication		2000							
study									1996-
report		1994							02-29

Materials and methods

Test type

two-generation study

Limit test

no

Test guideline

Qualifier Guideline

Deviations

according to	other guideline: EC Dangerous Substances Directive (67/548/EEC), Annex V, Part B; 1987	no data
according to	EPA OTS 798.4700 (Reproduction and Fertility Effects)	no data

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate (DINP) - Substance type: technical material - Physical state: not reported - Analytical purity: >99.7% verified by gas chromatography and infrared analysis - Impurities (identity and concentrations): did not contain any contaminants that could affect the outcome of the study - Composition of test material, percentage of components: N/A - Purity test date: not stated - Lot/batch No.: not stated - Expiration date of the lot/batch: not stated - Stability under test conditions: stability data confirmed that DINP was stable in feed for at least 14 days - Storage condition of test material: not stated - Other:

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Breeding Laboratories, Stone Ridge, NY, USA - Age at study initiation: (P) x wks not reported; (F1) x wks not reported - Weight at study initiation:

(Means given; two gen study): (P) Males: 202.1 - 204.1g; Females: 158.4 - 159.6g; (F1) Males: 247.8 - 249.2g; Females: 178.0 - 180.3g; (F2) Males: 184.6 - 211.1g; Females: 150.3 - 167.5g - Fasting period before study: N/A - Housing: individually housed in suspended stainless steel and wire mesh cages prior to mating - Diet (e.g. ad libitum): ad libitum (free access) - Water (e.g. ad libitum): ad libitum (free access) - Acclimation period: 2 weeks ENVIRONMENTAL CONDITIONS - Temperature (°C): 68 - 76°C - Humidity (%): 40 - 70 % - Air changes (per hr): not reported - Photoperiod (hrs dark / hrs light): 12 hours dark/12 hours light IN-LIFE DATES: Not reported

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on exposure

DIET PREPARATION - Rate of preparation of diet (frequency): biweekly - Mixing appropriate amounts with (Type of food): control (0.0%), 0.2, 0.4 or 0.8% in Certified Rodent Chow (PMI Feeds, Richmond, IN, USA) - Storage temperature of food: not reported VEHICLE N/A

Details on mating procedure

- M / F ratio per cage: 1:1 - Length of cohabitation: up to evidence of mating by either observation, copulatory plug or sperm in a vaginal rinse or up to about 3 weeks - Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0 of pregnancy - After successful mating each pregnant female was caged : not reported - Any other deviations from standard protocol: N/A

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Dietary analysis over the course of the study indicated concentration values of DINP to be $0.193 \pm 0.008\%$, $0.390 \pm 0.011\%$, and $0.782 \pm 0.038\%$ in comparison to target levels of 0.2, 0.4 and 0.8%.

Duration of treatment / exposure

Constant exposure up to about 10 weeks prior to mating, continuing throughout the mating period. Males were sacrificed after mating but treatment of the females continued throughout gestation and lactation until weaning of the offspring on postnatal day (PND) 21.

Frequency of treatment

constant exposure 7 days/week

Details on study schedule

- Selection of parents from F1 generation when pups were 21 days of age. - Age at mating of the mated animals in the study: not reported

Doses / concentrations

0.2

Basis nominal in diet % DINP

0.4

Basis nominal in diet % DINP

0.8

Basis nominal in diet % DINP

No. of animals per sex per dose

30 per sex per group

Control animals

yes, plain diet

Further details on study design

- Dose selection rationale: results from a preliminary one generation study demonstrated increased adult liver and kidney weights at dietary levels 0.5%, and body weight gain was reduced at 1% and 1.5%. It was anticipated that the body weight gain of both adults and offspring would be significantly reduced at the 0.8% level. - Rationale for animal assignment (if not random): randomly allocated

Positive control

N/A

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes - Time schedule: daily DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: once per week; during gestation females were examined on gestation day 0, 7, 14 and 21 and postnatal days 0, 4, 7, 14 and 21. BODY WEIGHT: Yes - Time schedule for examinations: once per week; during gestation and lactation female bodyweights were recorded on gestation day 0, 7, 14 and 21 and postnatal days 0, 4, 7, 14 and 21. FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Parental food consumption was measured weekly except during gestation when female food consumption was measured at three to four day intervals. - Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: No data - Compound intake calculated as time-weighted averages from the consumption and body weight gain data: No

Estrous cyclicity (Parental animals)

N/A

Sperm parameters (Parental animals)

N/A

Litter observations

STANDARDISATION OF LITTERS - Performed on day 4 postpartum: yes - If yes, maximum of 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 determined for pups born or found dead

Postmortem examinations (Parental animals)

SACRIFICE - Male animals: All surviving animals following delivery of the last litter it sired. - Maternal animals: All surviving animals after the last litter of each generation was weaned GROSS NECROPSY - Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera HISTOPATHOLOGY / ORGAN WEIGHTS The relevant tissues were prepared for microscopic examination and weighed, respectively.

Postmortem examinations (Offspring)

SACRIFICE - The F1 offspring not selected as parental animals and all F2 offspring were

sacrificed at [?] days of age. Not reported - These animals were subjected to postmortem examinations macroscopic and microscopic examination as follows: GROSS NECROPSY - Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera and brain. HISTOPATHOLOGY / ORGAN WEIGHTS The tissues were prepared for microscopic examination and weighed, respectively. Organ weights of the following in all animals: Liver, kidneys (paired), testes (individual), prostate, seminal vesicles, epididymides (individual), ovaries (individual), and brain. Microscopic examinations of the following in the control and high level DINP (0.8%): Pituitary, testes, epididymides, prostate, seminal vesicles, vagina, uterus, ovaries, mammary gland and gross lesions.

Statistics

Quantitative continuous variables (body weights, food consumption, organ weights, and relative organ weights) were analyzed for statistical significance. Bartlett's test of homogeneity of variance was used to determine if the groups had equal variance at the 1% level of significance. If the variances were equivalent, the groups were compared by using a standard one-way analysis of variance (ANOVA). If significant differences among the means were indicated, Dunnet's test was performed to determine which treated groups differed from control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. If the groups did not have equivalent variances at the 1% level, then a Kruskal-Wallis test (nonparametric) was used to assess differences in group means. If the means were different, Dunn's Rank Sum test (nonparametric) was used to determine which treatment groups differed significantly from control. In addition to the Kruskal-Wallis test, Jonckheere's test (nonparametric) for ordered response was also performed. Pup weight was analyzed by a mixed model of covariance with pups nested within dams, dams nested within dose, and total litter size as the covariant. The mathematical model incorporates the litter size as an independent variable related to pup weight. If body weight differences in groups were identified, the least squares means and the least significant difference test were used to determine which groups differed from the control group. Pup weights were analyzed separately by sex. Parental reproductive and offspring survival incidence data were evaluated by chi-square test analysis to determine if the proportions of incidences differed between the groups tested. Each treatment group was compared to the controls by using a 2x2 Fisher Exact test. Armitage's test for linear trend in the dosage groups was also performed. All tests were reported at the 5% or 1% level of significance.

Reproductive indices

Mating, male and female fertility, fecundity, gestational index or length of gestation were not significantly different from control values in either generation of the two-generation reproductive toxicity study at the highest concentration of DINP tested (0.8% in the diet, ~ 500 mg/kg/day).

Offspring viability indices

P1 effects: There was no apparent effect of treatment on survival. There were no important or dose-dependent clinical signs of toxicity in either P1 males or females or in any of their offspring. F1 effects: Sex ratios and survival during lactation were unaffected by treatment.

Mean litter sizes of the three treated groups were statistically significantly greater than the control group. Each treatment group contained 15 to 20% more pups than the controls between birth and culling (PND 4). At PND 0, weights were significantly reduced in male offspring from the 0.8% group but were within the historical control range. Significant reductions in body weights were also noted in the 0.4% and 0.8% groups on PND 7 and 14 and in all three treatment groups at PND 21, but all of the weights were within the historical control range. F2 effects: PND 0 offspring weights in the second generation were not significantly different from controls when litter size differences were taken into account, but were below the historical control range in the 0.8% group. Offspring body weights during lactation were significantly reduced in the 0.4% and 0.8% groups by comparison to control values. No gross effects related to treatment were recorded at necropsy.

Results and discussions

Effect levels

Endpoint	Generation	Sex	Effect level	Basis for effect level / Remarks
NOAEL	P	male/female	ca. 500 mg/kg bw/day (nominal)	body weight gain
NOAEL	F1	female	≥ 200 — ≤ 260 mg/kg bw/day (nominal)	bodyweight gain
NOAEL	P	male/female	1000 mg/kg bw/day (nominal)	Fertility - No adverse effects observed.
BMC05	F1	male/female	≥ 200 — ≤ 260 mg/kg bw/day	The 95% lower confidence limit for a 5% reduction in the predicted body weights ranges from 0.16 to 0.21 % in the diet.

Observations: parental animals

Clinical signs (parental animals)

no effects

Body weight and food consumption (parental animals)

yes

Test substance intake (parental animals)

no data

Reproductive function: estrous cycle (parental animals)

not examined

Reproductive function: sperm measures (parental animals)

not examined

Reproductive performance (parental animals)

no effects

Organ weights (parental animals)

yes

Gross pathology (parental animals)

no effects

Histopathology (parental animals)

yes

Details on results (parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS) There were no apparent effect of treatment on survival. All but two parental (P1) animals (one male from the 0.4% group and one female from the 0.2% group). There were no important or dose-dependent clinical signs of toxicity in either P1 males or females. BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS) Body weights were unaffected during premating and mating and, among the females, gestation. However, dams in the 0.8% group had significantly reduced body weights at postpartum days 14 and 21. Although food consumption was recorded there is no data reporting any effects on this parameter. TEST SUBSTANCE INTAKE (PARENTAL ANIMALS) No data REPRODUCTIVE FUNCTION: ESTROUS CYCLE (PARENTAL ANIMALS) Not examined REPRODUCTIVE FUNCTION: SPERM MEASURES (PARENTAL ANIMALS) Not examined REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS) There was no statistically significant differences in male mating, male fertility, female fertility, female fecundity or gestational indices. Additionally, there were no differences among groups in the mean length of gestation. ORGAN WEIGHTS (PARENTAL ANIMALS) Statistically significant elevations were found in absolute liver weights: 0.8% DINP levels Males and 0.4 and 0.8% DINP levels Females. Statistically significant elevations were found in absolute kidney weights: 0.4 and 0.8% DINP levels Males and 0.2, 0.4 and 0.8% DINP levels Females Ovarian weight was reduced in females at the 0.8% DINP level. but the difference was only in the left ovary. Relative weights were not statistically different. Absolute weights of testes, epididymides, prostate and seminal vesicles were unaffected. GROSS PATHOLOGY

(PARENTAL ANIMALS) No effects noted HISTOPATHOLOGY (PARENTAL ANIMALS) Microscopic examination of the livers revealed a minimal to moderately increased cytoplasmic eosinophilia in males and females from all DINP treated groups. OTHER FINDINGS (PARENTAL ANIMALS) None

Observations: offspring

Viability (offspring)

no effects

Clinical signs (offspring)

no effects

Body weight (offspring)

yes

Sexual maturation (offspring)

no effects

Organ weights (offspring)

yes

Gross pathology (offspring)

no effects

Histopathology (offspring)

yes

Details on results (offspring)

VIABILITY (OFFSPRING) No effects reported CLINICAL SIGNS (OFFSPRING) No effects reported BODY WEIGHT (OFFSPRING) The mean body weights of males in the 0.4% DINP group were 5-6% below control values but only achieved statistical significance sporadically. The mean female body weights in the same group were about 6% below control values but not significantly different from controls at any time after the first week. At the 0.8% level, the mean body weights of the males were significantly below control values throughout the pre-mating period, but the difference narrowed from ca. 13 to 7%. Among the females at the 0.8% level, the

mean body weights were ca. 10% below and statistically different from control values at Week 0 and remained below control values during pre-mating, although the differences were not significantly different after the third week. This pattern continued through gestation. During lactation, the body weights of the dams were significantly below control values in the 0.8% group. SEXUAL MATURATION (OFFSPRING) There were no effects on any of the classic reproductive indices. ORGAN WEIGHTS (OFFSPRING) Increased mean kidney weights (statistically different from control means) were observed in males at the 0.8% DINP diet and statistically significant increased mean liver weights in females at the 0.8% level. GROSS PATHOLOGY (OFFSPRING) No gross effects were noted during autopsy. HISTOPATHOLOGY (OFFSPRING) Examination of the kidneys revealed an increased incidence of minimal to moderate renal pelvis dilatation (primarily right kidney) in the males at 0.4% and 0.8% groups. This was not observed in females and is considered to be likely related to the induction of alpha 2u-globulin. No histological changes were seen in the testes or accessory organs or any of the other organs from either generation. OTHER FINDINGS (OFFSPRING) None reported

Remarks on results including tables and figures

Applicant's summary and conclusion

Conclusions

This study showed that DINP treatment does not affect fertility or male reproductive development at doses of up to approximately 500 mg/kg body weight/day.

Executive summary

The potential reproductive toxicity of di-isononyl phthalate (DINP) was assessed in a two-generation reproductive toxicity study. Groups of 30 male and female CRL : CD(SD)BR rats were given DINP via dietary administration at levels 0.0, 0.2, 0.4 or 0.8%. There were no changes in any of the classic reproductive parameters, i.e. mating, male or female fertility, fecundity, gestational index, or length of gestation. The overall NOAELs for these effects were the highest dietary level (%) tested, equivalent to approximately 500 mg/kg/day. There were no testicular effects in either the P1 males, exposed as juveniles and young adults or the P2 (F1) offspring exposed in utero, through lactation, and continuously to terminal sacrifice. Adult survival was unaffected at any level of dietary DINP. Liver and kidney weights were elevated at dietary level (%) above ca. 110 mg/kg/day, consistent with evidence from other studies of peroxisomal proliferation at these levels. The LOAEL of 159 mg/kg/day is conservative by nature since the dose of

interest actually consists of a range, 159 – 395 mg/kg/day, leading to the possibility that the NOAEL is on the border of the range or even within it. To address the issue of only having a LOAEL for decreases in pup body weight, the data were evaluated by benchmark dose procedures (Waterman et al., 2000). Benchmark dose modeling is a useful alternative since it

makes no particular assumption about the nature of toxicological dose-responses, other than that the change in response generally does not decrease with higher doses. In particular, there is no specific assumption of the relationship between a putative no-effect level in the dose-response and the benchmark dose. The 95% lower confidence limit for a 5% reduction in the predicted body weights ranged from 0.16-0.21 % in

the diet (~200 – 260 mg/kg/day) and is appropriate for use in risk assessments. Therefore the value of 200 mg/kg/day will be used for risk characterisation.

In this study, neither fertility or male reproductive development was affected when DINP was administered to rats at a dietary dose level of up to approximately 500 mg/kg/day.

Dossier > *Document*

 Dissemination Dossier

 Developmental Endpoint.001

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag supporting study

Study result type read-across from supporting substance (structural analogue or surrogate)

Reliability 1 (reliable without restriction)

Rationale for reliability OECD guideline study; GLP

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1995-04-05
publication	Hellwig, J. et al.	1997	Toxicity of Branched Phthalate Esters in Rats	Food Chem. Toxicol., 35, 501-512					

Materials and methods

Test guideline

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

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

DINP2, at least 95% of the main alcohol component is from alkyl-substituted octanol or heptanol

Test animals

Species

rat

Strain

Wistar

Administration / exposure

Route of administration

oral: gavage

Vehicle

olive oil

Duration of treatment / exposure

day 6 through 15 post coitum (p.c.)

Frequency of treatment

daily

Duration of test

up to day 20 p.c.

Doses / concentrations

40, 200, 1000 mg/kg/d

Basis

No. of animals per sex per dose

9-10

Control animals

yes, concurrent vehicle

Further details on study design

Sex: female

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Basis for effect level / Remarks
NOEL	maternal toxicity	200 mg/kg bw/day	
NOAEL	teratogenicity	1000 mg/kg bw/day	

Remarks on results including tables and figures

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument5703.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-c47ce875-3b97-4cf4-b30a-1a5f3b398de6%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > Document

 Dissemination Dossier

 Developmental Endpoint.002

Administrative Data

Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	16.03.1994 to 22.04.1994
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study undertaken to a standard considered to be equivalent to international test guidelines for a developmental toxicity study .		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1994							1994-11-30
publication	Waterman SJ, Ambrosio JL, Keller LH, Trimmer GW, Nikiforova AI, Harris SB.	1999	Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats.	Reprod Toxicol 13:1-6					

Materials and methods

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 414 (Prenatal Developmental Toxicity Study)	no

Principles of method if other than guideline

There is no explicit statement in the report that the study was undertaken in accordance with a specific guideline, although there are references to the US EPA 'Test guidelines for developmental toxicity' and the EU Annex V methods for the determination of toxicity, 'Teratogenicity '

GLP compliance

yes

Test materials**Test material equivalent to submission substance identity**

yes

Details on test material

- Name of test material (as cited in study report): Di-isononyl phthalate - Substance type: Technical material - Physical state: Colourless liquid - Stability under test conditions: No data - Storage condition of test material: Room temperature

Test animals**Species**

rat

Strain

Sprague-Dawley

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories Inc. - Age at study initiation: Approximately 10 weeks - Weight at study initiation: 212 to 270g - Fasting period before study: No - Housing: Single, except during mating, in suspended stainless steel cages, with wire mesh and absorbent paper below. - Diet (e.g. ad libitum): ad libitum - Water (e.g. ad libitum): ad libitum - Acclimation period: 15 days ENVIRONMENTAL CONDITIONS - Temperature (°C): 20 to 24.4 °C - Humidity (%): 40-70% RH - Air changes (per hr): No data - Photoperiod (hrs dark / hrs light): 12 hours dark/12 hours light IN-LIFE DATES: From: 12 March 1994 To: 22 April 1994

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Details on exposure

PREPARATION OF DOSING SOLUTIONS: -The undiluted test material was thoroughly mixed in carrier (corn oil) prior to dispensing. The test material/carrier mixture was prepared weekly. The test material was soluble in the carrier at the concentrations required for this study. VEHICLE - Justification for use and choice of vehicle (if other than water): No data - Concentration in vehicle: Nominal, 20, 100 or 200 mg/ml - Amount of vehicle (if gavage): 5 ml/kg - Lot/batch no. (if required): DEC1594B, DEC1494B - Purity: No data

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The stability and homogeneity of the test material in corn oil was demonstrated by the testing laboratory before or at the start of treatment. Homogeneity was investigated at 0.8% and 20% w/v. The coefficient of variance ranged from 0 to 0.820%, indicating excellent uniformity. Stability was confirmed for 8 days at room temperature. The concentrations of test material in the carrier were checked for Weeks 1 and 3, and were all within 8% of nominal concentrations.

Details on mating procedure

- Impregnation procedure: co-housed - M/F ratio per cage: 1:1 - Length of cohabitation: Overnight - Verification of same strain and source of both sexes: yes - Proof of pregnancy: vaginal plug/sperm in vaginal smear referred to as Day 0 of pregnancy - Any other deviations

from standard protocol: No

Duration of treatment / exposure

Gestation Day 6 through Day 15

Frequency of treatment

Daily

Duration of test

One month

Doses / concentrations

0 (Control), 100, 500 and 1000 mg/kg/day
Basis nominal conc.

No. of animals per sex per dose

25

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale: No data - Rationale for animal assignment (if not random): Females were assigned to dose groups in the order of mating

Examinations

Maternal examinations

CAGE SIDE OBSERVATIONS: Yes - Time schedule: Twice daily during the treatment period and once daily at other times. DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: females daily during gestation BODY WEIGHT: Yes - Time schedule for examinations: Gestation Day 0, 6, 9, 12, 15, 18 and 21 FOOD CONSUMPTION : Yes - Food consumption for each female concurrently with bodyweight. WATER CONSUMPTION: No POST-MORTEM EXAMINATIONS: Yes - Sacrifice on gestation day 21 - Organs examined: Uterus

Ovaries and uterine content

The ovaries and uterine content was examined after termination: Yes Examinations included: - Gravid uterus weight: Yes (with ovaries) - Number of corpora lutea: Yes - Number of implantations: Yes - Number of early resorptions: Yes - Number of late resorptions: Yes - Other: Live, dead and externally malformed fetuses counted -Uteri of apparently non-pregnant dams: stained to confirm pregnancy status.

Fetal examinations

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

Statistics

Reported in the 'any other information on materials and methods' field below.

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Basis for effect level / Remarks
NOAEL	maternal toxicity	500 mg/kg bw/day	Suppression of bodyweight gain and reduced food intake during GD 6-15 at 1000 mg/kg bw/day.
NOAEL	developmental toxicity	1000 mg/kg bw/day	There were no findings considered biologically important at the highest dosage of 1000 mg/kg bw/day
NOAEL	teratogenicity	1000 mg/kg bw/day	There were no findings considered to be biologically important at the highest dose of 1000 mg/kg bw/day
no NOAEL identified	embryotoxicity	1000 mg/kg bw/day	There were no findings considered to be biologically important at the highest dose of 1000 mg/kg bw/day

Maternal toxic effects

yes

Details on maternal toxic effects

Statistically significant mean bodyweight gain suppression and reduction in mean food consumption were observed in the high dose female animals compared with controls during the

treatment period (GD 6-15)

Embryotoxic / teratogenic effects

no effects

Details on embryotoxic / teratogenic effects

There were no statistically significant differences in mean fetal bodyweight and no statistically significant increases in total or individual external, visceral or skeletal malformations between treated and control animals. There were several statistically significant increases in the incidences of fetal variation both on an individual and litter basis in the treated groups compared with the controls. However, all of these incidences were within the historic control range of the laboratory or were regarded as alternative normal patterns and were not regarded as harmful developmental effects. These differences are tabulated in the results section below.

Remarks on results including tables and figures

Statistically significant increases in fetal variations from controls were as follows:

Fetal parameter	Dose mg/kg	Fetal incidence (%)	Litter incidence (%)	Historic range
Total with visceral variations	0	0.5	4.3	1.1
	100	3.7*	12.0	
	500	4.0*	16.7	4.4
	1000	5.1**	30.4*	
Dilated renal pelvis	0	0.0	0.0	1.1
	100	3.7**	12.0**	
	500	4.0**	16.7**	4.4
	1000	4.5**	26.1**	
Total with skeletal variations	0	16.8	62.5	29.8
	100	15.0	64.0	
	500	28.4**	91.7*	76.0
	1000	43.7**	87.0	
Rudimentary lumbar ribs	0	3.7	25.0	3.8
	100	5.4	20.0	
	500	18.6**	54.2	29.2
	1000	34.5**	78.3**	
Rudimentary cervical ribs	0	1.6	12.5	0.0
	100	1.6	12.0	
	500	1.0	8.3	4.3
	1000	5.7*	30.4	

*Statistically significant $p=0.05$ (Fisher exact test)

F on a per Fetus basis

** Statistically significant $p=0.01$ (Fisher exact test)

L on a per Litter basis

There was a dose-related increase in total fetuses with visceral and skeletal variations on a per fetus basis and a dose-related increase in total fetuses with skeletal variations on a per litter basis. Statistically significant increases in total fetuses with visceral variations were observed in all treated groups when compared with controls. The high dose group also was statistically significant on a per litter basis. Statistically significant increases in total fetuses with skeletal variations were observed in the mid and high dose groups when compared with controls. The mid dose group also was statistically significantly increased for skeletal variations on a per litter basis.

Statistically significant increases in dilated renal pelves were observed in all treated groups when compared with controls on a per fetus basis and in the high dose group when compared with controls on a per litter basis. The increase in dilated renal pelves on a per fetus basis occurred in a dose-related fashion. Rudimentary lumbar ribs were significantly increased ($p \leq 0.01$) in the mid and high dose groups when compared with controls on a per fetus basis and in the high dose group when compared with controls on a per litter basis. These increases occurred in a dose-related fashion on both a per fetus and litter basis. A statistically significant increase in fetuses with rudimentary cervical ribs also was observed in the high dose group when compared with controls.

Applicant's summary and conclusion

Conclusions

Maternal toxicity was evident at 1000 mg/kg/day, as suppression of bodyweight gain and reduced food consumption during the dosing period. Signs of embryotoxicity were not apparent. Accordingly, the maternal NOAEL was established as 500 mg/kg/day and the developmental NOAEL was established as 1000 mg/kg/day. The results indicated that DINP was not a selective developmental toxicant and was not embryotoxic or teratogenic under the conditions of this study.

Executive summary

In this developmental toxicity study, mated female rats received di-isononyl phthalate (DINP) at dosages of 0 (vehicle control), 100, 500 or 1000 mg/kg b.w./day, once daily by oral gavage, from gestation Day 6 through 15.

Maternal toxicity was evident at 1000 mg/kg/day, as suppression of bodyweight gain and reduced food consumption during the dosing period. Signs of embryotoxicity were not apparent. Accordingly, the maternal NOAEL was established as 500 mg/kg/day and the developmental NOAEL was established as 1000 mg/kg/day. The results indicated that DINP was not a selective developmental toxicant and was not embryotoxic or teratogenic under the conditions of this study.

The toxicity study is considered acceptable for classification, and satisfies the guideline

requirements for rat developmental toxicity studies.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument83fc.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-25eee427-2345-4790-8b83-f1434d1973c6%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Spec invest Endpoint.001

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	Acceptable, well documented publication which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2001							2001-03-27
other: Amendment to the report		2001							2001-11-22

Materials and methods

Type of effects studied

biochemical or cellular interactions

Type of method

in vivo

Endpoint addressed

repeated dose toxicity: oral

Principles of method if other than guideline

DINP was administered to groups of 8 male and 8 female mice at dietary concentrations of 0, 500, 1500, 4000 and 8000 ppm for 1 and 4 weeks. Food consumption and body weights were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations were conducted once a week. In the animals treated for 4 weeks, cyanide-insensitive palmitoyl-CoA-oxidation in the liver was examined. DNA-replication (S - Phase response) and apoptosis in the liver was examined in the animals treated for 1 and 4 weeks.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): DINP - Supplier: Exxon Chemical Holland, Inc. - Physical state: Liquid/colorless - Analytical purity: 99.9% - Lot/batch No.: 9116SB34 - Stability under test conditions: stable according to material safety data sheet - Storage condition of test material: Room temperature, under exclusion of light, under N2

Test animals

Species

mouse

Strain

B6C3F1

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Centre d'Elevage R, Janvier, France - Age at study initiation: males 12-13 weeks and females 12-14 weeks - Weight at study initiation: males 20.5-26.9 g and females 16.2-23.8 g - Fasting period before study: 16-20 hr - Housing: singly - Diet (e.g. ad libitum): 9433 LL meal, Eberle Nafag AG, Gossau, Switzerland - 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

Details on exposure

PREPARATION OF DOSING SOLUTIONS: The test substance was weighed out and thoroughly mixed with a small amount of food. Then corresponding amounts of food, depending on the dose groups, were added to this premix in order to obtain the desired concentrations, and mixing was carried out for 10 minutes in a laboratory mixer. DIET PREPARATION - Rate of preparation of diet (frequency): weekly - Storage temperature of food: frozen

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The concentration analyses of each formulation demonstrated values within 10% of target concentration.

Duration of treatment / exposure

1 or 4 weeks

Frequency of treatment

daily, 7 days per week

Post exposure period

none

Doses / concentrations

115, 352, 951, 2022 mg/kg bw/d for males and 142, 441, 1192, 2509 mg/kg bw/d for females
Basis actual ingested 4 weeks treatment

96, 309, 755, 1775 mg/kg bw/d in males and 129, 414, 1121, 2511 mg/kg bw/d in females
Basis actual ingested 1 week treatment

500, 1500, 4000, 8000 ppm
Basis nominal in diet 1 and 4 weeks treatment

No. of animals per sex per dose

8

Control animals

yes, concurrent no treatment

Further details on study design

- Dose selection rationale: In order to correlate peroxisome proliferation with data obtained in the previously conducted long term study (Butala et al. The Toxicologist 36: 173, 1997), concentrations of 500, 1500, 4000 and 8000 ppm in the diet were used.

Examinations

Examinations

Animals were observed twice daily for the clinical signs. Body weight was measured at day 0 and weekly thereafter. Food and compound consumption was determined at weekly interval. Before sacrifice, animals were labeled with BrdUrd for 7 days using ALZET osmotic pumps. The animals were sacrificed by decapitation under CO₂ anaesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Terminal weight assessment was carried out on all animals sacrificed. The weights of the following organs were determined: Liver, Kidneys, Brain. The livers were sliced into two strips 5 mm thick (right, lateral and medial lobes), fixed in 4% formaldehyde solution for 24-48 hours, and afterwards stored in ethanol (70%). A section of jejunum was included as positive control. Hepatocellular proliferation and apoptosis evaluation was performed with immunohistology using BrdU and TUNEL stains (S-phase response and apoptosis). The remainder of the liver was immediately frozen at approximately -70°C for biochemical analysis (cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA), total protein in liver homogenates).

Results and discussions

Details on results

Three animals died during the study, which included one male of 1500 ppm (4 weeks treatment) group, and two males of 8000 ppm (4 weeks treatment) group. As these animals did not show abnormal clinical symptoms prior to death, these deaths were assessed as being spontaneous and not treatment-related. No abnormal clinical symptoms were observed. Body weight was significantly increased in males of the 4000 ppm group (1 week treatment). Body weight change was significantly decreased in females of the 1500 ppm (1 week treatment) group, 8000 ppm (1 week treatment) group, 4000 ppm (4 weeks treatment) group on day 21, and 8000 ppm (4 weeks treatment) group from day 14 to day 28. This may or may not have been a treatment-related effect. This minor finding was considered not relevant for the aim of the study. On day 28, food consumption was significantly increased in males of the 8000 ppm (4 weeks treatment) group, in males of the 1500 ppm (1 week treatment), in males of the 8000 ppm (1 week treatment) group, in females of the 8000 (4 weeks treatment) group and in females of the 8000 ppm (1 week treatment) group. This may or may not have been a treatment-related effect. This minor finding was considered not relevant for the aim of the study. The approximate mean daily test substance intake (in mg/kg body weight per day) over the entire study period for 500, 1500, 4000, 8000 ppm (4 weeks treatment) dietary levels was 115, 352, 951, 2022 mg/kg bw/d in males, respectively, and 142, 441, 1192, 2509 mg/kg bw/d in females, respectively. The approximate mean daily test substance intake (in mg/kg body weight per day) over the entire study period for 500, 1500, 4000, 8000 ppm (1 weeks treatment) dietary levels was 96, 309, 755, 1775 mg/kg bw/d in males, respectively, and 129, 414, 1121, 2511 mg/kg bw/d in females, respectively. After 4 weeks of test substance administration dose-related significant increases in cyanide-insensitive palmitoyl-CoA-oxidation (PAL-CoA) were observed in males treated with 500 ppm and higher, and in females treated with the 1500 ppm and higher. Significant dose-dependent liver weight increase in males and females was recorded after 1 week of treatment with 1500 (females only), 4000 and 8000 ppm of DINP and after 4 weeks of treatment (male and female dose groups 1500, 4000 and 8000 ppm). No significant liver weight changes were recorded for the low dose group (500 ppm). Significant decrease in the terminal body weight of the females of the high dose group (4 weeks treatment only) was also observed. The absolute weights of the kidneys were statistically significantly decreased in the males of the 4000 ppm group (-10%) and in males and females of the 8000 ppm groups (males: -16%, females: -9%). This was noted for the 4 weeks treatment groups only. In addition, the relative weights of the kidneys were statistically significantly decreased in the 4 weeks treated males of the 4000 ppm (-11 %) and of the 8000 ppm groups (-16%). No treatment related gross lesions were noted. After 1 week treatment, a centrilobular hypertrophy of hepatocytes up to moderate degree was observed in the 1500 ppm (males 5/8, females 1/8), 4000 ppm (males and females 8/8) and 8000 ppm (males and females 8/8) groups. Although not dose-dependent, treatment related increase of mitotic figures was observed in all treated groups. After 4 weeks treatment, centrilobular hypertrophy of hepatocytes up to moderate degree was observed in the 500 ppm (only males 1/8), 1500 ppm (males 5/8, females 2/8), 4000 ppm (males and females 8/8) and 8000 ppm (males 6/8, females 8/8) groups. After 4 weeks of treatment, mitotic figures were barely or not increased in their amount, especially when compared with the values after a 1-week treatment. In males, the in all slight increase was seen to be treatment-related. In males, all treated groups showed a significant increase of cell proliferation. The increase was dose dependent and 2 up to 7 fold after 1 week of treatment. After 4 weeks of treatment, an increase was still observed, but at a lower level (1.5 to a significant 2.1 fold) and not in a dose-dependent pattern. In females, the groups treated with 1500, 4000 and 8000 ppm showed as well an increase after 1 week of treatment. The increase

ranged from 1.7 up to 2.4 fold with a significance found for the 4000 and 8000 ppm values. After 4 weeks of treatment, only the 1500 and 4000 ppm groups exhibited still a slight increase, reaching as highest 1.6 fold. Only the zone 3 values of the 1500 ppm group showed a significant increase. The lowest overall values (LI of 71%) are found in the high dose group treated with 8000 ppm. Only a low number of apoptotic cells could be detected in control and treated animals. However, in the male treated groups an increase of apoptotic cells was observed in the 4000 and 8000 ppm groups, resulting in a significant higher value for the top dose group after 1 week of treatment. In females, a slight trend for an increase was found in all dose groups, with a significant higher value in the 1500 ppm group. After 4 weeks of treatment, apoptotic cell values were within the range of the control or lower. Only in females of the 500 ppm group, a slight trend for an increase was recognized.

Remarks on results including tables and figures

Mean cyanide-insensitive palmitoyl-CoA-oxidation (PAL-CoA) in males and females after 4 weeks treatment.

Dose level (ppm)	PAL-CoA (mU/mg protein)	
	Males	Females
0	4.03±0.91	5.28±0.41
500	6.05±1.42*	5.91±0.82
1500	9.31±2.00**	8.88±0.74**
4000	23.14±5.33**	22.05±1.60**
8000	43.37±2.18**	43.90±1.52**

* = $p \leq 0.02$; ** = $p \leq 0.002$

S-phase response (absolute and relative) for the 4 different zones in the liver of males

Adminis- tration period	Test group	Zone 1		Zone 2		Zone 3		Zone 1+2+3	
		LI	%	LI	%	LI	%	LI	%
	Control	0.11	100	1.04	100	0.08	100	0.41	100
1 week	500 ppm	0.19	173	2.18**	210	0.13	163	0.84*	205
	1500 ppm	0.35**	318	2.76**	265	0.27**	338	1.14**	278
	4000 ppm	0.47**	427	2.83**	272	0.75**	938	1.36**	332
	8000 ppm	1.33**	1209	5.64**	542	1.60**	2000	2.90**	707
4 weeks	500 ppm	0.21	191	2.22*	213	0.16*	200	0.87*	212
	1500 ppm	0.31**	282	1.29	124	0.24**	300	0.62	151
	4000 ppm	0.28	255	1.26	121	0.26*	325	0.60	146
	8000 ppm	1.11**	1009	1.19	114	0.14	175	0.83	202

S-phase response (absolute and relative) for the 4 different zones in the liver of females

Adminis- tration period	Test group	Zone 1		Zone 2		Zone 3		Zone 1+2+3	
		LI	%	LI	%	LI	%	LI	%
	Control	1.27	100	3.48	100	0.64	100	1.79	100
1 week	500 ppm	1.17	92	3.20	92	0.78	122	1.71	96

4 weeks	1500 ppm	2.37	187	5.18	149	1.52	238	3.04	170
	4000 ppm	2.99**	235	5.97**	172	2.12	331	3.69**	206
	8000 ppm	3.60**	283	7.55**	217	2.14	334	4.38**	245
	500 ppm	0.92	72	2.48	71	0.59	92	1.32	74
	1500 ppm	2.20	173	4.56	131	1.60	250	2.80	156
	4000 ppm	2.41	190	3.95	114	1.33	208	2.55	142
	8000 ppm	1.60	126	1.71	49	0.51	80	1.27	71

% = relative to control; LI = Labeling Index; * = $p \leq 0.05$; ** = $p \leq 0.01$

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentb0cc.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-8a02f278-7f3c-4f40-a8cb-c282d7be9ae0%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 [Spec invest Endpoint.002](#)

[Administrative Data](#) [Data source](#) [Materials and methods](#)

[Results and discussions](#)

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	Acceptable, well documented study report which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2001							2001-01-23

Materials and methods

Type of effects studied

biochemical or cellular interactions

Type of method

in vivo

Endpoint addressed

repeated dose toxicity: oral

Principles of method if other than guideline

DINP was administered to groups of 5 male and 5 female mice at dietary concentrations of 0, 500, 1500, 4000 and 8000 ppm for 4 weeks. Food consumption and body weights were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations were conducted once a week. The animals were sacrificed by perfusion fixation. Accumulation of liver peroxisomes was evaluated by light and electron microscopy.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): DINP - Supplier: Exxon Chemical Holland, Inc. - Physical state: Liquid/colorless - Analytical purity: 99.9% - Lot/batch No.: 9116SB34 - Stability under test conditions: stable according to material safety data sheet - Storage condition of test material: Room temperature, under exclusion of light, under N₂

Test animals

Species

mouse

Strain

B6C3F1

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS - Source: Centre d'Elevage R, Janvier, France - Age at study initiation: 11-12 weeks - Weight at study initiation: males 25.5-28.0 g and females 20.5-23.4 g - Fasting period before study: 16-20 hr - Housing: singly - Diet (e.g. ad libitum): 9433 LL meal, Eberle Nafag

AG, Gossau, Switzerland - 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

Details on exposure

PREPARATION OF DOSING SOLUTIONS: The test substance was weighed out and thoroughly mixed with a small amount of food. Then corresponding amounts of food, depending on the dose groups, were added to this premix in order to obtain the desired concentrations, and mixing was carried out for 10 minutes in a laboratory mixer. DIET PREPARATION - Rate of preparation of diet (frequency): weekly - Storage temperature of food: frozen

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

The concentration analyses of each formulation demonstrated values within 10% of target concentration.

Duration of treatment / exposure

4 weeks

Frequency of treatment

daily, 7 days per week

Post exposure period

none

Doses / concentrations

117, 350, 913, 1860 mg/kg bw/d for males and 168, 546, 1272, 2807 mg/kg bw/d for females
Basis actual ingested

500, 1500, 4000, 8000 ppm

Basis nominal in diet

No. of animals per sex per dose

5

Control animals

yes, concurrent no treatment

Further details on study design

- Dose selection rationale: In order to correlate peroxisome proliferation with data obtained in the previously conducted long term study (Butala et al. The Toxicologist 36: 173, 1997), concentrations of 500, 1,500, 4000 and 8000 ppm in the diet were used.

Examinations

Examinations

Animals were observed twice daily for the clinical signs. Body weight was measured at day 0 and weekly thereafter. Food and compound consumption was determined at weekly interval. The test animals were deeply anesthetized (Narcoren, about 4 ml/kg body weight) at the end of the study and sacrificed by perfusion fixation. The liver was removed for the light and electron microscopic examination. The liver was processed histotechnically for cytochemical presentation of catalase in peroxisomes, plastic embedding and semi-thin sectioning (FAHIMI, J Cell Biol 43: 275-288, 1969). The incubated semi-thin sections were examined by light microscopy and assessed for the presence of peroxisomes. The average number of peroxisomes of various sizes was estimated with a simple grading scheme (1 to 5). From all animals, blocks from semi-thin section where zone 3 and 2 regions are best recognized, were selected for further ultra-thin sectioning. The ultra-thin sections were examined by electron microscopy and morphometric measurements were carried out. Quantitative measurements of peroxisomes was performed in ultra-thin sections. From all measurements, the total number and total area of peroxisomes (μm^2) were determined. The total area of all peroxisomes was expressed in relation to the whole cytoplasmic region selected for measurement (in %). All peroxisomes were classified in 5 size classes (<0.1 , <0.3 , <0.5 , <0.75 and $>0.75 \mu\text{m}^2$). Mean values for the counted amount (for each size class) and the total areas of peroxisomes in % of the covered cytoplasmic area of the hepatocytes were calculated.

Results and discussions

Details on results

One female of dose the 4000 ppm group died on day 16. Due to the lack of a dose-response relationship, this was considered to have been incidental finding. No abnormal clinical symptoms were observed. Body weight was significantly decreased in males of the 8000 ppm group on day 21. Body weight change was significantly decreased in males of the 4000 ppm group on days 15 - 28 and in males of the 8000 ppm group on days 7 - 28. This may or may not have been a treatment-related effect. This minor finding was not considered relevant for the aim of the study. Food consumption was statistically significantly decreased in males of the 8000 ppm group on day 7. Due to the isolated occurrence, this was considered to have been incidental. The approximate mean daily test substance intake (in mg/kg body weight per day) over the entire study period for 500, 1500, 4000, 8000 ppm dietary levels was 117, 350, 913, 1860 mg/kg bw/d in males, respectively, and 168, 546, 1272, 2807 mg/kg bw/d in females, respectively. There were no gross lesions noted. In males and females, only very few or few (grade 1 or 2) peroxisomes were present in test animals of the control, 500 and 1500 ppm group. No lobular enhancement could be recognized. In the 4000 and 8000 ppm group, a moderate number (grade 3) or many (grade 4) peroxisomes were found. A centrilobular enhancement (zone 3) was clearly visible. Electron microscopic, morphometric measurements of the size of peroxisomes revealed a significant increase of total number and total area of peroxisomes in the 1500 ppm group and above. Males and females showed similar results in respect to the total area development of peroxisomes. The % change in development of peroxisomal area in males relative to control for the dose groups 500, 1500, 4000 and 8000 ppm were +12, +84, +447 and +886, respectively. The % change in development of peroxisomal area in females relative to control for the dose groups 500, 1500, 4000 and 8000 ppm were -24, +46, +284, +498, respectively.

Remarks on results including tables and figures

Quantitative evaluations of hepatocellular peroxisomes- mean values (males)

Dose group	Total area of peroxisomes (%)	Size class				
		1	2	3	4	5
0 (control)	1.93±0.63	12.1±4.5	10.7±3.7	1.0±0.4	0.1±0.1	0.1±0.1
500 ppm	2.16±0.29	18.2±3.5*	11.0±2.1	1.3±0.3	0.04±0.1	0.0±0.0
1500 ppm	3.56±0.64**	55.0±17.6**	15.5±3.5	0.8±0.2	0.1±0.1	0.04±0.04
4000 ppm	10.57±3.49**	49.6±17.2**	47.3±18.4**	10.9±2.9**	1.1±0.5**	0.1±0.1
8000 ppm	18.68±3.63**	18.3±7.5**	35.5±8.6**	27.9±5.8**	13.9±3.0**	2.1±0.9**

Quantitative evaluations of hepatocellular peroxisomes- mean values (females)

Dose group	Total area of peroxisomes (%)	Size class				
		1	2	3	4	5
0 (control)	2.48±0.53	24.6±12.9	12.1±1.5	1.4±0.6	0.1±0.1	0.0±0.0
500 ppm	1.88±0.17	22.3±4.4	9.3±1.5	0.5±0.3	0.02±0.0	0.0±0.0
1500 ppm	3.63±0.67*	32.4±12.7	19.9±4.5**	1.2±0.7	0.02±0.0	0.0±0.0
4000 ppm	9.52±2.02**	31.3±10.1	37.2±9.4**	11.1±3.2**	2.1±1.4**	0.1±0.3
8000 ppm	14.84±6.50**	26.4±3.8	35.5±6.1**	18.6±7.3**	9.7±8.0**	1.8±2.1

*: P= 0.05

** : P= 0.01

A clear dose-dependent development of the peroxisomal area in the cytoplasm of hepatocytes was observed covering the dietary range of 1500 to 8000 ppm. In males, a nearly 9-fold increase, and in females a 5-fold increase were observed. No significant influence on the peroxisomal area development was found for males and females in the 500 ppm group. A comparison of the development of differently sized peroxisomes (5 classes), showed a clear statistical significant increase of class 1 peroxisomes ("microperoxisomes") in males in the 1500 to 8000 ppm groups. A small but statistically significant increase was also noted for the 500 ppm group. No statistically significant increase of class 1 peroxisomes was recorded in any dose group in the females, as comparable numbers of microperoxisomes appeared in the control and all treated groups. Class 2 peroxisomes showed in males a significant increase in total counts only in the 4000 and 8000 ppm groups. In females, a significant increase is observed only in the 1500 to 8000 ppm groups. Class 3 and 4 peroxisomes were found to be significantly increased in both sexes only in the 4000 and 8000 ppm groups. Class 5 peroxisomes were noted infrequently and only slightly increased in the top dose group.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument23b7.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-950fea21-ad29-4ea6-bbc9-3e56def9a014%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > *Document*

 Dissemination Dossier

 Spec invest Endpoint.003

Administrative Data Data source Materials and methods

Results and discussions

Administrative Data

Purpose flag	supporting study
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability	Acceptable, well documented study which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1996							

Materials and methods

Type of effects studied

biochemical or cellular interactions

Type of method

in vivo

Endpoint addressed

repeated dose toxicity: oral

Principles of method if other than guideline

This study was designed to provide information on the ability of Di (isononyl)phthalate (DINP) to cause hepatocellular proliferation or effect on DNA concentration, when administered to mice in the diet for 13 weeks. A group of 12 male mice received the test substance at dietary level of 10000 ppm. In addition, 12 male mice received the basal diet only and served as the control group and 12 male mice received positive control substance (WY 14463) and served as positive control group. Criteria evaluated in these animals included survival, body weight, food consumption, organ weights, gross pathology and microscopic pathology (liver specific), hepatocellular proliferation and DNA content.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

no

Details on test material

- Name of test material (as cited in study report): Di(isononyl)phthalate - Physical state: clear colorless liquid - Analytical purity: >99% - Lot/batch No.: QCL 9004-273 - Lot/batch No.: QCL 9004-273

Test animals

Species

mouse

Strain

B6C3F1

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS - Source: Charles River Laboratories, Inc., Raleigh, North Carolina - Weight at study initiation: 23.3 g (mean) - Housing: 2 per cage - Diet (e.g. ad libitum): Purina Certified

Rodent Chow 5002 - Water (e.g. ad libitum): tap water ENVIRONMENTAL CONDITIONS -
Temperature (°C): 20-26 - Humidity (%): 32-59 - Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration

oral: feed

Details on exposure

PREPARATION OF DOSING SOLUTIONS: The dose level was prepared by placing feed into an appropriately sized glass beaker. A depression was made in the center of the feed and the weighed test material was added to the depression. This test material-feed combination was mixed with approximately 200 g of feed for 2-3 minutes in a Waring blender to form a homogeneous premix. Additional premixes were needed as the amount of test material increased at the higher dose levels. The premix(es) were then added to 5 kg of feed in a Hobart blender and mixed for 10 minutes. The premix from the Hobart blender was added to the required amount of feed for the dose level and mixed in a Patterson-Kelly Twin Shell mixer (2 cu. ft.) fitted with an intensifier bar for 1 min/kg. At no time during the mixing procedure was plastic allowed to come in contact with the test material. DIET PREPARATION - Rate of preparation of diet (frequency): weekly - Storage temperature of food: 2-6°C

Analytical verification of doses or concentrations

yes

Duration of treatment / exposure

13 weeks

Frequency of treatment

daily, 7 days per week

Post exposure period

none

Doses / concentrations

10000 ppm

Basis nominal in diet

2323 mg/kg bw/d
Basis actual ingested

No. of animals per sex per dose

10

Control animals

yes, concurrent no treatment

Examinations

Examinations

Animals were observed daily for clinical signs. Body weight was measured at days 1, 7, 15, 29, 50, 78 and 92 of experiment. Food consumption and compound intake was weekly determined. Three days before sacrifice, animals were labeled with BrdUrd (20 mg/ml; 1 µl/hr) for 72 hours using ALZET osmotic pumps. Six animals from each group were sacrificed at day 7 and at the termination of the experiment (day 92). Terminal body weights were measured. Liver was removed and weighed. Tissues from liver (standard tissue samples from left and right median, and right anterior lobes) and duodenum from each animal were preserved in 10% neutral-buffered formalin and examined microscopically. The remainder of the liver was immediately frozen at approximately -70°C for biochemical analysis. For the cell proliferation portion of the study, three to five micron paraffin embedded sections from the left lateral; median and right lobes of the livers as well as samples from the duodenum were taken and processed for immunohistochemistry. Each slide was prepared with sections from both liver and duodenum so that the duodenum (a proliferating organ) could be used as an internal control for delivery of label and immunohistochemical staining. The slides were stained for determination of cell proliferation as measured by incorporation of BrdUrd into DNA using Biogenex antibodies with peroxidase-conjugated streptavidin and a 3,3-diaminobenzidine tetrahydrochloride (DAB) chromogen and hematoxylin counterstain. The percentage of nuclei incorporating label in the liver was determined microscopically at approximately 400 to 600X magnification. Three slides from the left lateral lobe were finally analyzed as no differences were in labeling existed between lobes. S-phase nuclei labeling indices (LI) for each animal were calculated as follows: Labeled S-phase nuclei (LI) = no. of labeled hepatocyte nuclei x 100/ total no. of hepatocytes counted. DNA content: Samples of livers were rinsed in cold homogenization buffer (tris HCl, pH 8.3, 154 mM KCl), minced and homogenized. After homogenization, the samples were precipitated in the presence of ice cold trichloroacetic acid (TCA) and centrifuged. The precipitate was washed twice with 14% cold TCA and then washed with 95% ethanol. The washed precipitate was suspended in 7% TCA and heated to approximately 90 °C for 15 minutes. The samples were then centrifuged, washed once with cold 5% TCA and the supernatants/DNA collected. The supernatants/DNA were stored at -70°C for later use (quantification).

Positive control

WY 14463 [4-Chloro-6-(2,3-xylylidino)-2-pyrimidinylthio]acetic acid was administered at dose level of 1000 ppm in similar manner as the test compound.

Results and discussions

Details on results

No mortality was observed in control and treated groups. In the positive control (PC) group, 3 unscheduled deaths were reported at days 50, 85 and 92. No clinical signs were observed in all groups, except in 1 animal of positive control group who was observed with hunched posture, dyspnea, hypoactive behaviour and few feces. No treatment related changes in body weight were observed in control and treated groups. Absolute food consumption was significantly increased in the treated group at day 85 of the experiment. No difference was observed in the total food consumption of the 3 groups. Mean overall compound consumption (mg/kg bw/d) for weeks 1-13 for 10000 ppm dietary levels was 2323 mg/kg bw/day. Absolute and relative liver weight was significantly increased in the treated and PC groups. Grossly, pale areas was observed in the liver of 3 treated animals sacrificed at day 7. At 92 days of sacrifice, liver was enlarged in all 6 animals, and presence of pale areas and mass was observed in 2 animals. Histologically, hepatocellular enlargement occurred and was accompanied by pigment accumulation, cytoplasmic eosinophilia and focal necrosis. Significant decrease in cell proliferation (indicated by LI) was observed in the treated group at terminal sacrifice (day 92). No effect was observed at interim sacrifice (day 7). The DNA content was significantly decreased in the treated group at interim and terminal sacrifice. Significantly increased cell proliferation and decreased DNA content was observed in the positive control group at both intervals (interim and terminal sacrifice).

Remarks on results including tables and figures

Mean $\pm$ SD S-phase response (LI) and DNA concentration after interim and terminal sacrifice

Dose group	Labeling Index (LI)		mg DNA/g liver	
	Interim sacrifice	Terminal sacrifice	Interim sacrifice	Terminal sacrifice
Control	0.43 $\pm$ 0.30	0.21 $\pm$ 0.07	2.57 $\pm$ 0.17	2.73 $\pm$ 0.14
Positive control	17.4 $\pm$ 13.0*	4.6 $\pm$ 2.7*	1.27 $\pm$ 0.18*	1.84 $\pm$ 0.15*
Treated (1000 ppm DINP)	0.52 $\pm$ 0.29	0.06 $\pm$ 0.06*	1.56 $\pm$ 0.23*	2.06 $\pm$ 0.06*

* $p \leq 0.05$

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument2f4f.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-eb03fd80-31b8-47fa-9f55-d8af5b10ee12%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

Dossier > Document

 Dissemination Dossier

 Sensitization human Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study		
Study result type	experimental result	Study period	5.6.1995 to 15.9. 1995
Reliability	1 (reliable without restriction)		
Rationale for reliability	A GLP compliant study, using an established test protocol for human clinical investigation (Modified Draize Procedure).		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1995-10-13
publication	Medeiros AM, Devlin DJ, Keller LH.	1999	Evaluation of skin sensitization response of dialkyl (C6-C13) phthalate esters.	Contact Dermatitis. 1999 Nov;41(5):287-9.					

Materials and methods

Type of sensitisation studied

skin

Study type

study with volunteers

Test guideline

Qualifier

no guideline required

Guideline

Deviations

Principles of method if other than guideline

Modified Draize Procedure. J.H Draize, 'Dermal toxicity' in Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (FDA)

GLP compliance

yes

Test materials**Test material equivalent to submission substance identity**

yes

Details on test material

A transparent , thin liquid supplied to the research facility on 4 May, 1995. No other details reported.

Method**Type of population**

general

Controls

None

Route of administration

dermal

Results and discussions

Results of examinations

No dermal responses were observed from MRD 95-140 during either the preliminary or main human repeated insult patch tests.

Remarks on results including tables and figures

No dermal responses were observed from MRD 95-140 during the preliminary or main human patch test studies. The individual scores for the preliminary test and the group total reaction scores for the main test are appended in the 'attached background material' window below.

Applicant's summary and conclusion

Conclusions

No evidence of clinical sensitisation or irritation was observed in any of the subjects completing the study.

Executive summary

50% w/w MRD 95-140 (Di-isononyl phthalate) was applied as a 50% w/w preparation in petrolatum under occluded patches, to

the deltoid region of the upper arms of 128 human volunteers for 24 hours, three times a week, during a three week induction period (nine applications), and once, approximately two weeks later, during the challenge. No evidence of clinical sensitisation or irritation was observed in any of the 104 subjects completing the study.

The study is considered acceptable to support classification and risk assessment and satisfies the protocol requirements for human patch test studies.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumentbf06.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-55cccef6-9d65-42bb-91fe-13ab37cceed2%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Dissemination Dossier

 Exposure other Endpoint.001

Administrative Data Data source

Materials and methods

Results and discussions Applicant's summary and conclusion

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability	Acceptable, well-documented study report, GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		1995							1995-08-20

Materials and methods

Type of information

A study consisting of a single 24-hour application using an occluded patch was conducted to evaluate the potential to cause irritation. Two evaluations were made; one 30 minutes after patch removal and the second at 24 hours post-patch removal. Two control were also tested; a positive irritant control and a negative control.

Endpoint addressed

skin irritation / corrosion

Test guideline

Qualifier

Guideline

Deviations

no guideline followed

Principles of method if other than guideline

A single 24-hour exposure was used.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Details on test material

- Name of test material (as cited in study report): MRD 95-140 - Substance type: liquid -
Analytical purity: assumed 100 %

Method

Ethical approval

confirmed and informed consent free of coercion received

Details on study design

Healthy male and female volunteers were entered into the study. Each test patch received 0.1 – 0.3 ml of the assigned test article. The final quantity dispensed is dependent upon the nature of the test material. Each subject will receive a single 24 +/- 1 hour contact application to specific skin sites of the paraspinal region of the back. All applications and patch removal will be conducted by a technician at the laboratory. Sites are scored at 30 minutes post-patch removal and 24 hours post-patch removal. Scoring of irritation was conducted by an experienced technical staff member using a 0-3 grade scoring scale for erythema and edema (3 being the most severe).

Exposure assessment

no data

Details on exposure

TYPE OF EXPOSURE: Occluded patch EXPOSURE LEVELS: 0.3-0.5 ml EXPOSURE PERIOD: 24 hours POSTEXPOSURE PERIOD: 24 hours

Results and discussions

Results

The group mean score for irritation was 0.00

Applicant's summary and conclusion

Conclusions

Not irritating.

Executive summary

MRD 95-140 (CAS 68515-48-0) was applied undiluted (0.2 ml) for 24 hours to human volunteers (fourteen female and one male subject) using an occluded patch applied to specific skin sites of the paraspinal region of the back, and was followed by an observation period of 24 hours. Two controls were also tested: a positive irritant control of 0.5% sodium lauryl sulphate in distilled water and a negative control of 0.89% NaCl in distilled water. No responses were observed. Mild to moderate erythema was observed with the positive irritant control at the 30-minute or 24 hour evaluation.

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 [Other Endpoint.001](#)

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Administrative Data

Purpose flag supporting study

Study result type other: Summary data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Harris CA, Henttu P, Parker MG, Sumpter JP.	1997	The estrogenic activity of phthalate esters in vitro.	Environ Health Perspect. Aug;105(8):802-11					
publication	Zacharewski TR, Meek MD, Clemons JH, Wu ZF, Fielden MR, Matthews JB.	1998	Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters.	Toxicol Sci. 46(2):282-93					
publication	Borch J, Ladefoged O, Hass U, Vinggaard	2004	Steroidogenesis in fetal male rats is reduced by DEHP and	Reprod Toxicol. Jan-Feb;18(1):53-61.					

AM.			DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats.	
			Effects of maternal exposure to diisononylphthalate (DINP) and 1,1-dichloro-2,2-bis(p-chlorophenyl-ethylene (p,p'-DDE) on steroidogenesis in the fetal rat testis and adrenal gland.	
publication	Adamsson A, Salonen V, Paranko J, Toppari J	2009	Reproductive Toxicology (28): 66-74	

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument1762.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-afl615be-867a-49b6-921e-459c99f76554%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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Administrative Data

Purpose flag supporting study
Study result type other: Summary data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Valles EG, Laughter AR, Dunn CS, Cannelle S, Swanson CL, Cattley RC, Corton JC.	2003	Role of the peroxisome proliferator-activated receptor alpha in responses to diisononyl phthalate.	Toxicology. Sep 30;191(2-3):211-25.					
publication	Kaufmann W, Deckardt K, McKee RH, Butala JH, Bahnemann R.	2003	Tumor induction in mouse liver: diisononyl phthalate acts via peroxisome proliferation.	Regul Toxicol Pharmacol. Oct;36(2):175-83.					

publication	Smith JH, Isenberg JS, Pugh G Jr, Kamendul is LM, Ackley D, Lington AW, Klaunig JE.	2000	Comparative in vivo hepatic effects of Di-isononyl phthalate (DINP) and related C7-C11 dialkyl phthalates on gap junctional intercellular communication (GJIC), peroxisomal beta-oxidation (PBOX), and DNA synthesis in rat and mouse liver.	Toxicol Sci. Apr;54(2):3 12-21.
publication	Hasmall SC, James NH, Macdonal d N, West D, Chevalier S, Cosulich SC, Roberts RA.	1999	Suppression of apoptosis and induction of DNA synthesis in vitro by the phthalate plasticizers monoethylhexylph thalate (MEHP) and diisononylphthalate (DINP): a comparison of rat and human hepatocytes in vitro.	Arch Toxicol. 1999 Nov;73(8- 9):451-6.
publication	Barber ED, Astill BD, Moran EJ, Schneider BF, Gray TJ, Lake BG, Evans JG.	1997	Peroxisome induction studies on seven phthalate esters.	Toxicol Ind Health. Jun;3(2):7- 24.
publication	Kamendul is LM, Isenberg JS, Smith JH, Pugh G Jr, Lington AW, Klaunig JE.	2002	Comparative effects of phthalate monoesters on gap junctional intercellular communication and peroxisome proliferation in rodent and primate hepatocytes.	J Toxicol Environ Health A. 2002 Apr 26;65(8):56 9-88.

publicati on	Benford D. et al	198 6	Species differences in the response of cultured hepatocytes to phthalate esters	Food and Chemical Toxicology. 24(6,7):799- 800
publicati on	Shaw D, Lee R, Roberts RA.	200 2	Species differences in response to the phthalate plasticizer monoisononylphtha late (MINP) in vitro: a comparison of rat and human hepatocytes.	Arch Toxicol. Jun;76(5- 6):344-50.
publicati on	Hall M, Matthews A, Webley L, Harling R.	199 9	Effects of di- isononyl phthalate (DINP) on peroxisomal markers in the marmoset-DINP is not a peroxisome proliferator.	J Toxicol Sci. 1999 Aug;24(3):2 37-44.Links
publicati on	Ito Y, Yamanos hita O, Asaeda N, Tagawa Y, Lee CH, Aoyama T, Ichihara G, Furuhashi K, Kamijima M, Gonzalez FJ, Nakajima T.	200 7	Di(2- ethylhexyl)phthalate induces hepatic tumorigenesis through a peroxisome proliferator- activated receptor alpha-independent pathway.	J Occup Health. 2007 May;49(3):1 72-82.

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument9d6a.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-e28f8f74-8277-48d5-92b9-bc1e8efc62c8%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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 Other Endpoint.003

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Administrative Data

Purpose flag supporting study

Study result type other: Summray data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Risk Assessment	European Food Safety Authority	2005	Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Diisononylphthalate (DINP) for use in food contact materials	EFSA-Q-2003-194					
other: Risk Assessment		2003							
other: Risk Assessment	US Consumer Products	2001	Chronic Hazard Advisory Panel on Diisononyl						

nt	Safety Commissi on	Phthalate	
		1,2- Benzenedicarbox ylic acid, di-C9- 11-branched alkyl esters, C10- rich, CAS#:	Risk Assessmen
publicatio n	European Commissi on	200 3 68515-49-1, EINECS#: 271- 091-4.and:di- "isodecyl" phthalate (DIDP), CAS#: 26761-40- 0, EINECS#: 247-977-1	t Report Vol. 36 (EUR 20785 EN)

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocument3ec4.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGCIR-c90c4dfc-3215-44b2-bf9d-25d1f0bb4508%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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Administrative Data

Purpose flag supporting study
Study result type other: Summary data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Gray LE Jr, Ostby J, Furr J, Price M, Veeramachandeni DN, Parks L.	2000	Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat.	Toxicol Sci.					
publication	Hass U, Fllinska M, Kledal T.	2003	Antiandrogenic effects of diisononyl phthalate in rats	Reproductive Toxicology					
publication	Lee BM, Koo HJ.	2007	Hershberger assay for antiandrogenic effects of phthalates.	J Toxicol Environ Health A.					
publication	Masutomi N,	200	Impact of dietary	Toxicology.					

on	Shibutani M, 3 Takagi H, Uneyama C, Takahashi N, Hirose M	exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reprodu ctive systems in later life	2003 Nov 5;192(2- 3):149-70
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Applicant's summary and conclusion

Executive summary

<http://apps.echa.europa.eu/registered/data/DISS-828e03e1-4edc-1b7a-e044-00144fd73934/showdocumenteee4.html?treeUuid=DISS-828e03e1-4edc-1b7a-e044-00144fd73934%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934&uuid=AGGR-1bfc3f14-858a-4ce3-85d2-d5aea48d91b9%2FDISS-828e03e1-4edc-1b7a-e044-00144fd73934>

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First-aid measures

INHALATION Remove from further exposure. For those providing assistance, avoid exposure to yourself or others. Use adequate respiratory protection. If respiratory irritation, dizziness, nausea, or unconsciousness occurs, seek immediate medical assistance. If breathing has stopped, assist ventilation with a mechanical device or use mouth-to-mouth resuscitation. **SKIN CONTACT** Wash contact areas with soap and water. Remove contaminated clothing. Launder contaminated clothing before reuse. **EYE CONTACT** Flush thoroughly with water. If irritation occurs, get medical assistance. **INGESTION** First aid is normally not required. Seek medical attention if discomfort occurs.

Fire-fighting measures

EXTINGUISHING MEDIA Appropriate Extinguishing Media: Use water fog, foam, dry chemical or carbon dioxide (CO₂) to extinguish flames. Inappropriate Extinguishing Media: Straight streams of water **FIRE FIGHTING** Fire Fighting Instructions: Evacuate area. Prevent run-off from fire control or dilution from entering streams, sewers or drinking water supply. Fire-fighters should use standard protective equipment and in enclosed spaces, self-contained breathing apparatus (SCBA). Use water spray to cool fire exposed surfaces and to protect personnel. Hazardous Combustion Products: Smoke, Fume, Incomplete combustion products, Oxides of carbon

Accidental release measures

NOTIFICATION PROCEDURES In the event of a spill or accidental release, notify relevant authorities in accordance with all applicable regulations. **SPILL MANAGEMENT** Land Spill: Eliminate all ignition sources (no smoking, flares, sparks or flames in immediate area). Stop leak if you can do so without risk. Absorb or cover with dry earth, sand or other non-combustible material and transfer to containers. If liquid is too viscous for pumping, shovel it up into a suitable container for recycle or disposal. Recover by pumping or with suitable absorbent. Water Spill: Warn other shipping. Remove from the surface by skimming or with suitable absorbents. Seek advice of a specialist Water spill and land spill recommendations are based on the most likely spill scenario for this material; however, geographic conditions, wind, temperature, (and in the case of a water spill) wave and current direction and speed may greatly influence the appropriate action to be taken. For this reason, local experts should be consulted. Note: Local regulations may prescribe or limit action to be taken. **ENVIRONMENTAL PRECAUTIONS** Large Spills: Dyke far ahead of liquid spill for later recovery and disposal. Prevent entry into waterways, sewers, basements or confined areas.

Handling and storage

HANDLING Avoid contact with skin. Provide adequate ventilation if fumes or vapour are generated. Prevent small spills and leakage to avoid slip hazard. DO NOT handle, store or open near an open flame, sources of heat or sources of ignition. Protect material from direct sunlight. Material can accumulate static charges which may cause an electrical spark (ignition source). Loading/Unloading Temperature: [Ambient] Transport Temperature: [Ambient] Transport Pressure: [Ambient] Static Accumulator: This material is a static accumulator. Storage Do not store in open or unlabelled containers. Keep container closed. Handle containers with care. Open slowly in order to control possible pressure release. Store in a cool, well-ventilated area. Storage Temperature: [Ambient] Storage Pressure: [Ambient] Suitable Containers/Packing: Drums; Barges; Tank Cars Suitable Materials and Coatings: Carbon steel; Stainless steel; Polypropylene; Teflon; Aluminium; Nylon; Viton Unsuitable Materials and Coatings: Butyl rubber; Natural rubber; Vinyls **ENGINEERING CONTROLS** The level of protection and types of controls necessary will vary depending upon potential exposure conditions. Control measures to consider: Adequate ventilation should be provided so that exposure limits are not exceeded. **PERSONAL PROTECTION** Personal protective equipment selections vary based on potential exposure conditions such as applications, handling practices, concentration and ventilation. Information on the selection of protective equipment for use with this material, as provided below, is based upon intended, normal usage. **Respiratory Protection:** If engineering controls do not maintain airborne contaminant concentrations at a level which is adequate to protect worker health, an approved respirator may be appropriate. Respirator selection, use, and maintenance must be in accordance with regulatory requirements, if applicable. Types of respirators to be considered for this material include: Half-face filter respirator Type P filter material., European Committee for Standardization (CEN) standards EN 136, 140 and 405 provide respirator masks and EN 149 and 143 provide filter recommendations. For high airborne concentrations, use an approved supplied-air respirator, operated in positive pressure mode. Supplied air respirators with an escape bottle may be appropriate when oxygen levels are inadequate, gas/vapour warning properties are poor, or if air purifying filter capacity/rating may be exceeded. **Hand Protection:** Any specific glove information provided is based on published literature and glove manufacturer data. Glove suitability and breakthrough time will differ depending on the specific use conditions. Contact the glove manufacturer for specific advice on glove selection and breakthrough times for your use conditions. Inspect and replace worn or damaged gloves. The types of gloves to be considered for this material include: If prolonged or repeated contact is likely, chemical-resistant gloves are recommended. If contact with forearms is likely, wear gauntlet-style gloves. Neoprene, Nitrile, CEN standards EN 420 and EN 374 provide general requirements and lists of glove types. **Eye Protection:** If contact is likely, safety glasses with side shields are recommended. **Skin and Body Protection:** Any specific clothing information provided is based on published literature or manufacturer data. The types of clothing to be considered for this material include: If prolonged or repeated contact is likely, chemical, and oil resistant clothing is recommended. **Specific Hygiene Measures:** Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants. Discard contaminated clothing and footwear that cannot be cleaned. Practice good housekeeping. **STABILITY:** Material is stable under normal conditions. **CONDITIONS TO AVOID:** Excessive heat. High energy sources of ignition. **MATERIALS TO AVOID:** Strong oxidisers **HAZARDOUS DECOMPOSITION PRODUCTS:** Material does not decompose at ambient temperatures. **HAZARDOUS POLYMERIZATION:** Will not occur. Disposal recommendations based on material as supplied. Disposal must be in accordance with current applicable laws and regulations, and material characteristics at time of disposal. **DISPOSAL RECOMMENDATIONS** Suitable routes of disposal are supervised incineration, preferentially with energy recovery, or appropriate recycling methods in accordance with applicable regulations and

material characteristics at the time of disposal. REGULATORY DISPOSAL INFORMATION European Waste Code: 07 02 XX NOTE: These codes are assigned based upon the most common uses for this material and may not reflect contaminants resulting from actual use. Waste producers need to assess the actual process used when generating the waste and its contaminants in order to assign the proper waste disposal code(s). Empty Container Warning Empty Container Warning (where applicable): Empty containers may contain residue and can be dangerous. Do not attempt to refill or clean containers without proper instructions. Empty drums should be completely drained and safely stored until appropriately reconditioned or disposed. Empty containers should be taken for recycling, recovery, or disposal through suitably qualified or licensed contractor and in accordance with governmental regulations. DO NOT PRESSURISE, CUT, WELD, BRAZE, SOLDER, DRILL, GRIND, OR EXPOSE SUCH CONTAINERS TO HEAT, FLAME, SPARKS, STATIC ELECTRICITY, OR OTHER SOURCES OF IGNITION. THEY MAY EXPLODE AND CAUSE INJURY OR DEATH.

Land transport (ADR/RID/)

UN number

Not Regulated for Land Transport

Inland waterway transport (AND(R))

UN number

Not Regulated for Inland Waterways Transport

Marine transport (IMDG)

UN number

Not Regulated for Sea Transport according to IMDG-Code

Marine pollutant

no

Air transport (ICAO/IATA)

UN number

Not Regulated for Air Transport