BASF

The Chemical Company

Helping Make Products Better™

RECEIVED
OPPT DMC

07 JAN 31 AM 6:24

FED EX # 7990 7712 2595
7990 7712 2584

Date January 29, 2007

8 6 0 7 0 0 0 0 0 4 6

OPPT Document Control Office (DCO)
EPA East Room 6428
1201 Constitution Ave., N.W.
Washington DC, 20460
Attn: 8(d) Health and Safety Reporting Rule (Notification/Reporting)

Re : Submission of TSCA 8(d) Health and Safety Study reports – BASF Corporation

Dear 8(d) Coordinator:

In compliance with 40 CFR 716, BASF hereby submits requested Health and Safety Study reports for substances recently added to the list of subject chemicals (71 FR 47130).

This submission contains information for the following list of substances:

62-56-6	121-69-7	1738-25-6
83-41-0	127-68-4	3039-83-6
84-69-5	131-57-7	4316-73-8
96-22-0	137-20-2	61788-76-9
104-93-8	645-62-5	68188-18-1
110-18-9	939-97-9	68609-05-2
111-44-4	1111-78-0	68909-77-3

Should you have questions regarding this submission, please do not hesitate to contact me at 734-324-6593 or email kara.sparks@basf.com .

Sincerely,

BASF Corporation

Kara Sparks
Manager, North America
Product Regulatory Centre of Expertise

CONTAINS NO CBI

Contains No CBI

BASF Corporation
100 Campus Drive
Florham Park, NJ 07932
Tel: (800) 526-1072
www.basf.com/usa

302148

IUCLID

Data Set

Existing Chemical	ID: 84-69-5
CAS No.	84-69-5
EINECS Name	diisobutyl phthalate
EC No.	201-553-2
Molecular Weight	278.35 g/mol
Molecular Formula	C16 H22 O4

Producer Related Part

Company:	BASF AG
Creation date:	09-DEC-1992

Substance Related Part

Company:	BASF AG
Creation date:	09-DEC-1992

Memo:	master

Printing date:	17-JAN-2007
Revision date:	
Date of last Update:	**17-JAN-2007**

Number of Pages:	**27**

Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: TSCA 8d

1.0.1 Applicant and Company Information

-

1.0.2 Location of Production Site, Importer or Formulator

-

1.0.3 Identity of Recipients

-

1.0.4 Details on Category/Template

-

1.1.0 Substance Identification

Mol. Formula: C16 H22 O4
Mol. Weight: 278.35 g/mol

Flag: non confidential, TSCA 8d
23-SEP-2005

1.1.1 General Substance Information

Substance type: organic
Physical status: liquid
Purity: >= 99.5 - % w/w
Colour: nearly colourless, clear
Odour: hardly distinguishable

Method: GC.
Test substance: PALATINOL*IC:
Chemical nature: diisobutyl phthalate
Flag: non confidential, TSCA 8d
17-JAN-2007 (1)

Substance type: organic
Physical status: liquid
Colour: colourless
Odour: faint odour

Test substance: PALATINOL*IC:
Chemical nature: diisobutyl phthalate
Flag: non confidential, TSCA 8d
17-JAN-2007 (2)

1.1.2 Spectra

-

1.2 Synonyms and Tradenames

-

1.3 Impurities

CAS-No:	7732-18-5
EC-No:	231-791-2
EINECS-Name:	water
Mol. Formula:	H2 O
Contents:	<= .1 - % w/w
Method:	DIN 51777, part 1.
Test substance:	PALATINOL*IC: Chemical nature: diisobutyl phthalate
Flag:	non confidential, TSCA 8d

17-JAN-2007 (1)

1.4 Additives

-

1.5 Total Quantity

-

1.6.1 Labelling

-

1.6.2 Classification

-

1.6.3 Packaging

-

1.7 Use Pattern

-

1.7.1 Detailed Use Pattern

-

1.7.2 Methods of Manufacture

-

1.8 Regulatory Measures

-

1.8.1 Occupational Exposure Limit Values

-

1.8.2 Acceptable Residues Levels

-

1.8.3 Water Pollution

-

1.8.4 Major Accident Hazards

-

1.8.5 Air Pollution

-

1.8.6 Listings e.g. Chemical Inventories

-

1.9.1 Degradation/Transformation Products

-

1.9.2 Components

-

1.10 Source of Exposure

-

1.11 Additional Remarks

-

1.12 Last Literature Search

-

1.13 Reviews

-

2.1 Melting Point

-

2.2 Boiling Point

Value: = 184 - 188 degree C at 7 hPa

Method: other: DIN 53406
GLP: no data
Test substance: other TS

Method: Determine the percentage of distillate starting at 184°C / 7 hPa
Remark: key study
Result: Initial boiling point: 184°C

% (V/V)	
5	186
10	187
20	187
30	187
40	187
50	188
60	188
70	188
80	188
90	188
95	188

Final boiling point: 188°C

Distillate	97.5 vol%
Residue	2.5 vol%
Balance	0.0 vol%

Test substance: diisobutyl phthalate, pure
Reliability: (2) valid with restrictions
scientifically acceptable
Flag: Material Safety Dataset, TSCA 8d, key study
16-NOV-2006 (3)

2.3 Density

Type: density
Value: = 1.0392 g/cm³ at 20 degree C

Method: other: DIN 51757
GLP: no
Test substance: other TS

Remark: key study
Result:

Temp. (°C)	Density (g/cm3)
10	1.0475
20	1.0392
30	1.0309
40	1.0227
50	1.0146

Test substance: Palatinol IC, LZC/WT registration No. #16753
Reliability: (2) valid with restrictions
reliable
Flag: Material Safety Dataset, TSCA 8d, key study
20-NOV-2006 (4)

2.3.1 Granulometry

-

2.4 Vapour Pressure

Value: = .5 hPa at 121.9 degree C

Method: other (measured): dynamic
Year: 1982
GLP: no
Test substance: other TS

Remark: key study
Result: temperature (°C) / vapour pressure (hPa): 121.91/0.5; 133.2/1.00; 145.61/2.00; 163,66/5.00; 178.69/10.0; 195.25/20.0; 219.66/50.0; 240.68/100.0; 263.7/200.0; 298.9/500.0; 330.3/1002.6

The regression of the measured values results in the following Antoine equation (average deviation 0.28%):
ln (p/bar) = 10.3596 - 5093.34 / (161.69 + t/°C)
Test substance: Diisobutyl phthalate, purity: 99.9 % (GC)
Reliability: (2) valid with restrictions
scientifically acceptable
Flag: Material Safety Dataset, TSCA 8d, key study
16-NOV-2006 (5)

Value: = .7 hPa at 127.6 degree C

Method: other (measured)
Year: 1990
GLP: no
Test substance: other TS

Method: dynamic under argon atmosphere
Remark: key study
Result corresponds to those of studies carried out at an earlier date.
Result: temperature (°C) / vapour pressure (hPa): 127.55/0.70; 133.01/1.00; 163.35/5.00; 178.36/10.00; 219.2/50.00; 240.1/100.0; 298.7/500.0; 329.4/1000.0
Test substance: Diisobutyl phthalate, purity: 99.64 area% (GC), sample no. 1228 provided by CIW/E
Reliability: (2) valid with restrictions
Comprehensible and scientifically acceptable data
Flag: Material Safety Dataset, TSCA 8d, key study
16-NOV-2006 (6)

Value: = 1 hPa at 133.2 degree C

Method: other (measured)
Year: 1988
GLP: no
Test substance: other TS

Remark: key study
Result: temperature (°C) / vapour pressure (hPa): 133.15/1.00; 163.37/5.00; 178.43/10.00; 219.3/50.00; 240.1/100.0; 298.0/500.0; 327.1/1000.0
Test condition: dynamic under argon atmosphere
Test substance: Diisobutyl phthalate, purity: 99.1 % (GC)
Reliability: (2) valid with restrictions
scientifically acceptable
Flag: Material Safety Dataset, TSCA 8d, key study
16-NOV-2006 (7)

2.5 Partition Coefficient

2.6.1 Solubility in different media

Solubility in: Water
Value: = 11.5 mg/l at 25 degree C
pH value: = 6.8 - 7
Conc.: 11.5 mg/l at 25 degree C

Method: Directive 92/69/EEC, A.6
GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Stable: yes

Method: flask method
Remark: key study
Result: Average of 3 samples 11.5 mg/l
Accuracy: +/- 0.3 mg/l
Test condition: 3 mixtures of c. 0.25 g of test item and 50 ml water were stirred at 30°C for 24 h, 48 h and 72 h and conditioned at 25°C for more than one day. The water phase was separated, and after centrifugation, the WATER PHASE WAS EXTRACTED 3 TIMES WITH CHLOROFORM: AFTER DRYING OF CHLOROFORM WITH NaSO4 THE SOLUTION WAS EVAPORATED AND THE CONCENTRATION OF TEST ITEM IN THIS SOLUTION WAS DETERMINED BY MEANS OF GC USING THE METHOD OF EXTERNAL STANDARD.

Apparatus HP 5890 with autosampler
Measuring parameters:

Column	SP-5-capillary
	- internal diameter: 0.25 mm
	- film thickness: 0.25 microns
	- length: 30 m
Injection temperature	250°C
Injection volume	4 microliter
Detection	FID
Detector temperature	320°C
Carrier gas	nitrogen
Column head pressure	75 kPa
total flow	21 ml/min
Oven temperature	150°C to 320°C, 10 K/min

Test substance: Palatinol IC "B4118 Partie: 23/01
Reliability: (1) valid without restriction
a GLP-approved guideline study
Flag: Material Safety Dataset, TSCA 8d, key study
20-NOV-2006 (8)

2.6.2 Surface Tension

-

2.7 Flash Point

-

2.8 Auto Flammability

-

2.9 Flammability

-

2.10 Explosive Properties

-

2.11 Oxidizing Properties

-

2.12 Dissociation Constant

-

2.13 Viscosity

-

2.14 Additional Remarks

-

3.1.1 Photodegradation

-

3.1.2 Stability in Water

-

3.1.3 Stability in Soil

-

3.2.1 Monitoring Data (Environment)

-

3.2.2 Field Studies

-

3.3.1 Transport between Environmental Compartments

-

3.3.2 Distribution

-

3.4 Mode of Degradation in Actual Use

-

3.5 Biodegradation

Type: aerobic
Inoculum: predominantly domestic sewage
Concentration: 2 mg/l related to Test substance
Degradation: = 79 % after 28 day(s)
Result: readily biodegradable

Method: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year: 1981
GLP: no

Remark: Non-BASF study
Reliability: (2) valid with restrictions
Scientifically acceptable
Flag: TSCA 8d
16-JAN-2007 (9)

Type: aerobic
Inoculum: domestic sewage, non-adapted
Concentration: 41.8 mg/l related to Test substance
Degradation: = 94 % after 28 day(s)
Result: readily biodegradable
Kinetic:

7 day(s)	= 55 %
14 day(s)	= 78.9 %
21 day(s)	= 89.6 %
28 day(s)	= 94 %

Method: other: BODIS-Test for Insoluble Substances (BODIS) (ISO 10708, draft)
GLP: yes
Test substance: other TS: Vestinol IB

Remark: Non-BASF study
Reliability: (1) valid without restriction
Guideline study according to GLP
Flag: TSCA 8d
27-OCT-2006 (10)

3.6 BOD5, COD or BOD5/COD Ratio

-

3.7 Bioaccumulation

-

3.8 Additional Remarks

-

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: semistatic
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: 3

Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
GLP: yes
Test substance: other TS: Vestinol IB

Remark: Non-BASF study.
Reliability: (1) valid without restriction
a GLP-approved OECD 203 guideline study
Flag: TSCA 8d
17-JAN-2007 (11)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: = 2.5
LC50: = 4.2
LC100: 6

Method: other: DIN 38 412 Teil 15
Year: 1982
GLP: no
Test substance: other TS: Vestinol IB

Remark: 20 degree C, Marlowet EF emulsifier added
NON-BASF study.
Flag: TSCA 8d
15-NOV-2006 (12)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 2.5
EC50: = 7.4
EC100: = 20

Method: other: DIN 38 412 part 11
Year: 1982
GLP: no
Test substance: other TS: Vestinol IB

Remark: Crustacea, static procedure, 21 degree C, 4 x 5 animals/conc., Marlowet EF
Non-BASF study.
Flag: TSCA 8d
20-NOV-2006 (13)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: biomass
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: =
EC10: = .72
EC50: = 2.2

Method: other: Directive 92/69/EEC, part C
Year: 1992
GLP: yes
Test substance: other TS: Vestinol IB

Remark: Non-BASF study.
The results with respect to biomass are:
NOEC = 0.19 mg/l
EC10 = 0.27 mg/l
EC50 = 1 mg/l
Test substance: purity 97.5 % (peak area)
Flag: TSCA 8d
20-NOV-2006 (14)

4.4 Toxicity to Microorganisms e.g. Bacteria

-

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

-

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period: 21 day(s)
Unit: mg/l **Analytical monitoring:** no data
NOEC: = 1
LOEC: = 3

Method: other: Draft protocol German Umweltbundesamt
Year: 1984
GLP: no
Test substance: other TS: Vestinol IB

Remark: An emulsifier was used for preparation of the stock solution.
Non-BASF study.
Flag: TSCA 8d
20-NOV-2006 (15)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

-

4.6.2 Toxicity to Terrestrial Plants

-

4.6.3 Toxicity to Soil Dwelling Organisms

-

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

-

4.7 Biological Effects Monitoring

-

4.8 Biotransformation and Kinetics

-

4.9 Additional Remarks

-

5.0 Toxicokinetics, Metabolism and Distribution

-

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

-

5.1.4 Acute Toxicity, other Routes

-

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

-

5.2.2 Eye Irritation

-

5.3 Sensitization

-

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** female
Strain: Fischer 344
Route of administration: gavage
Exposure period: 14 days
Frequency of treatment: daily
Doses: 0, 50, 100, 200, 2000 mg/kg/day
Control Group: yes, concurrent vehicle
NOEL : = 50 mg/kg bw

Year: 1994
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: A separate group receiving 1500 mg DEHP/kg/day was used as a positive control.
As a supplement to the report of the toxicity study (SA-92/0063), enzyme activites in test animals liver fractions have been assessed (BT-92/0063 and BT-92/0063-1).
The combined abstract entitled "An investigation of the subacute toxicity of Di.isobutylphthalate (DIBP) over a 14-day period in female Fischer-344-rats (oral gavage study) and its effect on hepatic enzyme activities" includes the final reports SA-92/0063 ("A 14-days oral toxicity study with Di-isobutyl-phthalate (DIBP) in female Fischer 344 rats", 93 pages) dated 14.01.94 including its amendment dated 25.11.94, BT-92/0063 ("Enzyme activities in liver fractions from female Fischer-344 rats treated with di-isobutyl phthalate (14-day oral gavage study)", 36 pages) dated 19.10.93 and BT-92/0063-1 ("Dodecanoic acid 12-hydroxylase activity in liver microsomes from female Fischer-344 rats treated with di-isobutyl phthalate (14-day oral gavage study) - results of individual animals and statistical evaluation", 23 pages) dated 19.10.93.
Test substance: Vestinol IB, purity 99.1%, CAS 84-69-5
Reliability: (2) valid with restrictions
Flag: TSCA 8d
17-JAN-2007 (16)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: TA 1535, TA 1537, TA 1538, TA 98, TA 100
Concentration: 10 - 5000 µg/plate
Metabolic activation: with and without
Result: negative

Method: other: Ames et al., 1975
Year: 1988
GLP: no data
Test substance: other TS

Test substance: Vestinol IB (CAS 84-69-5); no further details available.
Reliability: (2) valid with restrictions
Flag: TSCA 8d
10-NOV-2006 (17)

Type: Ames test
System of testing: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
Concentration: 8 - 5000 µg/plate
Metabolic activation: with and without
Result: negative

Method: Directive 84/449/EEC, B.14
Year: 1993
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Test substance: Vestinol IB (Diisobutylphthalate, CAS 84-69-5, purity 99.1%)
Reliability: (1) valid without restriction
Flag: Material Safety Dataset, TSCA 8d, key study
10-NOV-2006 (18)

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5.8.1 Toxicity to Fertility

-

5.8.2 Developmental Toxicity/Teratogenicity

Species:	rat	**Sex:**	female
Strain:	Wistar		
Route of administration:	oral feed		
Exposure period:	days 6-20 p.c.		
Frequency of treatment:	continuously		
Doses:	0 ppm, 1000 ppm (approx. 88mg/kg/d), 4000 ppm (approx. 363 mg/kg/d), 11000 ppm (approx. 942 mg/kg/d)		
Control Group:	yes, concurrent no treatment		
NOAEL Maternal Toxity:	= 4000 ppm		
NOAEL Teratogenicity:	> 11000 ppm		
NOAEL Fetotoxicity :	= 4000 ppm		
Result:	Signs of prenatal developmental toxicity only occurred at a concentration, which was also clearly toxic to the dams. There were no indications for teratogenicity up to and including the top concentration, i.e. 11,000 ppm [942 mg/kg bw/day].		

Method: OECD Guide-line 414 "Teratogenicity"
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: It was dosed to the OECD limit (1000 mg/kg bw per day by diet.
Result: The mean dose of Diisobutylphthalate (DIBP) administered (day 6 through day 20 of pregnancy) was approx. 88 mg/kg body weight/day in the 1,000 ppm group, approx. 363 mg/kg body weight/day in the 4,000 ppm group and approx. 942 mg/kg body weight/day in the 11,000 ppm group (meeting the OECD limit dose).

The following substance-related findings were obtained:

11,000 ppm (about 942 mg/kg body weight/day):

statistically significantly decreased food consumption on several days of the treatment period (about 5% less food intake than in the concurrent controls if calculated for days 6-20 p.c.)
statistically significantly impaired body weight gain on days 10-13, 6-20 and 0-20 p.c. (about 11% below controls if calculated for the entire treatment period - days 6-20 p.c.)
statistically significantly lower corrected body weight gain (about 25% below controls)
statistically significantly reduced mean fetal body weights (about 5% below controls if both genders are combined)
4,000 ppm (about 363 mg/kg body weight/day):

no substance-related effects on dams, gestational parameters or fetuses
1,000 ppm (about 88 mg/kg body weight/day):

no substance-related effects on dams, gestational parameters or fetuses
Thus, under the conditions of this prenatal developmental toxicity study, the oral administration of Diisobutylphthalate to pregnant Wistar rats via the diet from implantation to one day prior to the expected day of parturition (days 6 - 20 p.c.) elicited clear signs of maternal toxicity exclusively at 11,000 ppm (942 mg/kg body weight/day). Maternal toxicity was substantiated by statistically significant impairments in food consumption and impaired absolute and corrected body weight gains. No signs of substance-induced maternal toxicity occurred at the low and the mid concentration (1,000 or 4,000 ppm [88 or 363 mg/kg body weight/day]).

There were no substance-related influences on the gestational parameters up to and including the highest concentration (11,000 ppm [942 mg/kg body weight/day]). Conception rate, mean number of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio or the values calculated for the pre- and the postimplantation losses were unaffected by treatment.

The only sign of substance-induced prenatal developmental toxicity was a statistically significant reduction in mean fetal body weight at the high concentration (11,000 ppm [942 mg/kg body weight/day]). There occurred no further indications of substance-induced embryo-/fetotoxicity at the 3 concentrations tested, i.e. 1,000; 4,000 and 11,000 ppm [88, 363 or 942 mg/kg body weight/day].

Based on the results of this prenatal developmental toxicity study, the no observed adverse effect level (NOAEL) for maternal and prenatal developmental toxicity is 4,000 ppm (363 mg/kg body weight/day). Thus, signs of prenatal developmental toxicity only occurred at a concentration, which was also clearly toxic to the dams. There were no indications for teratogenicity up to and including the top concentration, i.e. 11,000 ppm [942 mg/kg body weight/day].

Reliability: (1) valid without restriction
Well described and documented guideline study.

Flag: Material Safety Dataset, TSCA 8d, key study
09-NOV-2006 (19)

5.8.3 Toxicity to Reproduction, Other Studies

-

5.9 Specific Investigations

-

5.10 Exposure Experience

5.11 Additional Remarks

6.1 Analytical Methods

6.2 Detection and Identification

7.1 Function

-

7.2 Effects on Organisms to be Controlled

-

7.3 Organisms to be Protected

-

7.4 User

-

7.5 Resistance

-

8.1 Methods Handling and Storing

-

8.2 Fire Guidance

-

8.3 Emergency Measures

-

8.4 Possib. of Rendering Subst. Harmless

-

8.5 Waste Management

-

8.6 Side-effects Detection

-

8.7 Substance Registered as Dangerous for Ground Water

-

8.8 Reactivity Towards Container Material

-

(1) BASF AG (2005). Techical Information PALATINOL* IC, Sep 2005.

(2) BASF AG (2004). Safety data sheet according to 91/155/EEC, PALATINOL* IC, revised 20 Jan 2004.

(3) BASF AG (1990), ZSZ/Analytik - M320, Siedeanalyse Palatinol IC, Order No. 323472, 21 Feb 1990

(4) BASF AG (2000), LZC/WT - H200, Density of Palatinol, documented in LZC/WT - Laborjournal Dichte und Viskosität Nr. 4, Oktober 1988, 20 Nov 2000, unpublished

(5) BASF AG (1982), ZAM - Physikalische Chemie, unpublished study No. BRU 82.2, 08 Jan 1982

(6) BASF AG (1990), ZSZ/A Physikalische Chemie, Unpublished Report No. BRU 90.347, 02 MAR 1990

(7) BASF AG (1988), Physikalische Chemie, unpublished study No. BRU 88.213, 14.10.1988

(8) BASF AG (2001), ZAX Analytik, Physico-chemical properties of Palatinol IC, Study-No. 01L00168, 31 May 2001, unpublished

(9) Huels AG, GF-188, 9.4.96 (unpublished)

(10) Schoeberl (1993): Bestimmung der biologischen Abbaubarkeit von VESTINOL IB im BODIS-Test (BOD-Test for Insoluble Substances). Unveroeffentlichter Bericht vom 9.07.1993, Abschlussbericht BO-93/6, Huels AG, Marl, 19 S.

(11) Infracor, FK 1428, 16.05.2001

(12) Scheubel, J. B.; Scholz, N. (1989): Oekotoxikologische Pruefung des Produktes Vestinol IB auf seine Wirkung im Fischtest akut (DIN 38412 Teil 15) mit Goldorfen. Unveroeffentlichter Untersuchungsbericht vom 26.01.1989, Huels AG, Marl, Nr. F 985, 5 S., mit Laborprotokoll.

(13) Scheubel, J. B. (1986): Oekotoxikologische Pruefung des Produktes Weichmacher Vestinol IB (Huels) auf seine Wirkung im Daphnientest akut (DIN 38412 Teil 11) mit Daphnia magna STRAUSS. Unveroeffentlichter Untersuchungsbericht vom 21.02.1986, Huels AG, Marl, Nr. D 172, 5 S., mit Laborprotokoll.

(14) Huels report No. AW-338, 1993 (unpublished)

(15) Huels study (unpublished)

(16) Hüls AG, unpublished reports SA-92/0063 year 1994, BT-92/0063 year 1993, BT-92/0063-1 year 1993

(17) Hüls AG, 1988, internal report 88/18, study report 88/109, project X 41

(18) Hüls AG, 1993, internal study report AM-93/5, 25 pages

(19) BASF AG, 2003, internal report 32R0233/02018

10.1 End Point Summary

-

10.2 Hazard Summary

-

10.3 Risk Assessment

-

CAS Number	Product Name	Study Title	Report Date	Laboratory	Comment	Study Report attached?	Report No
84-69-5	Diisobutylphthalate	Bestimmung der biologischen Abbaubarkeit von VESTINOL IB im Geschlossenen Flaschentest	9-Apr-96	Huels AG	Non-BASF report	yes	3.1
84-69-5	Diisobutylphthalate	Bestimmung der biologischen Abbaubarkeit von VESTNOL IB im BODIS-Test	9 July 1993	Huels AG	Non-BASF report	yes	3.2
84-69-5	Diisobutylphthalate	Fish toxicity	16 may 01	Infracor	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Daphnia toxicity, long-term	10/26/1994	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Daphnia toxicity	2/21/1986	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Algae toxicity	12/22/1993	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Bacterial inhibition test (Robra)	3/30/1988	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Diisobutylphthalate - Prenatal Developmental Toxicity Study in Wistar Rats - Administration in the diet	10-Apr-03	BASF	BASF	yes	3.3
84-69-5	Diisobutylphthalate	An investigation of the subacute toxicity of Di-isobutylphthalate (DIBP) over a 14-day period in female Fischer-344-rats (oral gavage study) and its effect on hepatic enzyme activities	1994	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Mutagenitätsuntersuchung von Vestinol IB mit Hilfe des Salmonella typhimurium/Mikrosomen-Mutagenitäts-Tests nach Ames	15-Mar-88	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Bestimmung der Mutagenitat von Vestinol OB im Salmonella/Säuger-Mikrosomen-Mutagenitätstest nach Ames - Mutagenitätstest nach der Richtlinie 84/449/EWG B.14	17-Feb-93	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Amendment to Report SA-92/0063, Quality Assurance Statement according to GLP	25-Nov-94	Huels AG	Above mentioned study "An investigation of the subacute toxicity of Di-isobutylphthalate (DIBP) over a 14-day period in female Fischer-344-rats (oral gavage study) and its effect on hepatic enzyme activities" is a combined abstract of all reports (SA-92/0063, BT-92/0063, BT-92/0063-1) and is therefore considered to serve as a sufficient notification of those non-BASF studies	no	
84-69-5	Diisobutylphthalate	SA-92/0063, A 14-days oral toxicity study with di-isobutyl-phthalate (DIBP) in female Fischer 344 rats	14-Jan-94	Huels AG			
84-69-5	Diisobutylphthalate	BT-92/0063-1; added as amendment to Report BT-92/0063; Dodecanoic acid 12 hydroxylase activity in liver microsomes from female Fisher-344 rats treated wirht di-isobutyl phthalate (14-day oral gavage study) - results of individual animals and statistical evaluation	19. Oct 93	Huels AG			
84-69-5	Diisobutylphthalate	BT-92/0063; Enzyme activties in liver fractions from female Fischer-344 rats treated with di-isobutyl phthalate (14-day oral gavage study)	19. Oct 93	Huels AG			

CAS Number	Product Name	Study Title	Report Date	Laboratory	Comment	Study Report attached?	Report No.
84-69-5	Diisobutylphthalate	Ökotoxikologische Prüfung des Produktes Vestinol IB auf seine Wirkung im Fischtest akut (DIN 38412 teil 15) mit Goldorfen	1/26/1989	Huels AG	Non-BASF report	no	
84-69-5	Diisobutylphthalate	Di-iso-butylphthalate Determination of the acute toxicity for the fish Cyprinus carpio (Acute Fish Toxicity Test)	16-May-01	Infracor	Non-BASF report	no	

hüls

3.1

Hüls Aktiengesellschaft
Prüfinstitut für Biologie
DUV-PS/BT
Bau 9015

Postfach 13 20
D-45764 Marl
Tel.: 0 23 65 / 49-25 87
Fax.: 0 23 65 / 49 60 50

Abschlußbericht

GF-188

Bestimmung der biologischen Abbaubarkeit von

VESTINOL IB

im Geschlossenen Flaschentest
(EG-Richtlinie 84/449 C.6)

Auftraggeber:
Dr. Merget
Hüls AG
45764 Marl

Der Unterzeichner erklärt, daß die im folgenden Bericht beschriebenen Versuche die Ergebnisse und den Verlauf der Prüfung vollständig wiedergeben.

Marl, den 9.4.96

Dr. R. Diefenbach

Der Abschlußbericht umfaßt 11 Seiten.

Zusammenfassung

1. Test: Bestimmung der "Ready Biodegradability"
2. Prüfsubstanz: VESTINOL IB
3. Methode: Geschlossener Flaschen-Test entsprechend der EG-Richtlinie 84/449 C.6
4. Testdauer: 28 Tage (23.11.88 - 21.12.88)
5. Temperatur: 20 °C
6. Kontrollsubstanz: Natriumbenzoat, Microselect;> 99,5 % Fluka Analysen-Nr.: 325702/1 493
7. Ergebnisse
   - Theoretische Prüfsubstanzkonzentration (mg/l): 2,0
   - Theoretischer Sauerstoff-Bedarf: 2,24 mg/mg Prüfsubstanz
   - Sauerstoff-Bedarf (28 d): 3,55 mg/l Prüfsubstanz
   - Prozentualer Abbau (28 d): 79 % Der Stoff ist leicht abbaubar.

Einleitung

Der nachfolgende Report enthält eine Beschreibung der Methode und der damit erhaltenen Resultate zur Untersuchung der "Ready Biodegradability" von

VESTINOL IB

im Geschlossenen Flaschentest.

die Untersuchung wurde durch Dr. Merget in Auftrag gegeben und zwischen dem 23.11.88 und dem 21.12.88 durchgeführt.

Material und Methode

Testsubstanz

Name: VESTINOL IB

Chemiche Indentität: Diisobutylphthalat $C_{16}H_{22}O_4$

Proben Nr.: 779/881028

Referenzsubstanz Natriumbenzoat ($C_6H_5 \cdot COONa$) p.a.
Microselelect:> 99,5 % Fluka
Analysen-Nr.:325702/1 493

Inokulum

Art: Ablauf einer überwiegend kommunalen Kläranlage des Lippeverbandes (Marl-Ost)

Datum der Inoculumentnahme: 23.11.88

Behandlung des Inoculums: Die aerob gehaltene Probe wurde filtriert (Schwarzband-Papierfilter). Die ersten 200 ml des Filtrats wurden verworfen.

Kulturlösung Mineralsalzlösung

Methode

Gefäße: 300 ml volumengeeichte BSB-Glasflaschen mit Schliffstopfen

Prüfansätze:
a) Beimpfte Kulturlösung
b) Beimpfte Kulturlösung + 2 mg Prüfsubstanz/l
c) Beimpfte Kulturlösung + 2 mg Natriumbenzoat/l

Die Ansätze a, b, und c wurden mit 0,5 ml/l des Kläranlagenablauffiltrates beimpft.

Prüfverfahren: Die BSB-Glasflaschen wurden mit Hilfe eines Hebers gefüllt und gasblasenfrei verschlossen. Es wurde eine ausreichende Anzahl von Flaschen angesetzt, um nur eine einzige Sauerstoff-Messung nach 0, 5, 15, und 28 Tagen pro Flasche und Prüfansatz (Parallelansätze) durchführen zu müssen.
Die Flaschen wurden im Brutschrank bei 20 °C 0, 5, 15, bzw. 28 Tage lang inkubiert.

Analytik: In jedem Prüfansatz wurden nach 0, 5, 15, bzw. 28 Tagen die Sauerstoff-Konzentration mit einer Sauerstoff-Elektrode (WTW) gemessen.

Ergebnisse

Die Resultate der Sauerstoff-Messungen in den Prüf- und Referenzsubstanzen sowie in den unbeimpften und beimpften Kontrollansätzen am 0., 5., 15. und 28. Tag sind zusammen mit den prozentualen Abbaugraden Anlage 1 zusammengestellt worden.

Die Berechnung des theoretischen Sauerstoffbedarfes für die Oxidation der Prüf- und Referenzsubstanz sind im Anlage 3 aufgeführt.

VESTINOL IB wies am 28. Versuchstag einen Abbau von 7 % auf.

Natrium-Benzoat als Kontrollsubstanz wies am 28. Versuchstag einen Abbau von 86 % auf.

Der Test wird als valide angesehen, da die Sauerstoffzehrung in den beimpften Kontrollansätzen nach 28 Tagen ≤ 1,5 mg O_2/l (hier 0,72 mg O_2/l) beträgt.

Anlage 1: Abbaugrade

Experiment Nr.: GF-188

Prüfinstitut für Biologie

Prüfleiter: Dr. Schöberl

Testbeginn: 23.11.88

Testende : 21.12.88

Prüfsubstanz: VESTINOL IB

Chemische Bezeichnung: Diisobutylphthalat

Anfangskonzentration pro Flasche: 2 mg/l

ThSB oder CSB der Testsubstanz: 2,24 mg O_2/mg

Inokulum

Herkunft: Ablauf Klärteich-Ost

Vorgenommene Behandlung: Filtration über Filterpapier

Konzentration des Inokulums: 0,5 ml/l

Testergebnis

D = 79 % BSB, ausgedrückt in % ThSB nach 28 Tagen

Gültigkeit der Ergebnisse

Kontrollsubstanz:

Konzentration der

Kontrollsubstanzstammlösung (mg/l) : 1000

Anfangskonzentration pro Flasche (mg/l): 2

ThSB der Kontrollsubstanz (mg O_2/mg) : 1,67

Ergebnis: 86 % BSB, ausgedrückt in % ThSB nach 28 Tagen

Anlage 1

Prüfsubstanz: VESTINOL IB

Exp.-Nr.: GF-188

A: Sauerstoffbestimmungen:

	Kolben Nr.		Sauerstoff nach x Tg. (mg/l) 0	5	15	28
Min.Nährlösung ohne Prüfsubstanz und ohne Impfung	Sauerstoff-Kontrolle	c1	8,97	8,71	8,57	
		c2	8,93	8,81	8,48	
	Mittelwert	m0= (c1+c2)/2	8,95	8,76	8,53	0,00
Min. Nährlösung ohne Prüfsubstanz aber mit Impfung	1	c3	8,95	8,71	8,60	8,25
	2	c4	8,99	9,14	8,99	8,24
	Mittelwert Blindversuch	mb= (c3+c4)/2	8,97	8,93	8,80	8,25
Min.Nährlösung mit Prüfsubstanz und Impfung	1	a1	8,94	7,60	4,15	4,61
	2	a2	8,89	7,91	6,53	4,79
	Mittelwert Prüfsubstanz	mt= (a1+a2)/2	8,92	7,76	5,34	4,70

B:Sauerstoff-Abnahme (mg BSB/l) nach X Tagen

BODx=(m0-mtx)-(m0-mbx) (*)

(*) Diese Differenz ist für die Gültigkeitskontrolle des Tests von Bedeutung

mg BSB/l nach x Tagen		
5	15	28
1,17	3,46	3,55

C: Bewertung mg Subst./l: 2 ThSB: 2,24

$$Dt = \frac{\text{mg BSBx/l*100}}{\text{mg Subst./l*ThSB}}$$

	nach x Tagen		
	5	15	28
% BSB/ThSB	26	77	79

Anlage 2

m_{t0} = Wert in der Prüfflasche zur Zeit 0

m_{tx} = Wert in der Prüfflasche zur Zeit X

m_{b0} = Mittelwert Blindansatz zur Zeit 0

m_{bx} = Mittelwert Blindansatz zur Zeit X

Außerdem ist die Korrektur infolge evtl. auftretender Nitifikation vorzunehmen, sofern die Prüfsubstanz Stickstoff enthält.

Anlage 3: Brechnung des theoretischen Sauerstoffbedarfs von VESTINOL IB

$$\text{Formel: ThSB} = \frac{[2\ C + 1/2\ H - O\ ] * 16}{MG} = \text{mg}\ \ O_2/\text{mg AS}$$

MG = Molekulargewicht

VESTINOL IB Formel $C_{16}H_{22}O_4$ MG = 278,0 g/mol

$$\text{ThSB} = \frac{[2 * 16 + 0{,}5 * 22 - 4\ \ ] * 16}{278{,}0} = 2{,}24\ \text{mg}\ O_2\ /\ \text{mg AS}$$

Das Molekulargewicht wurde auf der Basis der Strukturformel errechnet.

Berechnung des theoretischen Sauerstoffbedarfs von:

Natriumbenzoat Formel: $C_7H_5O_2Na$

$$\text{Formel : ThSB} = \frac{(2\ C + 0{,}5\ H - O) * 16}{MG} = \text{mg}\ \ O_2/\text{mg AS}$$

MG = Molekulargewicht

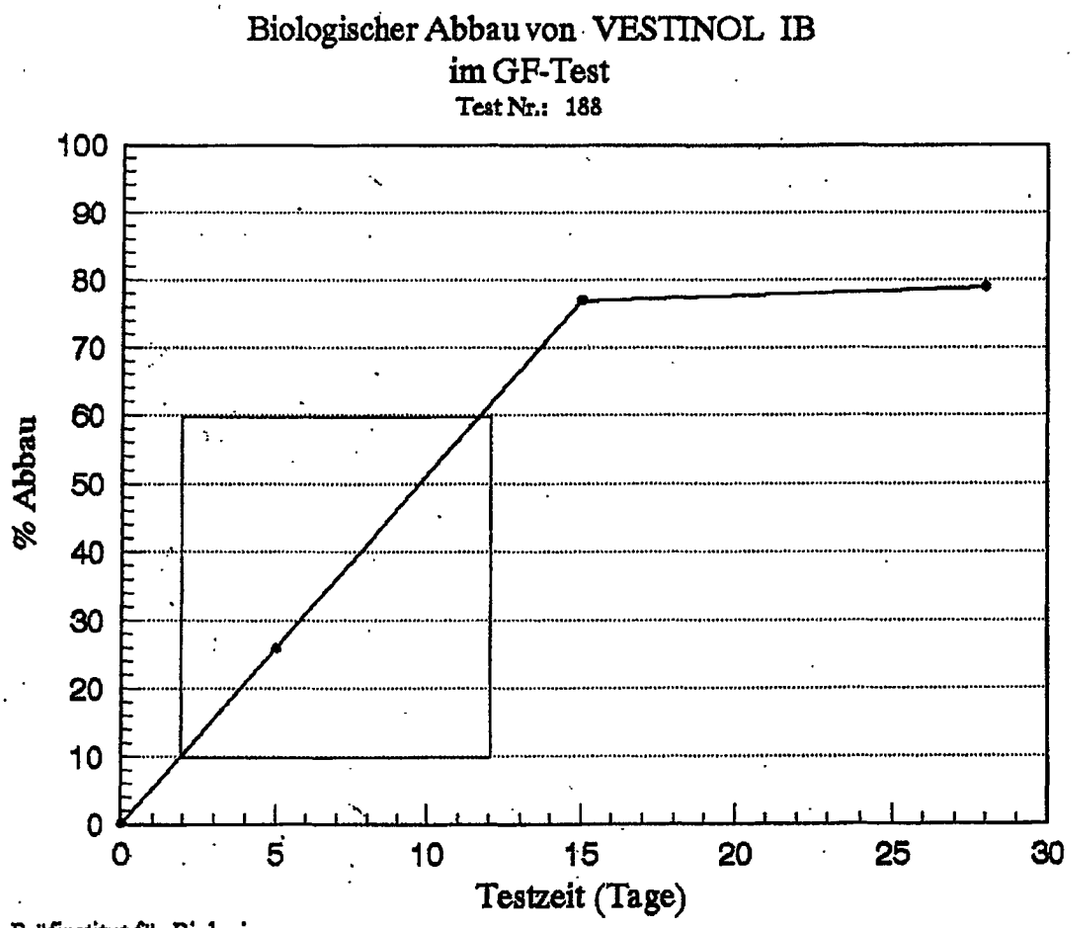
Mg von Natriumbenzoat = 144 g/mol

Hydrolysiert zu $C_7H_6O_2$ + NaOH

$$\text{ThSB} = \frac{(2 * 7 + 0{,}5 * 6 - 2\ ) * 16}{144} = 1{,}67\ \text{mg}\ O_2\ /\ \text{mg AS}$$

Das Molekulargewicht wurde auf der Basis der Strukturformel ermittelt.

Anlage 4: Graphische Darstellung des Sauerstoffverbrauchs



Prüfinstitut für Biologie
Hüls AG

Geschlossener Flaschentest

Prüfinstitut: PS/Biologie, Hüls AG

Prüfsubstanz: Vestinol 1B' (779/881028)

Experiment Nr.: 6/188

A: O_2-Bestimmungen:

	Kolben Nr.		mg O_2/l nach x Tagen 0	5	15	28
Min. Nährlösung ohne Testsubstanz und ohne Impfung	O_2-Kontrolle	c_1	8,97	8,71	8,57	—
		c_2	8,93	8,81	8,48	—
	Mittelwert	$m_0 = \frac{c_1 + c_2}{2}$	8,95	8,76	8,52	
Min. Nährlösung ohne Testsubstanz aber mit Impfung	1	c_3	8,95	8,71	8,60	8,25
	2	c_4	8,99	9,14	8,99	8,24
	Mittelwert Blindversuch	$m_b = \frac{c_3 + c_4}{2}$	8,97	8,92	8,79	8,24
Min. Nährlösung mit Testsubstanz und Impfung	1	a_1	8,94	7,60	4,15	4,61
	2	a_2	8,89	7,91	6,53	4,79
	Mittelwert Prüfsubstanz	$m_t = \frac{a_1 + a_2}{2}$	8,91	7,75	5,34	4,70

B: O_2-Abnahme (mg BSB/1) nach x Tagen

$BOD_x - (m_0 - m_{t_x}) - (m_0 - m_{b_x})$ (1)

mg BSB/1 nach x Tagen 5	15	28
1,17	3,45	3,54

(1) Diese Differenz ist für die Gültigkeitskontrolle des Tests von Bedeutung

C: Bewertung

$$D_t = \frac{\text{mg } BSB_x/l}{mg_{substanz}/l \cdot ThSB} \cdot 100 \quad \text{oder} \quad \% \, BSB_x/CSB = \frac{\text{mg } BSB_x/l}{mg_{substanz}/l \cdot CSB} \cdot 100$$

	nach x Tagen 5	15	28
% BSB/ThSB	26	~~75~~	79
% BSB_x/CSB		77	

Natriumbenzoat Abbauwerte zu finden bei Na-Benzoat vom 23.11.88

Änderung am 1.4.96 Frau mit HP nachgerechnet,

Biologischer Abbau: Geschlossener Flaschentest (Formblatt)

Prüfinstitut: PS/Biologie, Hüls AG

Untersuchungsleiter: Dr. Schöberl

Testbeginn am: 22.11.88 Experiment-Nr. 6/188

Prüfsubstanz: Vestinol IB (Diisobutylphthalat

Chemische Struktur: $C_{16}H_{22}O_4$

Analyse (~~Winkler-Verfahren oder~~ Sauerstoffelektrode): .....

ThSB oder CSB der Testsubstanz: 2,24 mg O_2/mg

Temperatur des Wassers nach Belüftung: 20

O_2-Konzentration des Wassers nach Belüftung und Absetzen vor Testbeginn: 8.95 mg O_2/l

---

Testergebnis

D_t = 79 BSB, ausgedrückt in % ThSB nach 28 Tagen oder

D_t = ... BSB, ausgedrückt in CSB nach 28 Tagen

Gültigkeit der Ergebnisse

Kontrollsubstanz: Na-Benzoat

Ergebnis: 86 BSB, ausgedrückt in % ThSB nach 28 Tagen

Bezugsexp.-Nr.: .....

Bemerkungen:

Geschlossener Flaschentest

Prüfinstitut: PS/Biologie Hüls AG

Prüfsubstanz: Na-Benzoat 23.11.88

Experiment Nr.: .....

A: O_2-Bestimmungen:

	Kolben Nr.		mg O_2/l nach x Tagen			
			0	5	15	28
Min. Nährlösung ohne Testsubstanz und ohne Impfung	O_2-Kontrolle	c_1	8,97	8,71	8,57	–
		c_2	8,93	8,81	8,48	–
	Mittelwert	$m_0 = \frac{c_1 + c_2}{2}$	8,95	8,76	8,53	
Min. Nährlösung ohne Testsubstanz aber mit Impfung	1	c_3	8,99	8,71	8,60	8,25
	2	c_4	8,95	8,14	8,99	8,24
	Mittelwert Blindversuch	$m_b = \frac{c_3 + c_4}{2}$	8,97	8,92	8,79	8,24
Min. Nährlösung mit Testsubstanz und Impfung	1	a_1	8,96	6,39	5,69	5,46
	2	a_2	8,94	6,55	5,66	5,48
	Mittelwert Prüfsubstanz	$m_t = \frac{a_1 + a_2}{2}$	8,95	6,47	5,67	5,47

B: O_2-Abnahme (mg BSB/1) nach x Tagen

$BOD_x = (m_0 - m_{t_x}) - (m_0 - m_{b_x})$ (1)

mg BSB/1 nach x Tagen		
5	15	28
2.45	3,12	2.77

(1) Diese Differenz ist für die Gültigkeitskontrolle des Tests von Bedeutung

C: Bewertung

$$D_t = \frac{\text{mg } BSB_x/l}{mg_{substanz}/l \cdot ThSB} \cdot 100 \quad \text{oder} \quad \% \; BSB_x/CSB = \frac{\text{mg } BSB_x/l}{mg_{substanz}/l \cdot CSB} \cdot 100$$

	nach x Tagen		
	5	15	28
% BSB/ThSB	76	97	98
% BSB_x/CSB			

Blindversuchsprodukt für
Produkt
Ethylhexenol (Octenal)
Veskinol TD
" iB

Biologischer Abbau: Geschlossener Flaschentest (Formblatt)

Prüfinstitut: PS/Biologie, Hüls AG

Untersuchungsleiter: Dr. Schöberl

Testbeginn am: 23.11.88 Experiment-Nr. .....

Prüfsubstanz: Na-Benzoat

Chemische Struktur: C_6H_5COONa

Analyse (~~Winkler-Verfahren oder~~ Sauerstoffelektrode): .....

ThSB oder CSB der Testsubstanz: 1.61 mg O_2/mg

Temperatur des Wassers nach Belüftung: 20 °C

O_2-Konzentration des Wassers nach Belüftung und Absetzen vor Testbeginn: 8.95 mg O_2/l

---

Testergebnis

D_t = ... BSB, ausgedrückt in % ThSB nach 28 Tagen oder

D_t = ... BSB, ausgedrückt in CSB nach 28 Tagen

Gültigkeit der Ergebnisse

Kontrollsubstanz: .....

Ergebnis: ..... BSB, ausgedrückt in % ThSB nach 28 Tagen

Bezugsexp.-Nr.: .....

Bemerkungen:

hüls

3.2

Hüls Aktiengesellschaft
Prüfinstitut für Biologie
UB W/U-Ps-Biologie
Bau 9015
Postfach 13 20
D-45764 Marl
Tel.: 0 23 65/49-48 87

Abschlußbericht BO-93/6

Bestimmung der biologischen Abbaubarkeit von

VESTINOL IB

im BODIS-Test
(BOD-Test for Insoluble Substances)

Auftraggeber: Dr. Menzel
GB 1.3/FEA 21/Pb16/1276
Hüls AG
Postfach 13 20
45764 Marl

Der Unterzeichner erklärt, daß die im vorliegenden Bericht beschriebenen Versuche unter seiner Verantwortung nach den Grundsätzen der Guten Laborpraxis (GLP) entsprechend dem Anhang 1 zum Chemikaliengesetz vom 14.03.1990 (OECD-Grundsätze der Guten Laborpraxis vom 04.02.1983) durchgeführt wurden und die Ergebnisse den Verlauf der Prüfung vollständig wiedergeben.

Marl, 9.7.97

.....
Dr. Schöberl (Prüfleiter)

Ø 7201/7240/12, 1260/02, 1276/16

Der Abschlußbericht umfaßt 19 Seiten.

abbo936.ga

Zusammenfassung der Ergebnisse

1. Test: Prüfung auf vollständige biologische Abbaubarkeit.

2. Prüfsubstanz: VESTINOL IB

   (chem. Bez.): Diisobutylphthalat

3. Methode: BODIS-Test (BOD-Test for Insoluble Substances); Entwurf in OECD eingebracht.

4. Testdauer: 28 d

5. Stabilisierungsphase: 7 d

6. Temperatur: 20,5 - 24,0 °C

7. Inoculum: Lippeverbands-Kläranlage "Marl-Ost"

8. Ergebnisse: Abweichend vom Prüfplan (Seite 9 Pkt.7.1) wurden die Berechnungen nicht im Programm "MULTIPLAN" sondern in "WORD PERFECT" durchgeführt.

   - ThOD: 2,242 mg O_2/mg Prüfsubstanz

   - Einsatzkonzentration: (Mittelwert aus 3 Prüfansätzen): 41,8 mg/l

   - Abbaugrad: 94 % nach 28 Tagen

     Da VESTINOL IB - gerechnet vom Zeitpunkt, zu dem der biologische Abbau 10% erreicht hatte - innerhalb von 10 Tagen zu ≥ 60% abgebaut wird, ist VESTINOL IB als "ready biodegradable" anzusehen.

Hüls Aktiengesellschaft
Qualitätssicherung
Gute Laborpraxis
Bau: 145

Tel.: 02365/49-61 28

Qualitätssicherungserklärung nach GLP

für den Abschlußbericht der Prüfung

BO-93/6

Titel: Bestimmung der biologischen Abbaubarkeit von

VESTINOL IB

im BODIS-Test

Hiermit wird bestätigt, daß diese Prüfung gemäß den Grundsätzen der Guten Laborpraxis durchgeführt wurde.

Die Qualitätssicherung-GLP der Hüls AG hat an folgenden Terminen die Prüfung überprüft:

Datum	Inspektion(en)/Audit(e)
10.05.1993	Prüfplan BO-93/6
02.06.1993	Herstellung der Prüf,- Kontroll- und Kontrollsubstanz-Ansätze zu Beginn der Prüfphase
30.06.1993	Sauerstoffmessungen zum Ende der Prüfung
13.07.1993	Abschlußbericht BO-93/6

Hierbei wurden keine wesentlichen Abweichungen zu den Anforderungen gemäß den Grundsätzen der Guten Laborpraxis (GLP) festgestellt.

Die überprüften Rohdaten sind vollständig, geben das Ergebnis richtig wieder und sind Bestandteil der archivierten Unterlagen.

Marl, 19.07.93

Dr. Krockenberger
(Leiter der Qualitätssicherung GLP)

Marl, 13.07.93

D.I. Koch
(Qualitätssicherung GLP)

qsBO9303.cb1

Inhaltsverzeichnis

	Seite
Zusammenfassung der Ergebnisse	2
Qualitätserklärung nach GLP	3
Inhaltsverzeichnis	4
Einleitung	5
Material und Methoden	6
Methode	7
Sauerstoffmessungen und Analysen	9
Ergebnisse	10
Darlegung der Ergebnisse, Aufzubewahrende Unterlagen	11

Anlagen:

Anlage 1:	Berechnung des theoretischen Sauerstoff-Bedarfs und erforderlicher Einwaage	12
Anlage 2:	Betriebsbedingungen während der Durchführung des BODIS-Tests	13
Anlage 3:	Berechnung der Versuchskonditionen der Prüfsubstanz und der Kontrollsubstanz	14
Anlage 4:	Sauerstoff-Messungen und Berechnung des Abbaugrades der Prüfsubstanz	15
Anlage 5:	Sauerstoff-Messungen und Berechnung des Abbaugrades der Kontrollsubstanz	16
Anlage 6:	Keimzahlbestimmungen	17
Anlage 7:	Graphische Darstellung des Abbauverlaufs	18
Anlage 8:	Kulturlösungszusammensetzung	19

Einleitung

Der nachfolgende Report enthält eine Beschreibung der Methode und der damit erhaltenen Resultate zur Untersuchung der vollständigen biologischen Abbaubarkeit von

VESTINOL IB

im BODIS-Test.

Die Untersuchung wurde durch Dr. Menzel/Werk Marl in Auftrag gegeben und zwischen dem 25.05.1993 und dem 30.06.1993 durchgeführt.

Material und Methoden

Prüfsubstanz

Produktname:	VESTINOL IB
Chemische Bezeichnung:	Diisobutylphthalat
CAS-Nr.:	84-69-5
Reinheit/Bestandteile:	99,1 Fl.% (GC-Analyse)
Stabilität:	< 1 Jahr bei RT (Haltbarkeitsuntersuchung durch KB FZ/AN2 im Juli 1993)
Homogenität:	flüssig, klar, farblos
Chargen-Nr.:	31035
Herstellungsdatum:	08.05.1992
Identitätsnummer:	3633/81435
Nummer der Prüfeinrichtung:	1363/921127

Inoculum: Belebtschlamm aus der überwiegend mit kommunalem Abwasser gespeisten Lippeverbands-Kläranlage "Marl-Ost"

Datum der Inoculumentnahme: 26.05.1993

Behandlung des Inoculums: Der aus der Kläranlage entnommene Belebtschlamm wurde mehrfach mit der für den Test verwendeten Kulturlösung (Anlage 8) gewaschen (Sedimentieren, Dekantieren, Auffüllen mit Kulturlösung). Der Trockensubstanzgehalt des verwendeten Inoculums belief sich auf 3,2 g/l.

Kulturlösung: Zubereitung und Zusammensetzung sind in der Anlage 8 zusammengestellt.

Methode

Testgefäße: Mit Schliffstopfen verschlossene Glasflaschen (Sauerstoff-Flaschen nach Winkler) mit bekanntem Volumen, die zu 2/3 mit Kulturlösung und zu 1/3 mit Luft gefüllt wurden.

Applikation der Prüfsubstanz: Die Prüfsubstanz wurde in ein Wägehilfsmittel (Reaktionsgefäß nach Eppendorf) eingewogen und mit diesem in die Prüfsubstanz-Ansätze überführt.

Versuchsansätze: Der Test wurde innerhalb des vorgeschriebenen Temperaturbereichs durchgeführt.

- Stabilisierungsphase: Die mit 200 ml beimpfter Kulturlösung (Inoculum 10 ml/l) und einem Magnetrührstab beschickten Testflaschen wurden belüftet (Fritte), mit einem Schliffstopfen verschlossen und schrägliegend auf einer Rundschüttelmaschine 1 Woche inkubiert. Innerhalb dieser 1 Woche erfolgte eine erneute Belüftung der Flaschen nach 2 Tagen.

- Prüfphase: Nach Abschluß der Stabilisierungsphase wurden folgende Versuchsansätze hergestellt:

a) Prüfansätze: Diese Ansätze (3 Parallelen) wurden mit Prüfsubstanz beaufschlagt.
Die Prüfsubstanzkonzentrationen in den drei Flaschen waren folgende:

Flasche a: 8,1 mg/Flasche
Flasche b: 8,2 mg/Flasche
Flasche c: 8,8 mg/Flasche

b) Kontrollansätze: Diese Ansätze (4 Parallelen) blieben unbeaufschlagt. 1 Flasche diente der Ermittlung der Ausgangskeimzahl. Die restlichen 3 Flaschen dienten der Bestimmung der Restatmung des Belebtschlammes.

c) Kontrollsubstanz-Ansätze: Diese Serie (3 Parallelen) wurde mit Diethylenglykol beaufschlagt und diente der Kontrolle der biologischen Aktivität der Belebtschlamm-Organismen. Das Diethylenglykol (Gehalt >99%/Fa.Schuchardt) wurde mit einer Injektionsspritze in Eppendorf-Reaktionsgefäße eingewogen und in diesen in die Flaschen überführt. Bevor die Prüfsubstanz bzw. die Kontrollsubstanz in die Flaschen gegeben wurde, fand eine gründliche Belüftung der Flüssigkeitsphase (Glasfritte) statt, um eine Sauerstoff-Sättigung dieser Phase zu erzielen. Der O_2-Gehalt wurde anschließend gemessen.

Sauerstoffmessungen

Die Sauerstoff-Messungen in der wäßrigen Phase aller Testflaschen wurden in wöchentlichem Abstand mit dem Sauerstoff-Meßgerät "OXY 2000" (Fa. WTW) durchgeführt. Die Sauerstoff-Messungen erfolgten nach Vortemperierung der Testflaschen bei 20 ± 0,5°C.
Nach jeder Sauerstoff-Bestimmung wurden die Flaschen mit Luft begast (Glasfritte), um die Flüssigkeitsphase erneut mit Sauerstoff zu sättigen. Der Sauerstoffgehalt wurde anschließend gemessen.

Analysen

Keimzahlbestimmung: Zu Beginn der Prüfung - nach Abschluß der Stabilisierungsphase - wurde in einem der 4 Kontrollansätze die Ausgangskeimzahl bestimmt. Dieser Ansatz wurde anschließend verworfen.

Nach Abschluß der Prüfung wurde der Dreifachansatz vereinigt und von dieser Mischung die End-Keimzahl bestimmt.

pH-Wert-Messung: Vor der Beschickung der Versuchsansätze mit Kulturlösung erfolgte eine pH-Messung der Kulturlösung.

Trockensubstanz-Bestimmung: Der Trockensubstanz-Gehalt des Inoculums wurde vor dessen Verwendung in einer Schnellbestimmung ermittelt. Nach Beimpfung der Kulturlösung erfolgte die eigentliche Trockensubstanz-Bestimmung des verwendeten Inoculums.

Ergebnisse

Die Einzeldaten der Sauerstoff-Analysen sind in der Anlage 4 und 5 zusammen mit der Berechnung des Abbaugrades aufgeführt.

Die Berechnung des biologischen Abbaugrades wurde auf der Basis folgender Formel durchgeführt:

$$\text{Abbaugrad A (\%)} = \frac{\text{Summe BOD} \times 100}{\text{mg Prüfsubstanz} \times \text{ThOD}}$$

Hierin bedeutet:

Summe BOD:	Kumulativer Sauerstoffbedarf in mg O_2 nach n Messungen.
ThOD:	Theoretischer Sauerstoffbedarf in mg O_2/mg Prüfsubstanz.

Die Berechnung der Ergebnisse wurde mit einem speziell hierfür erstellten Programm (WORD PERFECT) am PC durchgeführt.

Für

VESTINOL IB

lautet das im BODIS-Test erhaltene Ergebnis:

Abbaugrad A = 94 % nach 28 Tagen

Da VESTINOL IB - gerechnet vom Zeitpunkt, zu dem der biologische Abbau 10% erreicht hatte - innerhalb von 10 Tagen zu ≥ 60% abgebaut wird, ist VESTINOL IB als "ready biodegradable" anzusehen.

Darlegung der Ergebnisse

- Die Berechnung des theoretischen Sauerstoff-Bedarfs (ThOD) und erforderlicher Einwaage: Anlage 1
- Betriebsbedingungen: Anlage 2
- Berechnung der Versuchskonditionen der Prüfsubstanz und der Kontrollsubstanz: Anlage 3
- Sauerstoff-Messungen und Berechnung des Abbaugrades der Prüfsubstanz : Anlage 4
- Sauerstoff-Messungen und Berechnung des Abbaugrades der Kontrollsubstanz : Anlage 5
- Keimzahlbestimmungen: Anlage 6
- Graphische Darstellung des Abbauverlaufs: Anlage 7

Aufzubewahrende Unterlagen

Aufbewahrt werden:

- die handschriftlichen Originalaufzeichnungen,
- der Computer-Ausdruck mit testspezifischen Einzelangaben und der Ergebnisberechnung,
- das Temperaturaufzeichnungsprotokoll
- der Prüfplan
- dieser Abschlußbericht mit der von der Inspektionseinheit beigelegten Qualitätssicherungserklärung

Aufbewahrungsort:

Die oben genannten Unterlagen werden in einem zentral angelegten Archiv des Prüfinstituts für Biologie, Bau 9015, Raum 37, aufbewahrt.

Anlage 1: Berechnung des theoretischen Sauerstoff-Bedarfs und erforderlicher Einwaage

VESTINOL IB

$$C_{16}H_{22}O_4 + 39\ O \longrightarrow 16\ CO_2 + 11\ H_2O$$

278,35 g/Mol 624 g/Mol

Für 278,35 mg Substanz → 624,0 mg O

1 mg Substanz → 2,242 = ThOD

Erforderliche Einwaage pro Flasche:

$$\text{Einwaage} = \frac{20}{2{,}242} = 8{,}9 \text{ mg (Wert gerundet)}$$

Anlage 2: Betriebsbedingungen während der Durchführung des BODIS-Tests

Testbeginn: 25.05.1993 Testende: 30.06.1993

Prüfsubstanz: VESTINOL IB

pH-Wert (Kulturlösung) : 7,7

Inoculum : Kläranl. Marl-Ost(2l Belebtschlamm)

Datum der Inoculum-Entnahme: 26.05.1993

Schnelltrockengewichtsbestimmung:

1)	1,1820	g	2)	1,1622	g
	0,9853	g		0,9684	g
	0,1967	g/50 ml		0,1938	g/50 ml

Mittelwert: 0,1953 g/50 ml
TG: 3,905 g/l

Trockengewichtsbestimmung:

3)	1,0993	g	4)	1,0960	g
	0,9399	g		0,9386	g
	0,1594	g/50 ml		0,1574	g/50 ml

Mittelwert: 0,1584 g/50 ml
TG: 3,2 g/l

Anlage 3: Berechnung der Versuchskonditionen

Prüfsubstanz: VESTINOL IB

ThOD in mg Sauerstoff/ mg Prüfsubstanz	erforderliche Einwaage in mg	Kulturlösung in ml pro Flasche
2,242	8,92	200
Versuchskonditionen im Prüfgefäß		
Flaschenvolumen a	b	c
291,38	291,35	291,38
Gasphase in ml a	b	c
91,38	91,35	91,38
O_2-Kapazität mg O_2/Flasche a	b	c
27,39	27,38	27,39
Einwaage in mg a	b	c
8,1	8,2	8,8
mg theoret. O_2-Bedarf a	b	c
18,16	18,38	19,73

Kontrollsubstanz: Diethylenglykol

ThOD in mg Sauerstoff/ mg Prüfsubstanz	erforderliche Einwaage in mg	Kulturlösung in ml pro Flasche
1,509	13,25	200
Versuchskonditionen im Prüfgefäß		
Flaschenvolumen a	b	c
291,07	291,04	291,09
Gasphase in ml a	b	c
91,07	91,04	91,09
O_2-Kapazität mg O_2/Flasche a	b	c
27,30	27,29	27,31
Einwaage in mg a	b	c
13,2	13,2	13,3
mg theoret. O_2-Bedarf a	b	c
19,92	19,92	20,07

Anlage 4: Sauerstoff-Messungen und Berechnung des Abbaugrades

Prüfsubstanz: VESTINOL IB

Die Angabe "9,00" für den 0. Versuchstag ist ein theoretischer Wert, der lediglich zu Testbeginn ein Abbau von 0% anzeigen soll.

Sauerstoffkapazität der Testflaschen	a	b	c	ThOD-Wert	
	27,39	27,38	27,39	2,242	
Einwaage in mg	8,1	8,2	8,8		
Versuchstag	0	7	14	21	28
C_{bt}/MW Blindwerte	9,00	8,37	8,62	8,78	8,53
C_t= O_2-Konzentrationen der drei Testflaschen		5,03	7,17	8,33	8,28
		5,11	7,10	7,81	8,34
		4,75	7,20	8,24	8,21
$U_r = \frac{C_{bt} - C_t}{C_s}$		0,37	0,16	0,05	0,03
		0,36	0,17	0,11	0,02
		0,40	0,16	0,06	0,04
U_{rn}= Summe der O_2-Zehrung		0,37	0,53	0,58	0,61
		0,36	0,53	0,64	0,66
		0,40	0,56	0,62	0,66
$U_{rn} * O_c$		10,13	14,52	15,89	16,71
		9,86	14,51	17,52	18,07
		10,96	15,34	16,98	18,08
Abbau in %		55,78	79,96	87,50	92,01
		53,63	78,93	95,30	98,29
		55,55	77,75	86,06	91,64
Mittelwert in %		54,99	78,88	89,62	93,98

Anlage 5: Sauerstoff-Messungen und Berechnung des Abbaugrades

Kontrollsubstanz: Diethylenglykol

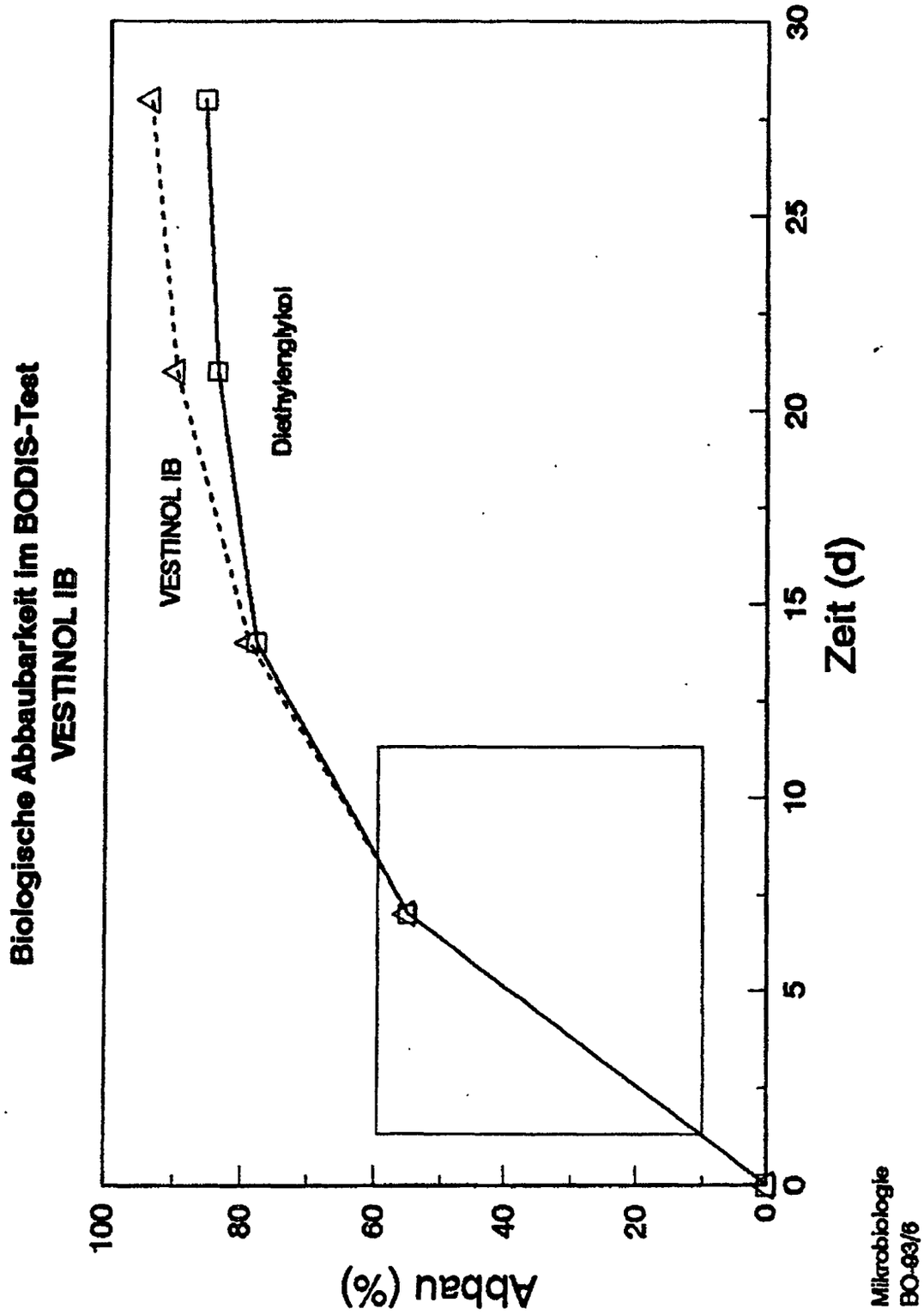
Die Angabe "9,00" für den 0. Versuchstag ist ein theoretischer Wert, der lediglich zu Testbeginn ein Abbau von 0% anzeigen soll.

Sauerstoffkapazität der Testflaschen	a	b	c	ThOD-Wert	
	27,30	27,29	27,31	1,509	
Einwaage in mg	13,2	13,2	13,3		
Versuchstag	0	7	14	21	28
C_{bt}/MW Blindwerte	9,00	8,37	8,62	8,78	8,53
C_t= O_2-Konzentrationen der drei Testflaschen		4,82	7,21	8,45	8,39
		4,71	6,95	8,39	8,38
		4,69	7,24	8,36	8,44
$U_r = \frac{C_{bt} - C_t}{C_s}$		0,39	0,16	0,04	0,02
		0,41	0,19	0,04	0,02
		0,41	0,15	0,05	0,01
U_{rn}= Summe der O_2-Zehrung		0,39	0,55	0,59	0,61
		0,41	0,60	0,64	0,66
		0,41	0,56	0,61	0,62
$U_{rn} * O_c$		10,65	15,02	16,11	16,65
		11,19	16,37	17,47	18,01
		11,20	15,29	16,66	16,93
Abbau in %		53,47	75,41	80,88	83,59
		56,18	82,18	87,71	90,42
		55,81	76,18	83,01	84,36
Mittelwert in %		55,15	77,92	83,87	86,12

Anlage 6: Keimzahlbestimmungen

Blindwert	(nach 0 Tagen) :	$18*10^3$
Blindwert	(nach 28 Tagen) :	$28*10^3$
Diethylenglykol	(nach 28 Tagen) :	$191*10^3$
Prüfsubstanz	(nach 28 Tagen) :	$74*10^3$

Anlage 7: Graphische Darstellung des Abbauverlaufs



Anlage 8: Kulturlösungszusammensetzung

Als Kulturlösung wird eine Nährsalzlösung, die sich aus den nachfolgend beschriebenen Lösungen a - d zusammensetzt. Diese Kulturlösung wird vor Gebrauch einen Tag im temperierten Versuchslabor belüftet.

a) Kaliumdihydrogenphosphat p.a. 8,5 g
Dikaliumhydrogenphosphat p.a. 21,75 g
Dinatriumhydrogenphosphat p.a. 33,4 g

in 1000 ml bidest. Wasser lösen (ca.pH 7,4/stellt sich selbst ein)

b) Magnesiumsulfat * 6 H_2O p.a. 22,5 g

in 1000 ml bidest. Wasser lösen

c) Calciumchlorid * 2 H_2O p.a. 27,5 g

in 1000 ml bidest. Wasser lösen

d) Ammoniumchlorid p.a. 15,0 g

in 1000 ml bidest. Wasser lösen

e) Eisen (III) chlorid * 6 H_2O p.a. 0,25 g

in 1000 ml bidest. Wasser lösen

Zur Herstellung der Kulturlösung werden folgende Mengen der verschiedenen Lösungen vereinigt:

Lösung a: 10 ml plus 800 ml Aqua bidest.

plus Lösung b: 1 ml
Lösung c: 1 ml
Lösung d: 1 ml
Lösung e: 1 ml

Anschließend wird mit Aqua bidest. auf 1 Liter aufgefüllt.

BASF

3.5

AKhe0093

STUDY TITLE

Report

DIISOBUTYLPHTHALATE – Prenatal Developmental Toxicity Study
in Wistar Rats
Administration in the diet

DATA REQUIREMENT

87/302/EEC
OECD Guidelines, Method No. 414
U.S. EPA Health Effects Test Guidelines OPPTS 870.3700

AUTHORS

Dr. S. Schneider (Study Director)
Dr. J. Hellwig
Dr. B. van Ravenzwaay

STUDY COMPLETED ON

April 10, 2003

PERFORMING LABORATORY

Experimental Toxicology and Ecology
BASF Aktiengesellschaft
67056 Ludwigshafen, Germany

LABORATORY PROJECT IDENTIFICATION

Project No.: 32R0233/02018

SPONSOR

BASF Aktiengesellschaft
67056 Ludwigshafen, Germany

VOLUME I of III
(REPORT SECTION AND SUMMARY TABLES)

This document contains manufacturing and trade secrets of BASF. It is the property of BASF and may be used only for that purpose for which it was intended by BASF. Every other or additional use, exploitation, reproduction, publication or submission to other parties require the written permission of BASF, with the exception of regulatory agencies acting within the limits of their administrative authority.

THIS PAGE IS INTENTIONALLY LEFT BLANK

GLP COMPLIANCE STATEMENT

This study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP provisions of the German "Chemikaliengesetz" (Chemicals Act).

April 10, 2003

Dr. med. vet. S. Schneider
(Study Director)

THIS PAGE IS INTENTIONALLY LEFT BLANK

SIGNATURE PAGE

Study Director: April 10, 2003
Dr. med. vet. S. Schneider

Assessment and Reporting: April 10, 2003
Dr. med. vet. J. Hellwig

Management: 10. April, 2003
Dr. rer. nat. B. van Ravenzwaay

STATEMENT

of the Quality Assurance Unit

The Quality Assurance Unit (QAU) inspected the study and reported any inspection results to the Study Director and to Management.

The final report reflects the raw data.

Phase of study	Date of inspection (mm-dd-yyyy)	Reported to Study Director and to Management (mm-dd-yyyy)
Study Plan:	08-27-2002	08-27-2002
Conduct of study:	10-17-2002 10-28-2002	10-17-2002 10-28-2002
Report:	02-12-2003	02-12-2003

Ludwigshafen, Apr. 10, 2003

Sträßner

RheinlandPfalz

Landesanstalt
für Pflanzenbau und Pflanzenschutz

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

BASF Aktiengesellschaft
Experimentelle Toxikologie und Ökologie
D-67056 Ludwigshafen

Prüfungen nach Kategorien / Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

.....1,2,3,4,5,8,9.....

Datum der Inspektion / Date of Inspection

.....15.05.2001 und vom 21. bis 26.06.2001.....

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility/test site is included in the national GLP-Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Schwetmann 24. Sept. 2001

Unterschrift, Datum / Signature, Date

(Name und Funktion der verantwortlichen Person / Name and function of responsible person)

Landesanstalt für Pflanzenbau und Pflanzenschutz Rheinland-Pfalz 1 (Siegel) Mainz

Landesanstalt für Pflanzenbau und Pflanzenschutz, Essenheimer Str. 144, D-55128 Mainz

CONTENTS OF VOLUME I (REPORT SECTION AND SUMMARY TABLES)

		Page
TITLE PAGE		I-1
BLANK PAGE		I-2
GLP COMPLIANCE STATEMENT		I-3
BLANK PAGE		I-4
SIGNATURE PAGE		I-5
STATEMENT OF THE QUALITY ASSURANCE UNIT		I-6
STATEMENT OF GLP COMPLIANCE		I-7
CONTENTS OF VOLUME I, II, and III		I-8
1.	SUMMARY	I-14
1.1.	METHODS	I-14
1.2.	RESULTS	I-14
1.3.	CONCLUSION	I-15
2.	INTRODUCTION AND DOSE SELECTION	I-16
2.1.	AIM OF THE STUDY	I-16
2.2.	SELECTION OF DOSES/CONCENTRATIONS	I-16
2.3.	TEST GUIDELINES	I-16
3.	MATERIAL AND METHODS	I-17
3.1.	TEST SUBSTANCE	I-17
3.2.	TEST ANIMALS	I-18
3.2.1.	Species and strain	I-18
3.2.2.	Animal identification	I-18
3.2.3.	Reason for species selection	I-18
3.3.	HOUSING AND DIET	I-18
3.4.	TEST GROUPS AND CONCENTRATIONS	I-19
3.5.	PREPARATIONS OF THE MIXTURES OF FOOD AND TEST SUBSTANCE	I-19

		PAGE
3.6.	ANALYSES	I-20
3.6.1.	Analyses of the test substance preparations	I-20
3.6.2.	Analytical methods	I-20
3.6.3.	Food analyses	I-20
3.6.4.	Drinking water analyses	I-20
3.7.	EXPERIMENTAL PROCEDURE AND TIME SCHEDULE	I-21
3.8.	EXAMINATIONS OF THE DAMS	I-22
3.8.1.	Clinical examinations	I-22
3.8.1.1.	Mortality	I-22
3.8.1.2.	Clinical symptoms	I-22
3.8.1.3.	Food consumption	I-22
3.8.1.4.	Body weight data	I-22
3.8.1.5.	Intake of test substance	I-22
3.8.1.6.	Corrected body weight gain (net maternal body weight change)	I-23
3.8.2.	Examinations of the dams at termination	I-23
3.9.	EXAMINATION OF THE FETUSES	I-25
3.9.1.	Examination of the fetuses after dissection from the uterus	I-25
3.9.2.	Soft tissue examination of the fetuses	I-25
3.9.3.	Skeletal examination of the fetuses	I-25
3.9.4.	Evaluation criteria for assessing the fetuses	I-26
3.10.	STATISTICS	I-27
3.10.1.	Statistics of clinical, necropsy and fetal examinations	I-27
3.11.	RETENTION OF RECORDS	I-28
4.	RESULTS AND ASSESSMENT OF FINDINGS	I-29
4.1.	ANALYSES	I-29
4.1.1.	Stability analysis of the test substance preparations	I-29
4.1.2.	Homogeneity analysis of the test substance preparations	I-29
4.1.3.	Concentration control analyses of the test substance preparations	I-29
4.1.4.	Food analyses	I-29
4.1.5.	Drinking water analyses	I-29

		PAGE
4.2.	EXAMINATIONS OF THE DAMS	I-30
4.2.1.	Clinical examinations	I-30
4.2.1.1.	Mortality	I-30
4.2.1.2.	Clinical symptoms	I-30
4.2.1.3.	Food consumption	I-31
4.2.1.4.	Body weight data	I-32
4.2.1.5.	Intake of test substance	I-33
4.2.1.6.	Corrected body weight gain (net maternal body weight change)	I-34
4.2.2.	Examinations of the dams at termination	I-34
4.2.2.1.	Uterus weight	I-34
4.2.2.2.	Necropsy findings	I-35
4.2.2.3.	Reproduction data of dams	I-35
4.3.	EXAMINATIONS OF THE FETUSES	I-36
4.3.1.	Examinations of the fetuses after dissection from the uterus	I-36
4.3.1.1.	Sex distribution of fetuses	I-36
4.3.1.2.	Weight of placentae	I-36
4.3.1.3.	Weight of fetuses	I-36
4.3.1.4.	External examination of the fetuses	I-37
4.3.2.	Soft tissue examination of the fetuses	I-38
4.3.3.	Skeletal examination of the fetuses	I-38
4.3.4.	Abstract of fetal external, soft tissue and skeletal observations and their final assessment	I-41
5.	DISCUSSION AND CONCLUSION	I-43
6.	REFERENCES	I-44
7.	APPENDIX (SUMMARY TABLES)	I-46
7.1.	LIST TO THE ABBREVIATIONS USED IN VOLUMES IA AND IB	I-46

Part A:
EXAMINATIONS OF THE DAMS - CLINICS AND GROSS NECROPSY (MEAN VALUES AND SUMMARY TABLES)

	Tables
Summary of maternal clinical observations	IA-001
Mean maternal food consumption	IA-002 - IA-003
Mean maternal body weights	IA-004
Mean maternal body weight change	IA-005 - IA-006
Mean maternal substance intake	IA-007 - IA-008
Mean gravid uterine weights and net maternal body weight change	IA-009
Summary of maternal necropsy observations	IA-010
Summary of reproduction data	IA-011 - IA-013

Part B:
EXAMINATIONS OF THE FETUSES (MEAN VALUES AND SUMMARY TABLES)

	Tables
Mean placental and fetal body weights (on a litter basis)	IB-001
Summary of all classified fetal external observations	IB-002 - IB-004
Summary of fetal external unclassified observations	IB-005
Summary of all classified fetal soft tissue observations	IB-006 - IB-008
Summary of fetal soft tissue unclassified observations	IB-009
Summary of all classified fetal skeletal observations	IB-010 - IB-024
Summary of fetal skeletal unclassified cartilage observations	IB-025 - IB-027
Summary of all classified fetal external, soft tissue and skeletal observations	IB-028

The tables with the individual values/observations are to be found in Volume II. Further information (detailed analytical results and historical control data) is included in Volume III (Supplement).

CONTENTS OF VOLUME II (TABLES SECTION; INDIVIDUAL VALUES AND OBSERVATIONS)

Table of contents Page II-2

Part A:
EXAMINATIONS OF THE DAMS - CLINICS AND GROSS NECROPSY (INDIVIDUAL VALUES)

List of abbreviations used in Tables IIA and IIB Page II-3

	Tables
Individual maternal clinical observations	IIA-001 - IIA-008
Individual maternal food consumption	IIA-009 - IIA-012
Individual maternal body weights	IIA-013 - IIA-016
Individual maternal body weight change	IIA-017 - IIA-020
Individual maternal substance intake	IIA-021 - IIA-023
Individual uterine weights and net maternal body weight change	IIA-024 - IIA-027
Individual maternal necropsy observations	IIA-028 - IIA-035
Individual reproduction data	IIA-036 - IIA-039

Part B:
EXAMINATIONS OF THE FETUSES (INDIVIDUAL VALUES)

	Tables
Individual fetal status and uterine location	IIB-001 - IIB-004
Individual placental weights	IIB-005 - IIB-008
Individual fetal body weights	IIB-009 - IIB-012
Individual fetal external, visceral, skeletal and/or cartilage observations	IIB-013 - IIB-087

CONTENTS OF VOLUME III (SUPPLEMENT)

	Page
Table of contents	III-2
1. Analyses of the test substance preparations	III-3
• **Stability** Analysis of DIISOBUTYLPHTHALATE in Feed	IIIA-001 - IIIA-011
• **Homogeneity** and **Concentration Control** Analysis of DIISOBUTYLPHTHALATE in Feed	IIIA-012 - IIIA-013
2. Historical control data	III-4
Tables	IIIB-001 - IIIB-018

This report consists of Volumes I, II and III.

1. SUMMARY

1.1. METHODS

DIISOBUTYLPHTHALATE was tested for its prenatal developmental toxicity in Wistar rats. The test substance was administered to 25 time-mated female Wistar rats/group as a constant homogeneous addition to the food at concentrations of 0; 1,000; 4,000 and 11,000 ppm on day 6 through day 20 post coitum (p.c.). Between 22 - 23 females/group had implantation sites at terminal sacrifice.

Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked each day.

On day 20 post coitum, all females were sacrificed and assessed by gross pathology (including weight determinations of the unopened uterus and the placentae). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external findings. Thereafter, nearly one half of the fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal (incl. cartilage) findings.

The mean dose of **DIISOBUTYLPHTHALATE** administered (during the administration period) was approx. 88 mg/kg body weight/day in the 1,000 ppm group, approx. 363 mg/kg body weight/day in the 4,000 ppm group and approx. 942 mg/kg body weight/day in the 11,000 ppm group.

1.2. RESULTS

The following substance-related findings were obtained:

11,000 ppm (about 942 mg/kg body weight/day):

- statistically significantly decreased food consumption on several days of the treatment period (about 5% less food intake as in the concurrent controls if calculated for days 6–20 p.c.)
- statistically significantly impaired body weight gain on days 10–13, 6–20 and 0-20 p.c. (about 11% below controls if calculated for the entire treatment period – days 6–20 p.c.)
- statistically significantly lower corrected body weight gain (about 25% below controls)
- statistically significantly reduced mean fetal body weights (about 5% below controls if both genders are combined)

4,000 ppm (about 363 mg/kg body weight/day):

- no substance-related effects on dams, gestational parameters or fetuses

1,000 ppm (about 88 mg/kg body weight/day):

- no substance-related effects on dams, gestational parameters or fetuses

1.3. CONCLUSION

Thus, under the conditions of this prenatal developmental toxicity study, the oral administration of **DIISOBUTYLPHTHALATE** to pregnant Wistar rats via the diet from implantation to one day prior to the expected day of parturition (days 6 - 20 p.c.) elicited **clear signs of maternal toxicity** exclusively at 11,000 ppm (942 mg/kg body weight/day). **Maternal toxicity** was substantiated by statistically significant impairments in food consumption and impaired absolute and corrected body weight gains. No signs of substance-induced **maternal toxicity** occurred at the low and the mid concentration (1,000 or 4,000 ppm [88 or 363 mg/kg body weight/day]).

There were no substance-related influences on the **gestational parameters** up to and including the highest concentration (11,000 ppm [942 mg/kg body weight/day]). Conception rate, mean number of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio or the values calculated for the pre- and the postimplantation losses were unaffected by treatment.

The only sign of substance-induced **prenatal developmental toxicity** was a statistically significant reduction in mean fetal body weight at the high concentration (11,000 ppm [942 mg/kg body weight/day]). There occurred **no further indications of substance-induced embryo-/fetotoxicity** at the 3 concentrations tested, i.e. **1,000; 4,000 and 11,000 ppm [88, 363 or 942 mg/kg body weight/day].**

Based on the results of this prenatal developmental toxicity study, the **no observed adverse effect level (NOAEL)** for **maternal** and **prenatal developmental toxicity** is **4,000 ppm (363 mg/kg body weight/day).** Thus, signs of prenatal developmental toxicity did only occur at a concentration, which was also clearly toxic to the dams. There were **no indications for teratogenicity** up to and including the top concentration, i.e. **11,000 ppm [942 mg/kg body weight/day].**

2. INTRODUCTION AND DOSE SELECTION

2.1. AIM OF THE STUDY

The purpose of this study was to assess the effects of **DIISOBUTYLPHTHALATE** on embryonic and fetal development according to current test guidelines (see also below). Moreover, detailed information about influences of the test substance on the maternal organism was expected to be obtained.

DIISOBUTYLPHTHALATE was administered daily in the diet to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 20 p.c.).

2.2. SELECTION OF DOSES/CONCENTRATIONS

The following concentrations in the diet were chosen for the present full-scale toxicity study in Wistar rats:

1,000 ppm:	as the expected no observed adverse effect level
4,000 ppm:	as intermediate concentration
11,000 ppm:	as the high concentration which should induce some developmental and/or maternal toxicity but not death or severe suffering

Risk assessments undertaken for several phthalates indicate the oral uptake by food to be the major route of exposure for the general public. Thus, in the present prenatal developmental toxicity study in Wistar rats administration in the diet was selected. In general, oral administration of a test substance has proven to be appropriate for the detection of a toxicological risk.

2.3. TEST GUIDELINES

The study was carried out according to or exceeding the requirements of the following test guidelines:

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

3. MATERIAL AND METHODS

3.1. TEST SUBSTANCE

Name of test substance:	DIISOBUTYLPHTHALATE
Test substance No.:	02/0233-1
CAS - No.:	84-69-5
Batch No.:	2527407 A
Date of production:	June 6 – 24, 2002
Physical state/appearance:	Liquid/colorless – clear
Purity:	99.77 % (method: GC, order No.: 31/05/2002, non-GLP-analysis by PROVIRON)
Homogeneity:	Homogeneous
Stability:	The stability under storage conditions was confirmed by reanalysis (GLP-analysis by Analytical Department of BASF Aktiengesellschaft)
Storage conditions:	Room temperature

3.2. TEST ANIMALS

3.2.1. Species and strain

Time-mated Wistar rats (CrlGlxBrlHan:WI) supplied by Charles River Laboratories, Germany which were free from clinical signs of disease, were used for the investigations.

3.2.2. Animal identification

The animals were mated by the breeder and supplied on day 0 post coitum (= detection of vaginal plug / sperm). They were assigned to the test groups by taken random selection from the transport box. After randomization the rats were identified uniquely by ear tattoo.

3.2.3. Reason for species selection

This strain was selected since extensive experience is available on Wistar rats and this strain has been proven to be sensitive to substances with a teratogenic potential.

3.3. HOUSING AND DIET

The rats were housed singly from day 0 - 20 p.c. in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, Germany (height: 15 cm, length: 37,5 cm, width: 21 cm; floor area about 800 cm^2).

The animals were accommodated in fully air- conditioned rooms in which central air conditioning guaranteed a range of temperature of 20 - 24°C and a range of relative humidity of 30 - 70%. There were no deviations from these limits.

The day/night rhythm was 12 hours (12 hours light from 6.00 a.m. to 6.00 p.m. and 12 hours darkness from 6.00 p.m. to 6.00 a.m.).

Before the study started, the animal room was completely disinfected using a disinfector ("AUTEX", fully automatic, formalin-ammonia - based terminal disinfection). In general, each week the walls and the floor were cleaned with water containing about 0.5% Mikro-Quat (supplied by ECOSAN GmbH, Germany).

The food used was ground Kliba maintenance diet rat/mouse/hamster meal, supplied by PROVIMI KLIBA SA, Kaiseraugst, Switzerland. Food was available to the animals ad libitum throughout the study (from the day of supply to the day of necropsy), as was drinking water of tap water quality from water bottles.

3.4. TEST GROUPS AND CONCENTRATIONS

Test group	Concentration in the diet (ppm)	Number of animals	Animal No.
0	0	25	1 – 25
1	1,000	25	26 – 50
2	4,000	25	51 – 75
3	11,000	25	76 – 100

3.5. PREPARATIONS OF THE MIXTURES OF FOOD AND TEST SUBSTANCE

For each concentration, the test substance was weighed out and thoroughly mixed with a small amount of food in a beaker. Then corresponding amounts of food, depending on the test group, were added to this premix in order to obtain the desired concentrations, and mixing was carried out for about 10 minutes in a laboratory mixer (Ruberg Mischtechnik KG, Paderborn, Germany).

The mixtures of test substance and food were prepared at the beginning of the administration period and thereafter at intervals which took into account the analytical results of the stability verification.

3.6. ANALYSES

All analyses mentioned under 3.6.1. were carried out at the Analytical Chemistry Laboratory of Experimental Toxicology and Ecology of BASF Aktiengesellschaft.

3.6.1. Analyses of the test substance preparations

Analytical verifications of the stability of the test substance in the diet for a period of at least 15 days at room temperature were carried out before the study was initiated.

Samples of the test substance preparations were sent to the analytical laboratory once during the study period (at the beginning) for verification of the concentrations. The samples which were taken for the concentration control analysis at the beginning of the administration period were also used to verify the homogeneity for the samples of the low and the high concentrations (1,000 and 11,000 ppm). 3 samples were taken for each of these concentrations.

3.6.2. Analytical methods

The methods used for the analytical investigations of the test substance preparations can be found in Volume III (Supplement).

3.6.3. Food analyses

The food used in the study was assayed for chemical as well as for microbiological contaminants.

3.6.4. Drinking water analyses

The drinking water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and by Technical Services of BASF Aktiengesellschaft as well as for the presence of microorganisms by a contract laboratory.

3.7. EXPERIMENTAL PROCEDURE AND TIME SCHEDULE

The female animals were supplied at an age of about 70 – 84 days on October 8, 9 and 10, 2002.

The animals were mated by the breeder ("time-mated") and supplied on day 0 post coitum (= detection of vaginal plug / sperm). The animals arrived on the same day (i.e. day 0 p.c.) at the experimental laboratory. The following day was designed "day 1" post coitum (p.c.). Between start of the study (beginning of the experimental phase) and first administration (day 6 p.c.) the animals were acclimated to the laboratory conditions.

Based on the pregnant animals the body weight on day 0 varied between 152.6 – 196.8 g.

The animals continuously received the test substance at the appropriate concentrations in the diet from implantation to one day prior to the expected day of parturition (day 6 to day 20 p.c.)

On day 20 p.c., all females were sacrificed in a randomized order and examined macroscopically. The fetuses were removed from the uterus and further investigated with different methods (for details see 3.9.).

Due to technical reasons, the study was carried out in 3 sections. Each test group was represented in each section. For further details, see Table 3.7.1.

Table 3.7.1.: Time schedule

Experimental starting date: October 8, 2002

	Arrival of the animals (day 0 p.c.)	**Beginning of treatment (day 6 p.c.)**	**End of treatment/ Sacrifice (day 20 p.c.)**
1st section	October 8, 2002	October 14, 2002	October 28, 2002
2nd section	October 9, 2002	October 15, 2002	October 29, 2002
3rd section	October 10, 2002	October 16, 2002	October 30, 2002

Experimental completion date: February 7, 2003

3.8. EXAMINATIONS OF THE DAMS

3.8.1. Clinical examinations

3.8.1.1. Mortality

A check was made twice a day on working days or once a day (Saturdays, Sundays or on public holidays) (days 0 - 20 p.c.).

3.8.1.2. Clinical symptoms

The animals were examined for clinical symptoms at least once a day, or more often when clinical signs of toxicity were elicited (days 0 - 20 p.c.).

3.8.1.3. Food consumption

With the exception of day 0, the consumption of food was determined on the same days as was body weight.

3.8.1.4. Body weight data

All animals were weighed on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c.. The body weight change of the animals was calculated from these results.

3.8.1.5. Intake of test substance

The intake of test substance was calculated from the amount of food consumed and expressed in mg/kg body weight per day.

The calculation of the group values/day was carried out according to the following formula:

$$IT_x = \frac{FC_x \times C}{BW_y}$$

IT_x = intake of test substance on day x in mg/kg body weight/day
FC_x = daily food consumption on day x in grams
C = concentration in ppm
BW_y = body weight on day y in grams (last weighing before day x)

The values listed in the Summary Tables are group means determined from the daily intakes of test substance by the individual animals.

3.8.1.6. Corrected body weight gain (net maternal body weight change)

Furthermore, the corrected body weight gain was calculated after terminal sacrifice (terminal body weight on day 20 p.c. minus weight of the unopened uterus minus body weight on day 6 p.c.).

3.8.2. Examinations of the dams at termination

On day 20 p.c., the dams were sacrificed in randomized order by cervical dislocation and the fetuses removed from the uterus.

After the dams had been sacrificed, they were necropsied and assessed by gross pathology in randomized order to minimize bias. The uterus and the ovaries were removed and the following data were recorded:

- Weight of the unopened uterus*
- Number of corpora lutea
- Number and distribution of implantation sites classified as:
  - live fetuses
  - dead implantations:
    - a) early resorptions (only decidual or placental tissues visible or according to SALEWSKI (Salewski, 1964) from uteri from apparently non-pregnant animals and the empty uterus horn in the case of single-horn pregnancy)
    - b) late resorptions (embryonic or fetal tissue in addition to placental tissue visible)
    - c) dead fetuses (hypoxemic fetuses which did not breathe spontaneously after the uterus had been opened)

* = After the weight of the uterus had been determined, all subsequent evaluations of the dams and the gestational parameters were conducted by technicians unaware of treatment group in order to minimize bias.

Furthermore, calculations of conception rate and pre- and postimplantation losses were carried out:

- The **conception rate** (in %) was calculated according to the following formula:

$$\frac{number\ of\ pregnant\ animals}{number\ of\ fertilized\ animals} x100$$

- The **preimplantation loss** (in %) was calculated[1] according to the following formula:

$$\frac{number\ of\ corpora\ lutea - number\ of\ implantations}{number\ of\ corpora\ lutea} x100$$

- The **postimplantation loss** (in %) was calculated[1] from the following formula:

$$\frac{number\ of\ implantations - number\ of\ live\ fetuses}{number\ of\ implantations} x100$$

[1] Calculation on the basis of each individual pregnant animal with scheduled sacrifice

3.9. EXAMINATION OF THE FETUSES

All fetal analyses were conducted by technicians unaware of treatment group in order to minimize bias.

3.9.1. Examination of the fetuses after dissection from the uterus

At necropsy each fetus was weighed, sexed and examined macroscopically for any external findings. The sex was determined by observing the distance between the anus and the base of the genital tubercle and was later confirmed in all fetuses fixed in Harrison's Fluid by internal examination. If there were discrepancies between the "external" and the "internal" sex of a fetus, the fetus was finally sexed according to the appearance of its gonads.

Furthermore, the viability of the fetuses and the condition of the placentae, the umbilical cords, the fetal membranes and fluids were examined. Individual placental weights were recorded.

Thereafter, the fetuses were sacrificed by subcutaneous injection of a pentobarbital (Narcoren®, Fa. Rhone Merieux GmbH, 88471 Laupheim, Germany; Dose: 0.1 ml/fetus).

After these examinations, approximately one half of the fetuses per dam were eviscerated, skinned and placed in ethyl alcohol, the other half was placed in Harrison's Fluid for fixation and further evaluation.

3.9.2. Soft tissue examination of the fetuses

The fetuses fixed in Harrison's Fluid were examined for any visceral findings according to the method of BARROW and TAYLOR (Barrow and Taylor, 1969). After this examination these fetuses were discarded.

3.9.3. Skeletal examination of the fetuses

The skeletons of the fetuses fixed in ethyl alcohol were stained according to a modified method of KIMMEL and TRAMMELL (Kimmel, C.A. and Trammell C., 1981). Thereafter, the skeletons of these fetuses were examined under a stereomicroscope. After this examination the stained fetal skeletons were retained individually.

3.9.4. Evaluation criteria for assessing the fetuses

There are differing opinions on classification and assessment of fetal findings (e.g. Beltrame and Giavini, 1990, Chahoud et al., 1999, Solecki et al., 2001). Moreover, according to WISE et al. (Wise et al., 1997) "nomenclature used to describe observations of fetal morphology often varies considerably among laboratories, investigators, and textbooks in the fields of teratology and developmental toxicity".

In the present study the glossary of WISE et al. (Wise et al., 1997) was used as much as possible to describe findings in fetal morphology.
Classification of these findings was based on the terms and definitions proposed by CHAHOUD et al. (Chahoud et al., 1999; Solecki et al., 2001):

- **Malformation**

  A permanent structural change that is likely to adversely affect the survival or health.

- **Variation**

  A change that occurs also in fetuses of control animals and is unlikely to adversely affect the survival or health. This includes delays in growth or morphogenesis that has otherwise followed a normal pattern of development.

Moreover, the terms **"unclassified observation"** or **"unclassified cartilage observation"** were used for those fetal findings, which could not be classified as malformations or variations (e.g. focal liver necrosis in fetuses, isolated cartilage findings without any impact on the respective bony structure).

According to the definitions specified before, the findings obtained in fetuses were classified and listed in the tables accordingly.

3.10. STATISTICS

3.10.1. Statistics of clinical, necropsy and fetal examinations

Statistical analyses were performed according to following tables:

Parameter	Statistical test	Markers in the tables	References
Food consumption[2], body weight, body weight change, corrected body weight gain (net maternal body weight change), carcass weight, weight of unopened uterus, number of corpora lutea, number of implantations, number of resorptions, number of live fetuses, proportions of preimplantation loss, proportions of postimplantation loss, proportions of resorptions, proportion of live fetuses in each litter, litter mean fetal body weight, litter mean placental weight	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 - 1121 DUNNETT, C.W. (1964): New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 - 491
Female mortality, females pregnant at terminal sacrifice, number of litters with fetal findings	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test (one-sided) for the hypothesis of equal proportions	* for $p \leq 0.05$ ** for $p \leq 0.01$	Siegel, S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York, 96 - 104
Proportions of fetuses with malformations, variations and/or unclassified observations in each litter	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	Nijenhuis, A.; Wilf, H.S. (1978): Combinatorial Algorithms. Academic Press New York, 32-33 Hettmansperger, T.P. (1984); Statistical Inference based on Ranks. John Wiley & Sons New York, 132-142

[2] For the parameter food consumption the "mean of means" was calculated and can be found in the relevant summary tables. The "mean of means" values allow a rough estimation of the total food consumption during different time intervals (pretreatment and treatment period); they are not exactly precise values, because the size of the intervals taken for calculation differs. For the "mean of means" values no statistical analysis was performed.

3.11. RETENTION OF RECORDS

GLP–relevant records and materials are stored at BASF Aktiengesellschaft for at least the period of time specified in the GLP principles. Details concerning responsibilities or locations of archiving can be seen from the respective SOPs and from the raw data.

4. RESULTS AND ASSESSMENT OF FINDINGS

4.1. ANALYSES

4.1.1. Stability analysis of the test substance preparations

The stability of the test substance in the diet was demonstrated for a period of 15 days at room temperature. For details see Volume III; Tables IIIA-001 – IIIA-011.

4.1.2. Homogeneity analysis of the test substance preparations

The homogeneity of the mixtures was verified. For details see Volume III; Tables IIIA-012 - IIIA-013.

4.1.3. Concentration control analyses of the test substance preparations

The concentration control analyses of all concentrations revealed that the values were in the expected range of the target concentrations. For details see Volume III, Tables IIIA-012 – IIIA-013. The mean values were found to be in the range of 95.7 – 99.1% of the nominal concentration.

4.1.4. Food analyses

On the basis of the duration of use and the analytical findings with respect to chemical and microbiological contaminants the food was found to be suitable. Fed. Reg. Vol. 44, No. 91 of May 9, 1979, p. 27354 (EPA), served as a guideline for maximum tolerable chemical contaminants. The amount of microorganisms did not exceed $5*10^5$/g feed.

The individual results are to be found in the archives of Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany.

4.1.5. Drinking water analyses

On the basis of the analytical findings, the drinking water was found to be suitable. German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetzblatt December 05, 1990) served as a guideline for maximum tolerable contaminants.

The individual results are to be found in the archives of Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany.

4.2. EXAMINATIONS OF THE DAMS

Summary tables of the results are given in the Appendix of Volume I; individual values and findings are given in Part A of Volume II.

4.2.1. Clinical examinations

Only **pregnant dams** were used for the calculations of mean maternal food consumption, body weight, body weight change and substance intake. Only **pregnant dams with scheduled sacrifice** (day 20 p.c.) were taken for the calculation of mean gravid uterine weights, mean net maternal body weight change (corrected body weight gain) and summary of reproduction data.

In this study the following females were partially or totally excluded from the above mentioned calculations:

Test group 0 (0 ppm):
- females Nos. 8 and 13 - not pregnant

Test group 1 (1,000 ppm):
- females Nos. 38, 47 and 49 - not pregnant

Test group 2 (4,000 ppm):
- females Nos. 52, 64 and 69 - not pregnant

Test group 3 (11,000 ppm):
- females Nos. 94 and 98 - not pregnant

4.2.1.1. Mortality

There were no substance-related or spontaneous mortalities in any of the groups.

4.2.1.2. Clinical symptoms
(Summary of findings: Tab. IA-001)

No clinical observations were noted in any of the dams of this study.

4.2.1.3. Food consumption

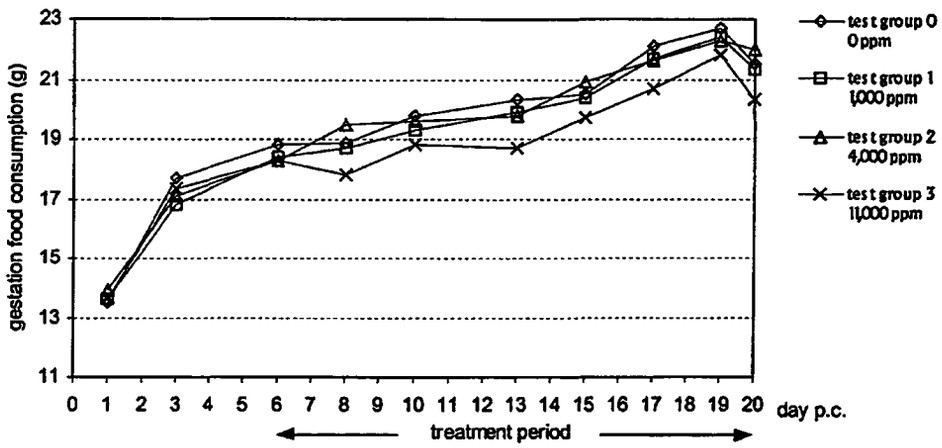
(Mean values: Tabs. IA-002 - IA-003; Fig. 4.2.1.3.1.)

The mean food consumption of the high concentration dams (11,000 ppm) was statistically significantly reduced on days 10-13 p.c. (about 8%) and 15-17 p.c. (about 6%) of the administration period. On all other intervals of the treatment phase (i.e. days 6-8, 8-10, 13-15, 17-19 and 19-20 p.c.) food uptake values of these dams was below the concurrent control values without attaining statistical significance. If calculated for the entire treatment phase (days 6-20 p.c.), the food consumption was about 5% below the concurrent control value.

The reduction in food consumption of the high concentration rats (11,000 ppm) is considered to be substance-induced. This effect is in line with the impaired absolute (see 4.2.1.4.) and corrected body weight gains (see 4.2.1.6.) of these females.

Food consumption at the mid and low concentration (4,000 and 1,000 ppm) was not affected by the test substance administration. Food consumption values of these rats were similar to or even exceeded control values.

Fig. 4.2.1.3.1.: Mean food consumption (g/animal/day)



4.2.1.4. Body weight data

(Mean values: Tabs. IA-004 - IA-006; Figs. 4.2.1.4.1. and 4.2.1.4.2.)

There were no statistically significant or biologically relevant differences between controls and the substance-treated dams in terms of mean body weights. The observable differences reflect the normal range of fluctuations for animals of this strain and age. Mean body weight gains of the 11,000 ppm dams, however, showed statistically significant impairments between days 10-13 p.c. (value about 26% below the concurrent control value). If calculated for the entire treatment phase (days 6-20 p.c.) or the entire study period (days 0-20 p.c.), body weight gain of these females was also statistically significantly lower (about 11%/10% below controls).

The substance-induced impairments on body weight gain at 11,000 ppm have both, a maternal and an embryo-/fetal component and are in line with the reductions in food consumption of these rats (see 4.2.1.3.). The corrected body weight gain (reflecting the maternal component – see 4.2.1.6.) and the mean fetal body weight (reflecting the embryo-/fetal component - see 4.3.1.3.) were both statistically significantly reduced at the highest concentration (11,000 ppm).

Mean body weight gains of the dams of test groups 1 and 2 (1,000 and 4,000 ppm) were similar to those of the concurrent controls.

Fig. 4.2.1.4.1. Mean body weight of pregnant animals

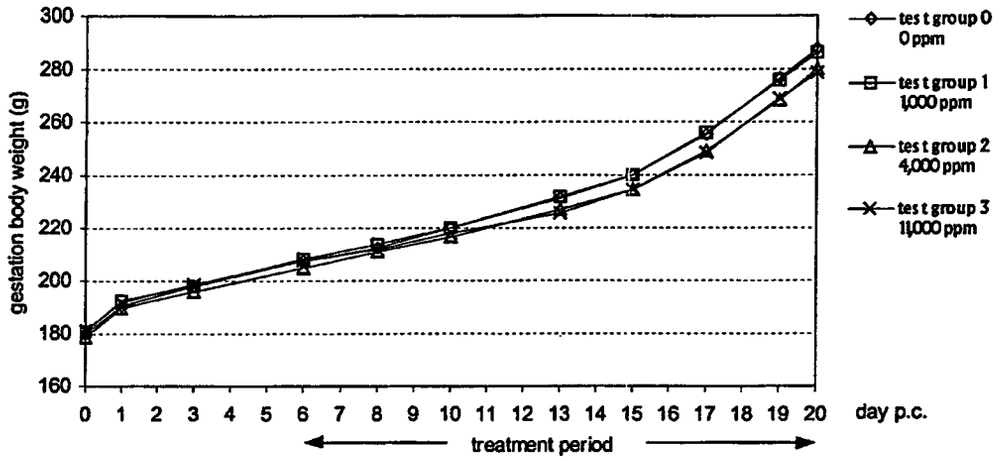
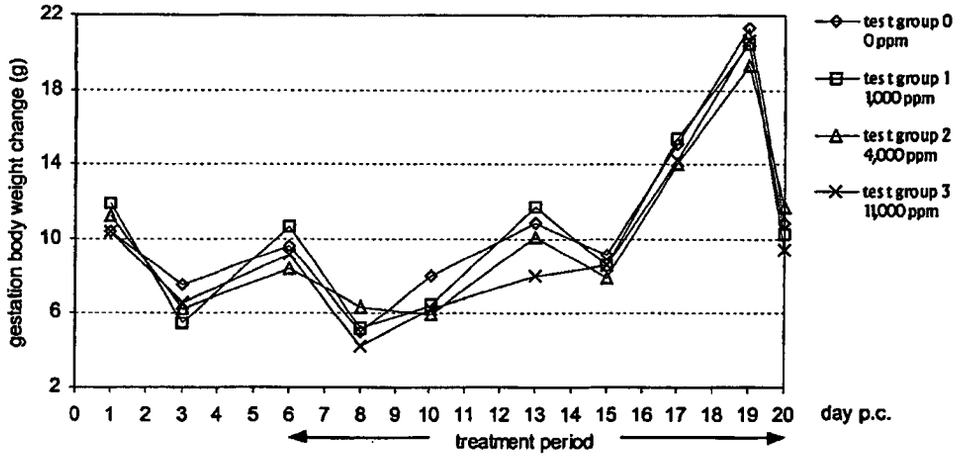


Fig. 4.2.1.4.2. Mean body weight change of pregnant animals



4.2.1.5. Intake of test substance

(Mean values: Tabs. IA-007 – IA-008)

The amount of test substance (in mg) which was consumed by the animals per kilogram body weight each day was calculated at the times at which food consumption was determined during the administration period (days 6 – 20 p.c.).

The overall approximate values are shown in Tab. 4.2.1.5.1.:

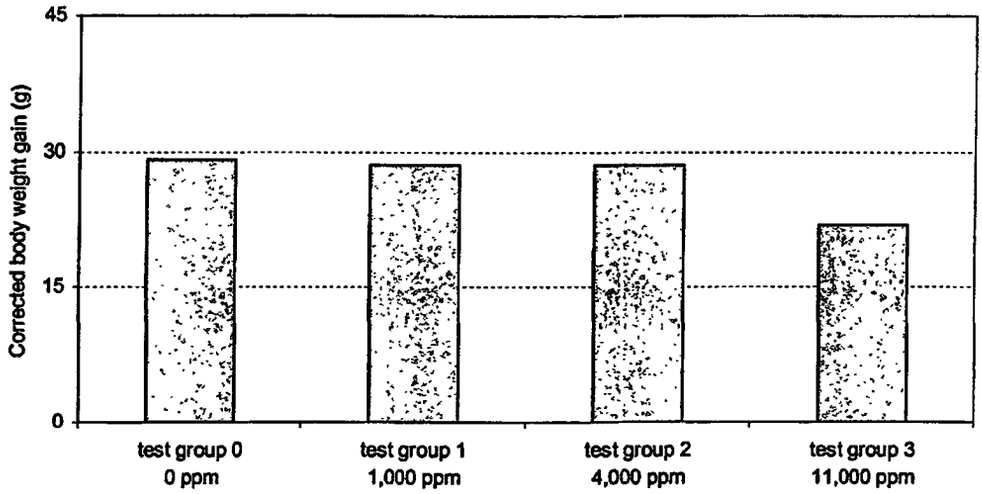
Tab. 4.2.1.5.1.:
Mean test substance intake (mg/kg body weight/day)

	Test group 1 (1,000 ppm)	Test group 2 (4,000 ppm)	Test group 3 (11,000 ppm)
Days 6 to 20 p.c.	87.6	363.4	942.1

4.2.1.6. Corrected body weight gain (net maternal body weight change)
(Mean values: Tab. IA-009; Fig. 4.2.1.6.1.)

The corrected body weight gains (terminal body weight on day 20 p.c. minus weight of the unopened uterus minus body weight on day 6 p.c.) of the dams of test groups 1 - 2 (1,000 and 4,000 ppm) revealed no differences of any biological relevance to the corresponding control group. The net weight change of the 11,000 ppm rats, however, was about 25% below the concurrent control value. As food consumption (see 4.2.1.3.) and absolute body weight gain (see 4.2.1.4.) of these rats were also diminished the effect on net body weight at the high concentration is considered to be a substance-related sign of straight maternal toxicity.

Fig. 4.2.1.6.1. Mean corrected body weight gain (net maternal body weight change) of pregnant animals



4.2.2. Examinations of the dams at termination

4.2.2.1. Uterus weight
(Mean values: Tab. IA-009)

The mean gravid uterus weights of the animals of test groups 1, 2 and 3 (1,000; 4,000 and 11,000 ppm) were not influenced by the administration of the test substance. The differences between these groups and the control group are considered to be without any biological relevance.

4.2.2.2. Necropsy findings
(Summary of findings: Tab. IA-010)

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

One low concentration animal (No. 47) showed a spontaneous diaphragmatic hernia. In one high concentration female (No. 78) a hemorrhagic thymus was recorded. It is very likely, that this by chance finding is related to the method how the rats were killed.

Furthermore, uni- or bilateral dilation of the renal pelvis occurred in one low concentration (No. 44) and one high concentration female (No. 83). This gross finding is also considered to be spontaneous in nature.

4.2.2.3. Reproduction data of dams
(Mean values: Tabs. IA-011 - IA-013)

The conception rate reached 88% in test groups 1 and 2 (1,000 and 4,000 ppm) and 92% in test groups 0 and 3 (0 and 11,000 ppm). All rats which became pregnant had implantation sites at necropsy. Thus a sufficient number of females for the purpose of the study was available (according to the test guidelines listed in chapter 2.3.).

There were no substance-related and/or biologically relevant differences between the different test groups in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the postimplantation losses, the number of resorptions and viable fetuses.

All differences observed appeared without a clear relation to dosing and reflect the normal range of fluctuations for animals of this strain and age; see also Volume III (Supplement) for historical control data. This includes the statistically significantly increased mean percentage of early resorptions at 4,000 ppm.

4.3. EXAMINATIONS OF THE FETUSES

Summary tables of the results are given in the Appendix of Volume I; individual values and findings are generally given in Part B of Volume II.

4.3.1. Examinations of the fetuses after dissection from the uterus

4.3.1.1. Sex distribution of fetuses
(Mean values: Tab. IA-013)

The sex distribution of the fetuses in test groups 1-3 (1,000; 4,000 and 11,000 ppm) was comparable with that of the control fetuses. The differences observed in comparison to the control were without any biological relevance.

4.3.1.2. Weight of placentae
(Mean values: Tab. IB-001)

The mean placental weights in test groups 1, 2 and 3 (1,000; 4,000 and 11,000 ppm) were not influenced by the test substance administration and were similar to the control values.

All differences observed are considered to reflect the normal range of fluctuations for animals of this strain and age; see also Volume III (Supplement) for historical control data.

4.3.1.3. Weight of fetuses
(Mean values: Tab. IB-001)

The mean fetal body weights in test group 3 (11,000 ppm) were statistically significantly reduced (about 5% below the concurrent control values if both genders are combined). This is assessed to be substance-induced.

The mean fetal body weights in test groups 1 and 2 (1,000 and 4,000 ppm) were not influenced by the test substance administration and were similar to the corresponding control values.

4.3.1.4. External examination of the fetuses
(Summary of findings: Tabs. IB-002 - IB-005)

As can be seen from Tab. 4.3.1.4.1. **external malformations** were observed in the form of bilateral anophthalmia and a severely malformed head in one fetus of test group 1 (1,000 ppm) only. The head of this low concentration female fetus (No. 1 from dam No. 40) showed externally a horn - like protrusion at the forehead and maxillary micrognathia and several associated skull malformations (see 4.3.3.).

Table 4.3.1.4.1. Individual external malformations

Test group	Dam No.-Fetus No., Sex	Malformation
0 (control)	none	
1 (1,000 ppm)	40 – 01, F	Anophthalmia, malformed head (+)
2 (4,000 ppm)	none	
3 (11,000 ppm)	none	

ppm = parts per million
M = male
F = female
(+) = fetus with associated skeletal malformations

In total, none of the 208 control fetuses from 23 litters, one out of 197 low concentration fetuses [= 0.5%] in one out of 22 litters [= 4.5%], none of the 182 mid concentration fetuses from 22 litters and none of the 211 high concentration fetuses from 23 litters showed external malformations. The mean percentages of affected fetuses/litter with external malformations amounted to 0.0, 0.5, 0.0 and 0.0%, respectively without attaining statistical significance in any of the test groups (0; 1,000; 4,000 and 11,000 ppm).

The isolated occurrence of the described external malformations in one low concentration fetus does not suggest any treatment relationship. Partly comparable findings of the head are also present in the historical control data (see Volume III, Tab. IIIB-006) at low incidences.

No **external variations** were recorded for any of the fetuses.

One so-called **unclassified external observation** was seen in the progeny of high concentration dam No. 96 (amniotic fluid discolored: fetus No. 3). This isolated finding has no toxicological relevance and is considered to be spontaneous in nature.

For overall assessment of the fetal findings, see 4.3.4.

4.3.2. Soft tissue examination of the fetuses
(Summary of findings: Tabs. IB-006 - IB-009)

No **soft tissue malformations** were recorded for any of the fetuses.

Two different **soft tissue variations** were detected in each group including the controls. Uni- or bilateral dilations of the renal pelvis - occasionally in association with unilaterally dilated ureter(s) - were found in fetuses of all test groups without any statistically significant or biologically relevant differences between the substance-treated and the actual control group. As can be seen from the respective historical control data set (see Volume III, Tab. IIIB-010) both aforementioned soft tissue variations appear also frequently in control rats of this strain.

The mean percentages of affected fetuses/litter with total soft tissue variations amounted to 10.2% (control), 6.6% (1,000 ppm), 11.3% (4,000 ppm) and 6.9% (11,000 ppm). All of these values are fully within the historical control range (4.4% – 22.2%) and do not show any relation to dosing. Thus, a substance-induced effect concerning the occurrence of soft tissue variations can be excluded with certainty.

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

For overall assessment of the fetal findings, see 4.3.4.

4.3.3. Skeletal examination of the fetuses
(Summary of findings: Tabs. IB-010 – IB-027)

As can be seen from Tab. 4.3.3.1. **malformations of the skeletons** were observed in single fetuses of all test groups (0; 1,000; 4,000 and 11,000 ppm) and affected skull, scapula, humerus, ribs and sternum. Low concentration female fetus No. 1 from dam No. 40 showed severely malformed skull bones (i.e. malpositioned and deformed nasal bone; absent incisive bone; small palatine bone; short, deformed and malpositioned maxilla); these skeletal findings match to the already described external malformations of this fetus (see 4.3.1.4).

Table 4.3.3.1. Individual fetal skeletal malformations

Test group	Dam No.-Fetus No., Sex	Malformation
0 (control)	02 – 05, F	Malpositioned and bipartite sternebra (unchanged cartilage), branched rib
1 (1,000 ppm)	31 – 05, F	Misshapen humerus
	40 – 01, F	Severely malformed skull bones (+)
	44 – 01, F	Misshapen humerus
	48 – 09, M	Malpositioned and bipartite sternebra (unchanged cartilage)
2 (4,000 ppm)	60 – 03, M	Shortened humerus
	60 – 05, M	Shortened humerus, shortened scapula
3 (11,000 ppm)	76 – 03, F	Misshapen humerus
	93 – 07, F	Misshapen humerus

ppm = parts per million
M = male
F = female
(+) = fetus with associated external malformations

In total, one out of 109 control fetuses [= 0.9%] in one out of 23 litters [= 4.3%], 4 out of 101 low concentration fetuses [= 4.0%] in 4 out of 22 litters [= 18%], 2 out of 97 examined mid concentration fetuses [= 2.1%] in one out of 22 litters [= 4.5%] and 2 out of 110 high concentration fetuses [= 1.8%] in 2 out of 23 litters [= 8.7%] showed skeletal malformations. The mean percentages of affected fetuses/litter with skeletal malformations amounted to 1.1, 3.6, 3.0 and 1.6%. All of the noted skeletal malformations appeared without a clear relation to dosing and some can be found at a comparable frequency in the historical control data (see Volume III (Supplement)). Therefore, the observable differences between the groups with regard to skeletal malformations are considered to be spontaneous in nature and without any biological relevance.

In all groups signs of **skeletal variations** with or without involvement of corresponding cartilaginous structures elicited. The observed variations were related to the skull (supraoccipital holes; incomplete ossification of basisphenoid, parietal, interparietal, supraoccipital, hyoid or all skull bones) the vertebral column (no ossification or incomplete, bipartite or dumbbell ossification of cervical, thoracic, lumbar and/or sacral vertebrae; misshapen sacral vertebrae; supernumerary thoracic vertebra; fused sacral centrum and arch), the ribs (supernumerary 14th, cervical or wavy ribs), the sternum (misshapen sternebra; unilateral, incomplete, missing or bipartite ossification of sternebra; extra sternebral ossification site) and the pelvic girdle (incomplete ossification of pubis). The mean percentages of affected fetuses/litter with skeletal variations amounted to 92.2, 85.4, 89.2 and 97.3% at 0; 1,000; 4,000 or 11,000 ppm. The vast majority of the noted skeletal variations appeared without a clear relation to dosing, without biologically relevant differences between the groups and/or can be found at a comparable frequency in the historical control data (see Volume III (Supplement)).

All skeletal variations with statistically significant differences on a fetus per litter basis between the control and the treated groups were compiled in Table 4.3.3.2.. All statistically significant differences which showed a dose-response relationship and/or were outside historical control ranges were marked in **bold types**.

Table 4.3.3.2. Occurrence of statistically significantly increased fetal skeletal variations (expressed as mean percentage of affected fetuses/litter)

Finding	Test group 0 0 ppm	Test group 1 1,000 ppm	Test group 2 4,000 ppm	Test group 3 11,000 ppm	HCD Mean % (range)
Incomplete ossification of thoracic centrum; unchanged cartilage	0.0	2.8*	2.7*	0.0	2.6 (0.0 – 7.2)
Incomplete ossification of sternebra; unchanged cartilage	38.4	42.7	46.5	68.7**	53.7 (41.3 – 74.6)
Unilateral ossification of sternebra; unchanged cartilage	0.0	0.0	0.0	**4.3***	1.5 (0.0 – 3.6)
Total fetal skeletal variations	92.2	85.4	89.2	97.3	93.8 (87.0 – 99.2)

ppm = parts per million
* = $p \leq 0.05$, ** = $p \leq 0.01$
HCD = Historical control data

The differences concerning skeletal variations of test groups 1 - 3 (1,000; 4,000 and 11,000 ppm) and the concurrent control are considered to be without any biological relevance and reflect the usual fluctuations in the strain of rats used for this study. This includes the statistically significant increase of unilateral ossification of sternebra (with unchanged cartilage) in the high concentration fetuses (as indicated in bold types in Tab. 4.3.3.2.). The respective fetus/litter value is only marginally above the upper historical control range and the overall rate of skeletal variations in this group (97.3%) is fully within the historical control range (87.0 – 99.2%). Moreover, the statistically significantly increased occurrence of some skeletal variations as listed in Tab. 4.3.3.2. represents common and transient findings on fetal morphology, which are not indicative for selective effects on the fetal organism.

Additionally, some isolated cartilage findings without any impact on the respective bony structures, which were designated as **unclassified cartilage observations**, occurred in all groups including the controls. The observed unclassified cartilage findings, which appeared without any statistically significant differences between the control and the substance-treated groups, were related to the skull, the vertebral column, the scapula, the ribs and the sternum. The mean percentages of affected fetuses/litter with these findings amounted to 34.3, 34.2, 35.1 and 36.7% at 0; 1,000; 4,000 and 11,000 ppm. The scattered occurrence of these cartilage findings without a clear dose-response relationship and/or at incidences which are fully within historical control ranges (see Volume III, Tab. IIIB-017) does not suggest any substance-induced origin.

For overall assessment of the above mentioned findings, see 4.3.4.

4.3.4. Abstract of fetal external, soft tissue and skeletal observations and their final assessment

The scattered occurrence of the few observed **external** (Tab. IB-003) and **skeletal malformations** (Tabs. IB-011 - IB-013) in single fetuses of all test groups including the controls (0; 1,000; 4,000 and 11,000 ppm) without a consistent pattern, without a clear dose-response relationship and/or at incidences, which are similar to historical control rates does not suggest any substance-induced origin of these findings. The most salient malformations affected head (anophthalmia; malformed head; severely malformed skull bones), limbs (shortened scapula; misshapen or shortened humerus), ribs (branched) and sternum (malpositioned and bipartite sternebra). **Soft tissue malformations** (Tab. IB-007), however, did not occur in this study.

If all different types of **malformations** (Tab. IB-028) are summarized, in total one of the 208 examined control fetuses [= 0.5%] in one out of 23 litters [= 4.3%], 4 of the 197 examined low concentration fetuses [= 2.0%] in 4 out of 22 litters [= 18%], 2 out of 182 mid concentration fetuses [= 1.1%] in one out of 22 litters [= 4.5%] and 2 out of 211 high concentration fetuses [= 0.9%] in 2 out of 23 litters [= 8.7%] showed malformations. The mean percentages of affected fetuses/litter with total malformations amounted to 0.5, 1.9, 1.5 and 0.9% at 0; 1,000; 4,000 or 11,000 ppm respectively. These incidences do not suggest any treatment-relationship.

External variations did not occur in any of the fetuses in this study (Tab. IB-004). **Soft tissue variations**, exclusively in the form of dilated renal pelvis and ureter (Tab. IB-008), occurred in all test groups including the controls without a clear relation to dosing and at incidences, which are fully within the historical control data range. From the broad spectrum of **skeletal variations** (Tabs. IB-014 – IB-024), which consisted primarily in transient delays in the ossification process, only one skeletal variation (i.e. of unilateral ossification of sternebra with unchanged cartilage) showed some relation to dosing and occurred at a statistically significantly increased rate in the high concentration fetuses (11,000 ppm) at an incidence, which was marginally above the upper historical control value.

Such slight retardations of the ossification process occur very frequently in gestation day 20 rat fetuses. This becomes obvious, if the overall rate of skeletal variations on a fetus/litter basis is taken into account: 92.2, 85.4, 89.2 and 97.3% at 0; 1,000; 4,000 or 11,000 ppm. Furthermore, this type of minor ossification delay has to be regarded as a transient phenomenon that is fully reversible postnatally. Thus, although the affected fetus/litter value for this finding exceeded slightly the historical control range it is considered as an incidental finding and not relevant in terms of developmental toxicity.

If all **variations** (Tab. IB-028) are summarized, in total 111 of the 208 examined control fetuses [= 53%] in all 23 litters [= 100%], 94 of the 197 examined low concentration fetuses [= 48%] in all 22 litters [= 100%], 94 out of 182 mid concentration fetuses [= 52%] in all 22 litters [= 100%] and 114 out of 211 high concentration fetuses [= 54%] in all 23 litters [= 100%] showed variations. The mean percentages of affected fetuses/litter with

total variations amounted to 53.6, 47.1, 52.7 and 54.1% at 0; 1,000; 4,000 or 11,000 ppm respectively without attaining statistical significance.

A spontaneous origin is also assumed for the observed **unclassified external and fetal skeletal observations** (Tabs. IB-005 and IB-025 – IB-027). Distribution and type of these findings do not suggest any relation to treatment.

Thus, the oral administration of **DIISOBUTYLPHTHALATE** with the diet up to a concentration of 11,000 ppm (approx. 942 mg/kg body weight/day) to pregnant Wistar rats **did not affect fetal morphology** and gave in particular **no indications for teratogenic properties**.

5. DISCUSSION AND CONCLUSION

DIISOBUTYLPHTHALATE was administered to pregnant Wistar rats/group as a constant homogeneous addition to the food at concentrations of 0; 1,000; 4,000 and 11,000 ppm on day 6 through day 20 post coitum (p.c.). The mean dose of **DIISOBUTYLPHTHALATE** administered was approx. 88 mg/kg body weight/day in the 1,000 ppm group, approx. 363 mg/kg body weight/day in the 4,000 ppm group and approx. 942 mg/kg body weight/day in the 11,000 ppm group.

Clear, substance-related **signs of maternal toxicity** occurred exclusively at 11,000 ppm (942 mg/kg body weight/day). Statistically significant impairments in food consumption were observed on several days of the treatment period (about 5% below control values if calculated for days 6 – 20 p.c.). The reductions in food intake were accompanied by statistically significantly impaired absolute body weight gains (about 11% below the control value if calculated for days 6 – 20 p.c.) and clear impairments of the corrected body weight gain (about 25% below the concurrent control value).

No signs of substance-induced **maternal toxicity** occurred at the low and the mid concentration (1,000 or 4,000 ppm [88 or 363 mg/kg body weight/day]).

The oral administration of **DIISOBUTYLPHTHALATE** to the dams at all 3 concentrations (1,000; 4,000 and 11,000 ppm) had **no influence on the gestational parameters**. Conception rate, mean number of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio or in the values calculated for the pre- and the postimplantation losses were unaffected by treatment.

The only sign of substance-induced **prenatal developmental toxicity** was a statistically significant reduction in mean fetal body weight at the high concentration (11,000 ppm). If both genders were combined mean fetal body weight was about 5% below the corresponding control value. Mean placental weights were not affected by treatment. The external, soft tissue and/or skeletal examinations of the fetuses revealed no differences between the control and the substance-treated groups, which might be related to the test substance administration. Number and type of fetal external, soft tissue and skeletal findings, which were classified as malformations and/or variations, did not show any differences of toxicological relevance between the groups. The few malformations and the broad spectrum of variations which occurred, appeared without a clear relation to the concentration administered and/or were seen at incidences previously found to occur spontaneously in control fetuses of this strain of rats. Additionally, no specific malformation pattern was found, which could be indicative of a selective teratogenicity.

Based on the results of this prenatal developmental toxicity study, the **no observed adverse effect level (NOAEL)** for **maternal** and **prenatal developmental toxicity** is **4,000 ppm (363 mg/kg body weight/day)**. Thus signs of prenatal developmental toxicity did only occur at a concentration, which was also clearly toxic to the dams. There were **no indications for teratogenicity** up to and including the top concentration, i.e. **11,000 ppm (942 mg/kg body weight/day)**.

6. REFERENCES

BARROW, M.V. and TAYLOR, W.J.
A rapid method for detecting malformations in rat fetuses.
J. Morph. 127, 291 - 306 (1969)

BELTRAME, D. and GIAVINI, E.
Morphological abnormalities in experimental Teratology: Need for a standardization of current terminology.
Cong. Anom. 30 (3), 187 - 195 (1990)

CHAHOUD, I., BUSCHMANN, J., CLARK, R., DRUGA, A., FALKE, H., FAQI; A., HANSEN, E., HEINRICH - HIRSCH, B., HELLWIG, J., LINGK, W., PARKINSON, M., PAUMGARTTEN, F., PFEIL, R., PLATZEK, T., SCIALLI, A., SEED, J., STAHL-MANN, R., ULBRICH, B., WU, X., YASUDA, M., YOUNES, M. and SOLECKI, R.
Classification terms in developmental toxicology: Need for harmonisation
Reproductive Toxicology 13, 77 - 82 (1999)

HOOD, R.D.
Handbook of Developmental Toxicology
CRC Press
Boca Raton, New York, London, Tokyo (1997)

KIMMEL, C.A. and TRAMMELL, C.
A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals.
Stain Technology 56, 271 - 273 (1981)

NIJENHUIS, A. and WILF, H.S.
Random permutation of n letters.
Combinatorial Algorithms, Academic Press, New York, San Francisco, London, 62 - 64 (1978)

SALEWSKI, E.
Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte.
Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. 247, 367 (1964)

SOLECKI, R., BÜRGIN H., BUSCHMANN, J., CLARK, R., DUVERGER M., FIALKOWSKI O., GUITTIN P., HELLWIG, J., HOFFMANN E., HOFMANN T., HÜBEL U., KHALIL S., LINGK, W., MANTOVANI A., MOXON M., MÜLLER S., PARKINSON, M., PAUL M., PAUMGARTTEN, F., PFEIL, R., PLATZEK, T., RAUCH-ERNST M., SCHEEVELENBOS A., SEED J., TALSNESS C.E., YASUDA, M., YOUNES, M. and CHAHOUD, I
Harmonisation of rat fetal skeletal terminology and classification. Report of the third workshop on terminology in developmental toxicology; Reproductive Toxicology 15, 713 - 721 (2001)

WILSON, J.G. and WARKANY, J.
Teratology: Principles and Techniques.
The University of Chicago Press.
Chicago and London (1965)

WISE, D., BECK, S., BELTRAME, D., BEYER, B., CHAHOUD, I., CLARK, R.L., CLARK, R., DRUGA, A., FEUSTON, M., GUITTIN, P., HENWOOD, S., KIMMEL, C., LINDSTROM, P., PALMER, A., PETRERE, J., SOLOMON, H., YASUDA, M. and YORK, R.
Terminology of developmental abnormalities in common laboratory mammals (Version 1)
Teratology 55, 249 - 292 (1997)

7. APPENDIX (SUMMARY TABLES)

7.1. LIST TO THE ABBREVIATIONS USED IN VOLUMES IA AND IB

Tables IA-001 – IA-013 (EXAMINATION OF THE DAMS - CLINICS AND GROSS NECROPSY)
Tables IB-001 – IB-028 (EXAMINATION OF THE FETUSES)

MEAN	=	mean value
MG/KG BW/D	=	milligram per kilogram body weight per day
N/#/NO.	=	number/number of animals or litters
S.D.	=	standard deviation
%	=	per cent
UNCLASS. CARTILAGE OBS.	=	unclassified cartilage observations
ppm	=	parts per million

All other abbreviations used are explained in the tables.

Part A:
EXAMINATIONS OF THE DAMS - CLINICS AND GROSS NECROPSY (MEAN VALUES AND SUMMARY TABLES)

	Tables
Summary of maternal clinical observations	IA-001
Mean maternal food consumption	IA-002 - IA-003
Mean maternal body weights	IA-004
Mean maternal body weight change	IA-005 - IA-006
Mean maternal substance intake	IA-007 - IA-008
Mean gravid uterine weights and net maternal body weight change	IA-009
Summary of maternal necropsy observations	IA-010
Summary of reproduction data	IA-011 - IA-013

The relevant individual values/findings are to be found in Volume II (Tables IIA-001 - IIA-039).

Part B:
EXAMINATIONS OF THE FETUSES (MEAN VALUES AND SUMMARY TABLES)

	Tables
Mean placental and fetal body weights (on a litter basis)	IB-001
Summary of all classified fetal external observations	IB-002 - IB-004
Summary of fetal external unclassified observations	IB-005
Summary of all classified fetal soft tissue observations	IB-006 - IB-008
Summary of fetal soft tissue unclassified observations	IB-009
Summary of all classified fetal skeletal observations	IB-010 - IB-024
Summary of fetal skeletal unclassified cartilage observations	IB-025 - IB-027
Summary of all classified fetal external, soft tissue and skeletal observations	IB-028

The relevant individual values or findings are to be found in Volume II (Tables IIB-001 - IIB-087).

23-JAN-03

02018

TABLE : IA- 001

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING GESTATION

	GROUP#	DAY OF GESTATION 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	TOTAL
# OF FEMALES EXAMINED	0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
	1	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
	2	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
	3	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
NORMAL																							
NOTHING ABNORMAL DETECTED	0	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	1	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	2	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	3	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25

23-JAN-03

02018

TABLE : IA- 002

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- GRAMS/ANIMAL/DAY

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAYS 0 TO 1	MEAN	13.5 D	13.6	13.9	13.6
	S.D.	1.33	1.31	1.90	2.09
	N	23	22	21	23
DAYS 1 TO 3	MEAN	17.7 D	16.8	17.1	17.3
	S.D.	1.35	1.85	1.41	1.32
	N	23	22	22	23
DAYS 3 TO 6	MEAN	18.8 D	18.4	18.3	18.3
	S.D.	1.43	1.59	1.30	1.38
	N	23	22	22	23
DAYS 6 TO 8	MEAN	18.9 D	18.7	19.5	17.8
	S.D.	1.60	1.63	1.63	1.66
	N	23	22	22	23
DAYS 8 TO 10	MEAN	19.8 D	19.3	19.6	18.8
	S.D.	1.81	1.79	1.25	1.40
	N	23	22	22	23
DAYS 10 TO 13	MEAN	20.3 D	19.9	19.8	18.7**
	S.D.	1.65	1.80	1.51	1.14
	N	23	22	22	23
DAYS 13 TO 15	MEAN	20.5 D	20.4	20.9	19.7
	S.D.	1.87	2.02	1.90	1.77
	N	23	22	22	23
DAYS 15 TO 17	MEAN	22.1 D	21.7	21.6	20.7*
	S.D.	1.60	2.08	1.97	1.51
	N	23	22	22	23
DAYS 17 TO 19	MEAN	22.7 D	22.4	22.3	21.8
	S.D.	1.68	2.26	1.98	1.57
	N	23	22	22	23
DAYS 19 TO 20	MEAN	21.5 D	21.3	22.0	20.3
	S.D.	1.07	2.39	1.87	2.94
	N	23	22	22	23

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

049

23-JAN-03

02018

TABLE : IA- 003

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- GRAMS/ANIMAL/DAY

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAYS 0 TO 6	MEAN OF MEANS	16.7	16.3	16.4	16.4
	S.D.	2.75	2.43	2.28	2.47
	N	3	3	3	3
DAYS 6 TO 20	MEAN OF MEANS	20.8	20.5	20.8	19.7
	S.D.	1.33	1.34	1.16	1.37
	N	7	7	7	7
DAYS 0 TO 20	MEAN OF MEANS	19.6	19.2	19.5	18.7
	S.D.	2.62	2.59	2.56	2.26
	N	10	10	10	10

23-JAN-03

02018

TABLE : IA- 004

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN MATERNAL BODY WEIGHTS DURING GESTATION -- GRAMS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAY 0	MEAN	179.9 D	180.4	178.7	181.4
	S.D.	10.59	8.85	8.91	7.51
	N	23	22	22	23
DAY 1	MEAN	190.2 D	192.4	189.9	191.8
	S.D.	10.60	8.14	9.44	6.97
	N	23	22	22	23
DAY 3	MEAN	197.7 D	197.8	196.2	198.3
	S.D.	10.86	8.90	8.42	7.28
	N	23	22	22	23
DAY 6	MEAN	207.3 D	208.5	204.5	207.4
	S.D.	10.41	9.60	8.89	7.55
	N	23	22	22	23
DAY 8	MEAN	212.3 D	213.7	210.8	211.5
	S.D.	9.88	10.44	9.54	7.42
	N	23	22	22	23
DAY 10	MEAN	220.3 D	220.1	216.7	217.7
	S.D.	10.19	10.47	9.74	8.53
	N	23	22	22	23
DAY 13	MEAN	231.1 D	231.7	226.8	225.7
	S.D.	10.12	11.53	11.31	7.77
	N	23	22	22	23
DAY 15	MEAN	240.2 D	240.2	234.7	234.3
	S.D.	10.37	11.27	12.50	8.21
	N	23	22	22	23
DAY 17	MEAN	255.3 D	255.6	248.8	248.5
	S.D.	11.18	12.64	14.39	8.95
	N	23	22	22	23
DAY 19	MEAN	276.6 D	276.1	268.1	269.2
	S.D.	12.37	15.08	16.55	10.91
	N	23	22	22	23
DAY 20	MEAN	287.4 D	286.5	279.8	278.6
	S.D.	13.38	15.22	18.01	11.34
	N	23	22	22	23

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

051

23-JAN-03

02018

TABLE : IA- 005

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION -- GRAMS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAYS 0 TO 1	MEAN	10.4 D	11.9	11.2	10.4
	S.D.	2.86	2.74	3.11	3.89
	N	23	22	22	23
DAYS 1 TO 3	MEAN	7.5 D	5.5	6.2	6.5
	S.D.	2.78	3.12	3.02	2.42
	N	23	22	22	23
DAYS 3 TO 6	MEAN	9.6 D	10.7	8.3	9.1
	S.D.	3.06	2.99	2.69	3.31
	N	23	22	22	23
DAYS 6 TO 8	MEAN	5.0 D	5.2	6.3	4.2
	S.D.	2.58	2.64	2.55	2.36
	N	23	22	22	23
DAYS 8 TO 10	MEAN	8.0 D	6.4	5.9	6.2
	S.D.	3.06	3.19	2.88	3.36
	N	23	22	22	23
DAYS 10 TO 13	MEAN	10.8 D	11.7	10.1	8.0*
	S.D.	2.41	3.81	4.34	3.11
	N	23	22	22	23
DAYS 13 TO 15	MEAN	9.1 D	8.5	7.9	8.5
	S.D.	3.06	2.76	3.58	3.20
	N	23	22	22	23
DAYS 15 TO 17	MEAN	15.1 D	15.4	14.0	14.2
	S.D.	3.69	3.69	3.65	2.45
	N	23	22	22	23
DAYS 17 TO 19	MEAN	21.3 D	20.5	19.3	20.7
	S.D.	3.54	4.50	4.95	4.36
	N	23	22	22	23
DAYS 19 TO 20	MEAN	10.8 D	10.3	11.7	9.4
	S.D.	2.95	3.60	3.13	4.77
	N	23	22	22	23

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

052

23-JAN-03

02018

TABLE : IA- 006

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION -- GRAMS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAYS 0 TO 6	MEAN	27.4 D	28.1	25.8	25.9
	S.D.	4.56	4.82	4.03	4.79
	N	23	22	22	23
DAYS 6 TO 20	MEAN	80.1 D	78.0	75.4	71.2*
	S.D.	8.33	10.54	14.04	7.82
	N	23	22	22	23
DAYS 0 TO 20	MEAN	107.5 D	106.1	101.1	97.2*
	S.D.	11.37	13.45	15.24	9.33
	N	23	22	22	23

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

23-JAN-03

02018

TABLE : IA- 007

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN MATERNAL SUBSTANCE INTAKE DURING GESTATION -- MG/KG BODY WEIGHT/DAY

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAYS 6 TO 8	MEAN	0.0	89.7	382.7	945.9
	S.D.	0.00	7.53	35.45	83.99
	N	23	22	22	23
DAYS 8 TO 10	MEAN	0.0	90.2	372.9	978.0
	S.D.	0.00	8.18	21.95	64.69
	N	23	22	22	23
DAYS 10 TO 13	MEAN	0.0	90.5	366.1	943.8
	S.D.	0.00	7.52	25.24	63.30
	N	23	22	22	23
DAYS 13 TO 15	MEAN	0.0	88.0	367.6	958.2
	S.D.	0.00	8.17	25.79	85.74
	N	23	22	22	23
DAYS 15 TO 17	MEAN	0.0	90.2	368.1	972.7
	S.D.	0.00	7.83	29.51	63.40
	N	23	22	22	23
DAYS 17 TO 19	MEAN	0.0	87.5	357.9	966.2
	S.D.	0.00	7.00	23.66	62.05
	N	23	22	22	23
DAYS 19 TO 20	MEAN	0.0	77.3	328.3	829.6
	S.D.	0.00	7.78	28.13	119.49
	N	23	22	22	23

23-JAN-03

02018

TABLE : IA- 008

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN MATERNAL SUBSTANCE INTAKE DURING GESTATION -- MG/KG BODY WEIGHT/DAY

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAYS 6 TO 20	MEAN OF MEANS	0.0	87.6	363.4	942.1
	S.D.	0.00	4.70	17.18	51.21
	N	7	7	7	7

23-JAN-03

02018

TABLE : IA- 009

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN GRAVID UTERINE WEIGHTS AND NET MATERNAL BODY WEIGHT CHANGE -- GRAMS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
GRAVID UTERUS	MEAN	50.9 D	49.5	46.7	49.3
	S.D.	6.70	6.52	11.40	7.23
	N	23	22	22	23
CARCASS	MEAN	236.5 D	237.0	233.1	229.3
	S.D.	12.46	13.47	14.74	9.70
	N	23	22	22	23
NET WEIGHT CHANGE FROM DAY 6	MEAN	29.2 D	28.5	28.6	21.9*
	S.D.	6.81	8.61	10.16	8.21
	N	23	22	22	23

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

CARCASS WEIGHT = TERMINAL BODY WEIGHT MINUS UTERINE WEIGHT
NET WEIGHT CHANGE FROM DAY 6 = CARCASS WEIGHT MINUS DAY 6 BODY WEIGHT

23-JAN-03

02018

TABLE : IA- 010

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF MATERNAL NECROPSY OBSERVATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
DAMS EXAMINED	N	25	25	25	25
NOTHING ABNORMAL DETECTED	N	25	23	25	23
	%	100	92	100	92
HEMORRHAGIC THYMUS	N	0	0	0	1
	%	0.0	0.0	0.0	4.0
DIAPHRAGMATIC HERNIA	N	0	1	0	0
	%	0.0	4.0	0.0	0.0
DILATED RENAL PELVIS	N	0	1	0	1
	%	0.0	4.0	0.0	4.0

057

23-JAN-03

02018

TABLE : IA- 011

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF REPRODUCTION DATA

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Females Mated	N	25	25	25	25
Pregnant	N	23	22	22	23
Conception Rate	%	92	88	88	92
Aborted	N	0	0	0	0
Premature Births	N	0	0	0	0
Dams with Viable Fetuses	N	23	22	22	23
Dams with all Resorptions	N	0	0	0	0
Female Mortality	N	0Fi	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at Terminal Sacrifice	N	23Fi	22	22	23
	%	92	88	88	92
Corpora Lutea	MEAN	10.7 D	10.5	10.4	10.5
	S.D.	1.11	1.41	1.71	1.41
	TOTAL	245	231	228	241
Implantation Sites	MEAN	9.5 D	9.5	9.0	9.7
	S.D.	1.34	1.26	2.08	1.15
	TOTAL	218	210	199	222
Preimplantation Loss	MEAN%	10.5 D	8.5	13.1	7.3
	S.D.	12.58	9.78	12.50	8.42
Postimplantation Loss	MEAN%	4.4 D	6.0	9.0	5.1
	S.D.	5.22	7.59	9.52	7.40

Statistics: D=Dunnett-test (two-sided) Fi =Fisher's exact test (one-sided)
* : p<=0.05 ** : p<=0.01

058

23-JAN-03

02018

TABLE : IA- 012

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF REPRODUCTION DATA

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Pregnant at Terminal Sacrifice	N	23	22	22	23
Resorptions: Total	MEAN	0.4 D	0.6	0.8	0.5
	S.D.	0.51	0.73	0.81	0.67
	TOTAL	10	13	17	11
	MEAN%	4.4 D	6.0	9.0	5.1
	S.D.	5.22	7.59	9.52	7.40
Early	MEAN	0.3 D	0.5	0.7	0.4
	S.D.	0.49	0.67	0.77	0.58
	TOTAL	8	12	16	9
	MEAN%	3.5 D	5.5	8.5*	4.0
	S.D.	4.90	6.78	9.21	5.94
Late	MEAN	0.1 D	0.0	0.0	0.1
	S.D.	0.29	0.21	0.21	0.29
	TOTAL	2	1	1	2
	MEAN%	1.0 D	0.5	0.5	1.2
	S.D.	3.20	2.37	2.13	3.87
Dead Fetuses	N	0	0	0	0

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

059

23-JAN-03

02018

TABLE : IA- 013

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF REPRODUCTION DATA

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Dams with Viable Fetuses	N	23	22	22	23
Live Fetuses	MEAN	9.0 D	9.0	8.3	9.2
	S.D.	1.30	1.25	2.16	1.37
	TOTAL	208	197	182	211
	MEAN%	95.6 D	94.0	91.0	94.9
	S.D.	5.22	7.59	9.52	7.40
Females	MEAN	4.6 D	4.4	3.8	4.9
	S.D.	1.50	1.59	1.74	1.28
	TOTAL	106	96	83	113
	MEAN%	48.4 D	46.5	40.6	50.9
	S.D.	13.76	17.65	16.18	11.62
Males	MEAN	4.4 D	4.6	4.5	4.3
	S.D.	1.24	1.99	1.77	1.57
	TOTAL	102	101	99	98
	MEAN%	47.2 D	47.5	50.5	43.9
	S.D.	12.74	17.25	16.25	15.50
PER CENT LIVE FEMALES		51.0	48.7	45.6	53.6
PER CENT LIVE MALES		49.0	51.3	54.4	46.4

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

060

23-JAN-03

02018

TABLE : IB- 001

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
MEAN PLACENTAL AND FETAL BODY WEIGHTS (ON A LITTER BASIS)

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
PLACENTAL WEIGHTS UNITS: GRAMS					
of all Viable Fetuses	MEAN	0.45 D	0.42	0.43	0.43
	S.D.	0.064	0.034	0.063	0.039
	N	23	22	22	23
of Male Fetuses	MEAN	0.46 D	0.44	0.44	0.44
	S.D.	0.066	0.038	0.060	0.037
	N	23	22	22	23
of Female Fetuses	MEAN	0.44 D	0.41	0.43	0.42
	S.D.	0.065	0.036	0.069	0.042
	N	23	22	22	23
FETAL WEIGHTS UNITS: GRAMS					
of all Viable Fetuses	MEAN	3.7 D	3.6	3.6	3.5**
	S.D.	0.22	0.23	0.19	0.17
	N	23	22	22	23
of Male Fetuses	MEAN	3.8 D	3.7	3.7	3.6**
	S.D.	0.24	0.26	0.20	0.21
	N	23	22	22	23
of Female Fetuses	MEAN	3.6 D	3.5	3.5	3.4**
	S.D.	0.24	0.25	0.21	0.19
	N	23	22	22	23

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 002

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF ALL CLASSIFIED FETAL EXTERNAL OBSERVATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	208	197	182	211
Live	N	208	197	182	211
Dead	N	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.5	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.5	0.0	0.0
	S.D.	0.00	2.13	0.00	0.00
TOTAL VARIATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0Fi	0	0	0
	%	0.0	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

062

5-FEB-03

02018

TABLE : IB- 003

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL EXTERNAL MALFORMATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	208	197	182	211
Live	N	208	197	182	211
Dead	N	0	0	0	0
MALFORMED HEAD					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.5	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.5	0.0	0.0
	S.D.	0.00	2.13	0.00	0.00
ANOPHTHALMIA					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.5	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.5	0.0	0.0
	S.D.	0.00	2.13	0.00	0.00
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.5	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.5	0.0	0.0
	S.D.	0.00	2.13	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL EXTERNAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	208	197	182	211
Live	N	208	197	182	211
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL VARIATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0Fi	0	0	0
	%	0.0	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 005

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL EXTERNAL UNCLASSIFIED OBSERVATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	208	197	182	211
Live	N	208	197	182	211
Dead	N	0	0	0	0
AMNIOTIC FLUID DISCOLORED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.5
Litter Incidence	N	0Fi	0	0	1
	%	0.0	0.0	0.0	4.3
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.7
	S.D.	0.00	0.00	0.00	3.48
TOTAL FETAL EXTERNAL UNCLASSIFIED OBSERVATIONS					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.5
Litter Incidence	N	0Fi	0	0	1
	%	0.0	0.0	0.0	4.3
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.7
	S.D.	0.00	0.00	0.00	3.48

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF ALL CLASSIFIED FETAL SOFT TISSUE OBSERVATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	99	96	85	101
Live	N	99	96	85	101
Dead	N	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0Fi	0	0	0
	%	0.0	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00
TOTAL VARIATIONS					
Fetal Incidence	N	10	7	9	7
	%	10	7.3	11	6.9
Litter Incidence	N	7Fi	5	8	5
	%	30	23	36	22
Affected Fetuses/Litter	MEAN%	10.2Wi	6.6	11.3	6.9
	S.D.	22.03	14.59	16.43	13.89

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

13-FEB-03

02018

TABLE : IB- 007

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SOFT TISSUE MALFORMATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	99	96	85	101
Live	N	99	96	85	101
Dead	N	0	0	0	0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0Fi	0	0	0
	%	0.0	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

067

13-FEB-03

02018

TABLE : IB- 008

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SOFT TISSUE VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	99	96	85	101
Live	N	99	96	85	101
Dead	N	0	0	0	0
DILATED RENAL PELVIS					
Fetal Incidence	N	10	7	9	7
	%	10	7.3	11	6.9
Litter Incidence	N	7Fi	5	8	5
	%	30	23	36	22
Affected Fetuses/Litter	MEAN%	10.2Wi	6.6	11.3	6.9
	S.D.	22.03	14.59	16.43	13.89
DILATED URETER					
Fetal Incidence	N	2	2	1	1
	%	2.0	2.1	1.2	1.0
Litter Incidence	N	2Fi	1	1	1
	%	8.7	4.5	4.5	4.3
Affected Fetuses/Litter	MEAN%	2.0Wi	1.8	1.1	0.9
	S.D.	6.53	8.53	5.33	4.17
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence	N	10	7	9	7
	%	10	7.3	11	6.9
Litter Incidence	N	7Fi	5	8	5
	%	30	23	36	22
Affected Fetuses/Litter	MEAN%	10.2Wi	6.6	11.3	6.9
	S.D.	22.03	14.59	16.43	13.89

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

068

13-FEB-03

02018

TABLE : IB- 009

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SOFT TISSUE UNCLASSIFIED OBSERVATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	99	96	85	101
Live	N	99	96	85	101
Dead	N	0	0	0	0
TOTAL FETAL SOFT TISSUE UNCLASSIFIED OBSERVATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0Fi	0	0	0
	%	0.0	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 010

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF ALL CLASSIFIED FETAL SKELETAL OBSERVATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N	1	4	2	2
	%	0.9	4.0	2.1	1.8
Litter Incidence	N	1Fi	4	1	2
	%	4.3	18	4.5	8.7
Affected Fetuses/Litter	MEAN%	1.1Wi	3.6	3.0	1.6
	S.D.	5.21	7.90	14.21	5.31
TOTAL VARIATIONS					
Fetal Incidence	N	101	87	85	107
	%	93	86	88	97
Litter Incidence	N	23Fi	22	22	23
	%	100	100	100	100
Affected Fetuses/Litter	MEAN%	92.2Wi	85.4	89.2	97.3
	S.D.	13.38	18.71	15.93	7.19

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

070

5-FEB-03

02018

TABLE : IB- .011

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL MALFORMATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
SEVERELY MALFORMED SKULL BONES					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.9	0.0	0.0
	S.D.	0.00	4.26	0.00	0.00
SHORTENED SCAPULA; Cartilage present					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	1.0	0.0
Litter Incidence	N	0Fi	0	1	0
	%	0.0	0.0	4.5	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	1.5	0.0
	S.D.	0.00	0.00	7.11	0.00
MALPOSITIONED AND BIPARTITE STERNEBRA; Unchanged cartilage					
Fetal Incidence	N	1	1	0	0
	%	0.9	1.0	0.0	0.0
Litter Incidence	N	1Fi	1	0	0
	%	4.3	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.1Wi	0.9	0.0	0.0
	S.D.	5.21	4.26	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

071

5-FEB-03

02018

TABLE : IB- 012

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL MALFORMATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
BRANCHED RIB					
Fetal Incidence	N	1	0	0	0
	%	0.9	0.0	0.0	0.0
Litter Incidence	N	1Fi	0	0	0
	%	4.3	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.1Wi	0.0	0.0	0.0
	S.D.	5.21	0.00	0.00	0.00
MISSHAPEN HUMERUS					
Fetal Incidence	N	0	2	0	2
	%	0.0	2.0	0.0	1.8
Litter Incidence	N	0Fi	2	0	2
	%	0.0	9.1	0.0	8.7
Affected Fetuses/Litter	MEAN%	0.0Wi	1.8	0.0	1.6
	S.D.	0.00	5.88	0.00	5.31
SHORTENED HUMERUS					
Fetal Incidence	N	0	0	2	0
	%	0.0	0.0	2.1	0.0
Litter Incidence	N	0Fi	0	1	0
	%	0.0	0.0	4.5	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	3.0	0.0
	S.D.	0.00	0.00	14.21	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

072

5-FEB-03

02018

TABLE : IB- 013

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL MALFORMATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	1	4	2	2
	%	0.9	4.0	2.1	1.8
Litter Incidence	N	1Fi	4	1	2
	%	4.3	18	4.5	8.7
Affected Fetuses/Litter	MEAN%	1.1Wi	3.6	3.0	1.6
	S.D.	5.21	7.90	14.21	5.31

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

073

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
SUPRAOCCIPITAL HOLE(S)					
Fetal Incidence	N	31	23	15	38
	%	28	23	15	35
Litter Incidence	N	15Fi	13	12	19
	%	65	59	55	83
Affected Fetuses/Litter	MEAN%	28.4Wi	21.7	15.8	35.1
	S.D.	28.30	21.92	17.09	26.04
INCOMPLETE OSSIFICATION OF BASISPHENOID					
Fetal Incidence	N	7	2	6	8
	%	6.4	2.0	6.2	7.3
Litter Incidence	N	3Fi	2	5	4
	%	13	9.1	23	17
Affected Fetuses/Litter	MEAN%	5.5Wi	2.3	5.6	6.7
	S.D.	16.16	7.36	11.45	14.91
INCOMPLETE OSSIFICATION OF INTERPARIETAL; Unchanged cartilage					
Fetal Incidence	N	24	11	22	13
	%	22	11	23	12
Litter Incidence	N	15Fi	7	12	9
	%	65	32	55	39
Affected Fetuses/Litter	MEAN%	21.4Wi	10.6	23.5	12.8
	S.D.	21.98	16.88	26.13	19.40

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 015

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF PARIETAL; Unchanged cartilage					
Fetal Incidence	N	16	13	22	13
	%	15	13	23	12
Litter Incidence	N	10Fi	9	13	7
	%	43	41	59	30
Affected Fetuses/Litter	MEAN%	14.1Wi	12.2	23.9	12.2
	S.D.	19.32	17.40	25.56	21.45
INCOMPLETE OSSIFICATION OF SUPRAOCCIPITAL; Unchanged cartilage					
Fetal Incidence	N	9	14	14	13
	%	8.3	14	14	12
Litter Incidence	N	7Fi	11	12	6
	%	30	50	55	26
Affected Fetuses/Litter	MEAN%	8.3Wi	13.8	15.0	12.2
	S.D.	14.89	17.15	16.02	28.76
INCOMPLETE OSSIFICATION OF SKULL; Unchanged cartilage					
Fetal Incidence	N	5	2	7	2
	%	4.6	2.0	7.2	1.8
Litter Incidence	N	4Fi	1	4	2
	%	17	4.5	18	8.7
Affected Fetuses/Litter	MEAN%	4.3Wi	2.3	9.0	1.7
	S.D.	10.37	10.66	23.45	5.76

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

075

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF HYOID; Cartilage present					
Fetal Incidence	N	1	0	1	1
	%	0.9	0.0	1.0	0.9
Litter Incidence	N	1Fi	0	1	1
	%	4.3	0.0	4.5	4.3
Affected Fetuses/Litter	MEAN%	0.7Wi	0.0	0.6	0.9
	S.D.	3.48	0.00	3.05	4.17
INCOMPLETE OSSIFICATION OF CERVICAL ARCH; Cartilage present					
Fetal Incidence	N	1	0	0	0
	%	0.9	0.0	0.0	0.0
Litter Incidence	N	1Fi	0	0	0
	%	4.3	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.1Wi	0.0	0.0	0.0
	S.D.	5.21	0.00	0.00	0.00
INCOMPLETE OSSIFICATION OF THORACIC CENTRUM; Unchanged cartilage					
Fetal Incidence	N	0	3	3	0
	%	0.0	3.0	3.1	0.0
Litter Incidence	N	0Fi	3	3	0
	%	0.0	14	14	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	2.8*	2.7*	0.0
	S.D.	0.00	7.34	7.14	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
DUMBBELL OSSIFICATION OF THORACIC CENTRUM; Unchanged cartilage					
Fetal Incidence	N	4	3	2	9
	%	3.7	3.0	2.1	8.2
Litter Incidence	N	3Fi	3	2	7
	%	13	14	9.1	30
Affected Fetuses/Litter	MEAN%	3.7Wi	3.0	1.8	8.3
	S.D.	10.25	7.66	5.88	15.10
DUMBBELL OSSIFICATION OF THORACIC CENTRUM; Dumbbell-shaped cartilage of centrum					
Fetal Incidence	N	14	13	13	17
	%	13	13	13	15
Litter Incidence	N	9Fi	9	13	14
	%	39	41	59	61
Affected Fetuses/Litter	MEAN%	12.4Wi	12.7	12.8	16.5
	S.D.	17.62	17.37	11.46	16.94
BIPARTITE OSSIFICATION OF THORACIC CENTRUM; Dumbbell-shaped cartilage of centrum					
Fetal Incidence	N	2	2	0	1
	%	1.8	2.0	0.0	0.9
Litter Incidence	N	2Fi	2	0	1
	%	8.7	9.1	0.0	4.3
Affected Fetuses/Litter	MEAN%	1.7Wi	2.0	0.0	1.4
	S.D.	5.76	6.67	0.00	6.95

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
SUPERNUMERARY THORACIC VERTEBRA					
Fetal Incidence	N	1	2	1	3
	%	0.9	2.0	1.0	2.7
Litter Incidence	N	1Fi	1	1	2
	%	4.3	4.5	4.5	8.7
Affected Fetuses/Litter	MEAN%	0.9Wi	1.8	0.8	3.8
	S.D.	4.17	8.53	3.55	14.33
UNOSSIFIED THORACIC CENTRUM; Dumbbell-shaped cartilage of centrum					
Fetal Incidence	N	1	0	0	0
	%	0.9	0.0	0.0	0.0
Litter Incidence	N	1Fi	0	0	0
	%	4.3	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.1Wi	0.0	0.0	0.0
	S.D.	5.21	0.00	0.00	0.00
DUMBBELL OSSIFICATION OF LUMBAR CENTRUM; Dumbbell-shaped cartilage of centrum					
Fetal Incidence	N	1	0	0	0
	%	0.9	0.0	0.0	0.0
Litter Incidence	N	1Fi	0	0	0
	%	4.3	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.1Wi	0.0	0.0	0.0
	S.D.	5.21	0.00	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 019

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF LUMBAR ARCH: Cartilage present					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	1.0	0.0
Litter Incidence	N	0Fi	0	1	0
	%	0.0	0.0	4.5	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	1.5	0.0
	S.D.	0.00	0.00	7.11	0.00
MISSHAPEN SACRAL VERTEBRA					
Fetal Incidence	N	1	1	1	4
	%	0.9	1.0	1.0	3.6
Litter Incidence	N	1Fi	1	1	4
	%	4.3	4.5	4.5	17
Affected Fetuses/Litter	MEAN%	0.9Wi	0.9	1.1	3.6
	S.D.	4.17	4.26	5.33	8.01
FUSED SACRAL CENTRUM AND ARCH: Unchanged cartilage					
Fetal Incidence	N	3	7	5	5
	%	2.8	6.9	5.2	4.5
Litter Incidence	N	2Fi	3	4	3
	%	8.7	14	18	13
Affected Fetuses/Litter	MEAN%	2.6Wi	8.0	4.7	4.3
	S.D.	9.15	23.64	11.97	11.99

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

079

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF SACRAL ARCH; Cartilage present					
Fetal Incidence	N	5	2	0	0
	%	4.6	2.0	0.0	0.0
Litter Incidence	N	4Fi	2	0	0
	%	17	9.1	0.0	0.0
Affected Fetuses/Litter	MEAN%	4.3Wi	1.7	0.0	0.0
	S.D.	10.37	5.42	0.00	0.00
UNOSSIFIED STERNEBRA; Unchanged cartilage					
Fetal Incidence	N	4	11	1	6
	%	3.7	11	1.0	5.5
Litter Incidence	N	4Fi	7	1	4
	%	17	32	4.5	17
Affected Fetuses/Litter	MEAN%	3.6Wi	10.1	1.5	5.7
	S.D.	8.01	17.00	7.11	14.54
INCOMPLETE OSSIFICATION OF STERNEBRA; Unchanged cartilage					
Fetal Incidence	N	42	44	44	75
	%	39	44	45	68
Litter Incidence	N	17Fi	17	19	22*
	%	74	77	86	96
Affected Fetuses/Litter	MEAN%	38.4Wi	42.7	46.5	68.7**
	S.D.	33.34	33.33	28.84	28.30

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

080

5-FEB-03

02018

TABLE : IB- 021

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
MISSHAPEN STERNEBRA: Unchanged cartilage					
Fetal Incidence	N	32	26	20	28
	%	29	26	21	25
Litter Incidence	N	19Fi	17	12	16
	%	83	77	55	70
Affected Fetuses/Litter	MEAN%	29.0Wi	26.3	23.2	26.5
	S.D.	19.71	21.52	27.61	26.08
UNILATERAL OSSIFICATION OF STERNEBRA: Unchanged cartilage					
Fetal Incidence	N	0	0	0	4
	%	0.0	0.0	0.0	3.6
Litter Incidence	N	0Fi	0	0	4
	%	0.0	0.0	0.0	17
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	4.3*
	S.D.	0.00	0.00	0.00	10.01
EXTRA STERNEBRAL OSSIFICATION SITE; Unchanged cartilage					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	1.0	0.0
Litter Incidence	N	0Fi	0	1	0
	%	0.0	0.0	4.5	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	2.3	0.0
	S.D.	0.00	0.00	10.66	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
BIPARTITE OSSIFICATION OF STERNEBRA; Unchanged cartilage					
Fetal Incidence	N	1	0	0	2
	%	0.9	0.0	0.0	1.8
Litter Incidence	N	1Fi	0	0	2
	%	4.3	0.0	0.0	8.7
Affected Fetuses/Litter	MEAN%	0.7Wi	0.0	0.0	2.0
	S.D.	3.48	0.00	0.00	6.53
SUPERNUMERARY RIB (14TH); Cartilage present					
Fetal Incidence	N	6	1	6	6
	%	5.5	1.0	6.2	5.5
Litter Incidence	N	5Fi	1	6	5
	%	22	4.5	27	22
Affected Fetuses/Litter	MEAN%	5.4Wi	0.9	6.1	5.8
	S.D.	11.17	4.26	10.74	11.94
SUPERNUMERARY RIB (14TH); Cartilage not present					
Fetal Incidence	N	50	33	40	62
	%	46	33	41	56
Litter Incidence	N	15Fi	15	17	22*
	%	65	68	77	96
Affected Fetuses/Litter	MEAN%	44.3Wi	31.7	39.5	57.4
	S.D.	40.03	28.73	32.28	31.70

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 023

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
CERVICAL RIB; Cartilage present					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	1.1	0.0	0.0
	S.D.	0.00	5.33	0.00	0.00
CERVICAL RIB; Cartilage not present					
Fetal Incidence	N	5	5	5	4
	%	4.6	5.0	5.2	3.6
Litter Incidence	N	5Fi	4	3	4
	%	22	18	14	17
Affected Fetuses/Litter	MEAN%	4.6Wi	5.7	4.5	3.9
	S.D.	8.91	13.21	13.71	9.14
WAVY RIB					
Fetal Incidence	N	6	2	10	9
	%	5.5	2.0	10	8.2
Litter Incidence	N	5Fi	2	4	9
	%	22	9.1	18	39
Affected Fetuses/Litter	MEAN%	5.9Wi	2.0	12.7	8.5
	S.D.	12.67	6.67	30.58	11.20

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

083

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF PUBIS; Cartilage present					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.9	0.0	0.0
	S.D.	0.00	4.26	0.00	0.00
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence	N	101	87	85	107
	%	93	86	88	97
Litter Incidence	N	23Fi	22	22	23
	%	100	100	100	100
Affected Fetuses/Litter	MEAN%	92.2Wi	85.4	89.2	97.3
	S.D.	13.38	18.71	15.93	7.19

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 025

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL UNCLASS. CARTILAGE OBS.

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
NOTCHED CARTILAGE BETWEEN BASISPHENOID AND BASIOCCIPITAL					
Fetal Incidence	N	2	0	0	1
	%	1.8	0.0	0.0	0.9
Litter Incidence	N	2Fi	0	0	1
	%	8.7	0.0	0.0	4.3
Affected Fetuses/Litter	MEAN%	1.6Wi	0.0	0.0	0.9
	S.D.	5.31	0.00	0.00	4.17
FUSED CERVICAL ARCH CARTILAGE					
Fetal Incidence	N	1	0	0	0
	%	0.9	0.0	0.0	0.0
Litter Incidence	N	1Fi	0	0	0
	%	4.3	0.0	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.9Wi	0.0	0.0	0.0
	S.D.	4.17	0.00	0.00	0.00
DUMBBELL-SHAPED CARTILAGE OF CERVICAL CENTRUM					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.9	0.0	0.0
	S.D.	0.00	4.26	0.00	0.00

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

085

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL UNCLASS. CARTILAGE OBS.

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
HOLE IN PROCESSUS CORACOIDEUS					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	1.1	0.0	0.0
	S.D.	0.00	5.33	0.00	0.00
BIPARTITE PROCESSUS XIPHOIDEUS					
Fetal Incidence	N	36	30	30	40
	%	33	30	31	36
Litter Incidence	N	14Fi	15	15	14
	%	61	68	68	61
Affected Fetuses/Litter	MEAN%	31.5Wi	29.0	31.3	35.8
	S.D.	33.29	28.13	29.05	35.32
NOTCHED MANUBRIUM					
Fetal Incidence	N	5	7	3	1
	%	4.6	6.9	3.1	0.9
Litter Incidence	N	5Fi	5	3	1
	%	22	23	14	4.3
Affected Fetuses/Litter	MEAN%	4.4Wi	7.3	3.6	0.9
	S.D.	8.67	14.78	9.40	4.17

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

5-FEB-03

02018

TABLE : IB- 027

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF FETAL SKELETAL UNCLASS. CARTILAGE OBS.

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	109	101	97	110
Live	N	109	101	97	110
Dead	N	0	0	0	0
FUSED RIB CARTILAGE					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	1.0	0.0
Litter Incidence	N	0Fi	0	1	0
	%	0.0	0.0	4.5	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	2.3	0.0
	S.D.	0.00	0.00	10.66	0.00
BRANCHED RIB CARTILAGE					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	1.0	0.0
Litter Incidence	N	0Fi	0	1	0
	%	0.0	0.0	4.5	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	2.3	0.0
	S.D.	0.00	0.00	10.66	0.00
TOTAL FETAL SKELETAL UNCLASS. CARTILAGE OBS.					
Fetal Incidence	N	39	35	32	41
	%	36	35	33	37
Litter Incidence	N	16Fi	17	16	15
	%	70	77	73	65
Affected Fetuses/Litter	MEAN%	34.3Wi	34.2	35.1	36.7
	S.D.	31.85	29.41	29.29	34.64

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

087

5-FEB-03

02018

TABLE : IB- 028

PROJECT NO. 32R0233/02018: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (DIET)
SUMMARY OF ALL CLASSIFIED FETAL EXTERNAL, SOFT TISSUE, AND SKELETAL OBSERVATIONS

		TEST GROUP 0 0 PPM	TEST GROUP 1 1,000 PPM	TEST GROUP 2 4,000 PPM	TEST GROUP 3 11,000 PPM
Litters Evaluated	N	23	22	22	23
Fetuses Evaluated	N	208	197	182	211
Live	N	208	197	182	211
Dead	N	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N	1	4	2	2
	%	0.5	2.0	1.1	0.9
Litter Incidence	N	1Fi	4	1	2
	%	4.3	18	4.5	8.7
Affected Fetuses/Litter	MEAN%	0.5Wi	1.9	1.5	0.9
	S.D.	2.61	4.17	7.11	2.93
TOTAL VARIATIONS					
Fetal Incidence	N	111	94	94	114
	%	53	48	52	54
Litter Incidence	N	23Fi	22	22	23
	%	100	100	100	100
Affected Fetuses/Litter	MEAN%	53.6Wi	47.1	52.7	54.1
	S.D.	8.93	11.13	12.44	6.90

Statistics: Fi =Fisher's exact test (one-sided) Wi =Wilcoxon-test (one-sided)
* : p<=0.05 ** : p<=0.01

088