

**ETHYLENEAMINES PRODUCT STEWARDSHIP DISCUSSION GROUP
AEEA TESTING CONSORTIUM**

8EHQ-1203-15167

December 5, 2003

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TSCA Section 8(e) Coordinator
Document Control Officer (MC-7407)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460-0001

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Re: Toxic Substances Control Act -- Section 8(e)



Dear TSCA Section 8(e) Coordinator:

This letter supplements the July 3, 2002, and September 26, 2002, letters submitted by the Ethyleneamines Product Stewardship Discussion Group (EPSDG) Aminoethylethanolamine (AEEA) Testing Consortium (Consortium), c/o Mr. William C. Hayes, The Dow Chemical Company, 1691 N. Swede Road, Midland, Michigan 48674, to the U.S. Environmental Protection Agency (EPA), pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA). The Consortium is comprised of the following companies: Akzo-Nobel Functional Chemicals, LLC, BASF Corporation, The Dow Chemical Company, and Huntsman Corporation.

The Consortium's July 2, 2002, letter submitted interim results of an Organization for Economic Cooperation and Development (OECD) 421 Reproduction/Developmental Toxicity Screening Test in Wistar rats (strain CrIGlxBrIHan:WI) with AEEA (CAS No. 111-41-1) (OECD 421 Study). The Consortium's September 26, 2002, letter agreed to provide the OECD 421 Study when it was issued in final in response to an August 28, 2002, letter from Mr. Richard H. Hefter, Chief, High Production Volume Chemicals Branch, EPA. The August 28, 2002, letter from EPA also requested "available information to assist EPA in assessing potential human and environmental exposures."

The Dow Chemical Company • Mr. William C. Hayes • 1691 N. Swede Road • Midland, Michigan 48674
Huntsman Ethyleneamines, Ltd. • Mr. Michael O. Nutt • 7114 North Lamar Boulevard • Austin, Texas 78752
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ETHYLENEAMINES PRODUCT STEWARDSHIP DISCUSSION GROUP
AEEA TESTING CONSORTIUM

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December 5, 2003

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With this letter, the Consortium submits the final OECD 421 Study. In addition, the Consortium is submitting three other documents: (1) an OECD 414 study with AEEA, *Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (Gavage)* (OECD 414 Study); (2) a histopathology study, *Additional Histopathological Examination of Pups from an OECD 421 Screening Study* (Histopathology Study); and (3) a synopsis document that summarizes the findings of the OECD 421 Study, OECD 414 Study, and Histopathology Study. The OECD 414 Study and Histopathology Study were performed by BASF Aktiengesellschaft, Ludwigshafen, Germany. The Consortium observed no significant adverse effects in the OECD 414 Study and there is, therefore, no requirement under TSCA Section 8(e) to submit these studies. In addition, the Histopathology Study examined the target tissue in two pups from the 421 study and there were no significant adverse effects that were not already reported when the Consortium submitted its July 2, 2003, letter under TSCA Section 8(e). The Consortium is nevertheless submitting these studies because of their relevance to the findings in the OECD 421 Study, as set forth in the synopsis document.

In addition, in response to EPA's request for "available information to assist EPA in assessing potential human and environmental exposures," the Consortium also submits the appended document describing the commercial uses of AEEA.

If you have any questions, please contact Lynn Bergeson at (202) 557-3801 or lbergeson@lawbc.com.

Sincerely,

William C. Hayes

William C. Hayes, Chair
Ethyleneamines Product Stewardship
Discussion Group AEEA Testing Consortium

Attachments

cc: Ethyleneamines Product Stewardship Discussion Group AEEA Testing Consortium (via e-mail) (Commercial Uses of Aminoethylethanolamine is the only attachment)

Commercial Uses of Aminoethylethanolamine

Aminoethylethanolamine (AEEA) is used almost exclusively as a chemical intermediate to make other chemicals. The largest global use of AEEA is in the production of cationic and amphoteric surfactants. Cationic surfactants are typically made by reacting AEEA with one or more moles of a fatty acid followed by quaternization of one of the amines. These products are used to impart softness and reduce static build-up in the production of textiles from fiber and in fabric softeners used in home washing machines and dryers.

Amphoteric surfactants made from AEEA are mild surfactants that have low irritation to eyes and are typically used in shampoos and body washes. These surfactants are made by reacting AEEA with fatty acids and are active over a wide pH range.

AEEA is also used to make chelants, which are products used to inactivate metal ions in a number of industries such as polymer latexes, agricultural nutrients, and food packaging.

AEEA is also used in many other applications such as epoxy curing, urethane polyols, textile additives and leather additives. In most of these cases, the AEEA is reacted with another raw material to make a new chemical entity.

In the case of the uses of AEEA, the concentration of chelants containing AEEA in final products is at very low levels. For the other applications, it is used in industrial applications or, in the case of epoxy curing applications, infrequently by home users.

STUDY TITLE

Report

N-(2-Aminoethyl)ethanolamine – Prenatal Developmental Toxicity Study
in Wistar Rats
Oral Administration (Gavage)

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. K. Deckardt
Dr. J. Hellwig
Dr. B. van Ravenzwaay

STUDY COMPLETED ON

June 10, 2003

PERFORMING LABORATORY

Experimental Toxicology and Ecology
BASF Aktiengesellschaft
67056 Ludwigshafen, Germany

LABORATORY PROJECT IDENTIFICATION

Project No.: 30R0019/01105

SPONSOR

BASF Aktiengesellschaft
67056 Ludwigshafen, Germany

VOLUME I of III
(REPORT SECTION AND SUMMARY TABLES)

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GLP COMPLIANCE STATEMENT

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

However, there was the following deviation from the requirements of the above mentioned principles:

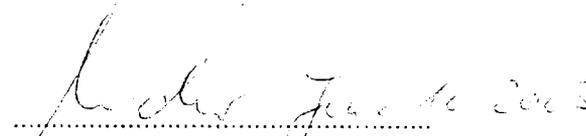
Trace element determinations did not correspond the formal GLP-requests, but were performed according "Good Scientific Practice".


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

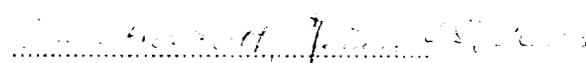
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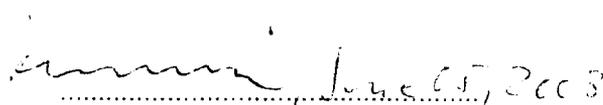
Study Director:


.....
Dr. med. vet. S. Schneider

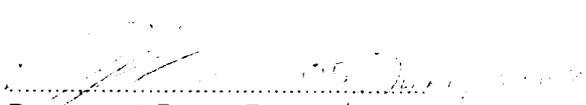
Clinical Pathology:


.....
Dr. phil. nat. K. Deckardt

Assessment and Reporting:


.....
Dr. med. vet. J. Hellwig

Management:


.....
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:	08-19-2002	08-19-2002
Conduct of study:	09-04-2002	09-04-2002
	09-10-2002	09-10-2002
Report:	02-24-2003	02-24-2003
	06-05-2003	06-05-2003

Ludwigshafen,

June 05, 2003

Hajok

Rheinland-Pfalz



**Landesanstalt
für Pflanzenbau und Pflanzenschutz**

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 18 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.

Schiering 24. Sep. 2001

Unterschrift, Datum / Signature, Date
(Name und Funktion der verantwortlichen Person /
Name and function of responsible person)



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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).

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**Part B:
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**Part C:
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This report consists of Volumes I, II and III.

¹ "Frankenthaler Leitungswasser" is tap water delivered by the town of Frankenthal.

² "Aqua bidest." is doubly distilled water.

1. SUMMARY

1.1. METHODS

N-(2-Aminoethyl)ethanolamine was tested for its prenatal developmental toxicity in Wistar rats. The test substance was administered as an aqueous solution to 25 time-mated female Wistar rats/group by stomach tube at doses of 0.5; 2.0; 10 and 50 mg/kg body weight on day 6 through day 19 post coitum (p.c.). A standard dose volume of 10 ml/kg body weight was used for each group. The control group, consisting of 25 females, was dosed with the vehicle only (doubly distilled water). Between 21 - 24 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 blood samples were taken for trace element analysis from 12 randomly selected females/group. Thereafter, 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. Subsequently, nearly one half of the fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal (incl. cartilage) findings.

1.2. RESULTS

There were no substance-related adverse effects on the dams concerning food consumption, body weight, body weight change, uterine weights, corrected body weight change or clinical and necropsy observations up to and including a dose of 50 mg/kg body weight/day.

There were no differences of toxicological relevance between the control and the substance-treated groups (0.5; 2.0; 10 and 50 mg/kg body weight/day) in 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.

Furthermore, the determination of copper, magnesium, manganese and zinc in the serum of dams revealed no treatment effects in the animals which received the test compound.

No substance-related differences were recorded for placental and fetal body weights. 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. In particular, no substance-induced effects on fetal cardio-vascular system occurred.

Thus, under the conditions of this comprehensive prenatal developmental toxicity study, the administration of **N-(2-Aminoethyl)ethanolamine** to pregnant female Wistar rats elicited **no signs of maternal toxicity**, had **no influence on gestational parameters** and induced **no signs of prenatal developmental toxicity** up to and including the high dose of 50 mg/kg body weight/day; especially, **no indications of teratogenic effects** occurred, which could be causally related to the test substance administration.

1.3. CONCLUSION

Based on the results of this prenatal developmental toxicity study, the **no observed adverse effect level (NOAEL)** for **maternal and prenatal developmental toxicity** is **50 mg/kg body weight/day**. There were **no indications for teratogenicity** up to and including the top dose of 50 mg/kg body weight/day. In particular, no substance-induced effects on fetal great vessels occurred, which were observed in a preceding reproduction/developmental toxicity screening test with N-(2-Aminoethyl)ethanolamine at doses of 50 and 250 mg/kg/day (BASF, 2003).

2. INTRODUCTION AND DOSE SELECTION

2.1. AIM OF THE STUDY

The purpose of this study was to assess the effects of **N-(2-Aminoethyl)ethanolamine** 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.

N-(2-Aminoethyl)ethanolamine was administered daily as an aqueous solution to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 19 p.c.).

2.2. SELECTION OF DOSES

The following doses, which were based on the results of a preceding reproduction/developmental toxicity screening test (BASF, 2003), were chosen for the present full-scale prenatal developmental toxicity study in Wistar rats:

0.5 mg/kg body weight/day: as the low dose level

2 mg/kg body weight/day: as the low mid dose level

10 mg/kg body weight/day: as the high mid dose level

50 mg/kg body weight/day: as the dose level which could induce some developmental and/or maternal toxicity but not death or severe suffering

The oral route was selected since this has proven to be suitable for the detection of a toxicological hazard.

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: N-(2-Aminoethyl)ethanolamine

Test substance No.: 01/0019-2

CAS No.: 111-41-1

Batch No.: Continuous production from BASF AG

Date of production: September 19, 2001

Physical state/color: Liquid/yellowish-clear

pH-value: 14.6

Purity: 99.8 area% (analytical report 01L00492)

Homogeneity: Homogeneous

Stability: The stability under storage conditions was confirmed by reanalysis

Storage conditions: Room temperature

Analytical laboratory: Analytical Department, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany

3.2. TEST ANIMALS

3.2.1. Species and strain

Time-mated Wistar rats (CrI:Glx(Br)Han: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 proved 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, FRG (height: 15 cm, length: 37,5 cm, width: 21 cm; floor area about 800 cm²).

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 disinfectant ("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, FRG).

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 DOSES

Test group	Dose mg/kg body weight/day	Concentration of the solutions (mg/100 ml)	Dose Volume ml/kg body weight	Number of animals	Animal No.
0	0	0	10 ¹⁾	25	1 – 25
1	0.5	5	10 ²⁾	25	26 – 50
2	2	20	10 ²⁾	25	51 – 75
3	10	100	10 ²⁾	25	76 – 100
4	50	500	10 ²⁾	25	101 – 125

1) doubly distilled water

2) test substance solutions in doubly distilled water

3.5. TEST SUBSTANCE PREPARATIONS

The test substance solutions in doubly distilled water were prepared at the beginning of the administration period and thereafter at intervals which took into account the analytical results of the stability verification. For the preparation of the solutions the test substance was weighed in a graduated measuring flask depending on the dose group, topped up with doubly distilled water and subsequently thoroughly mixed using a magnetic stirrer.

3.6. ANALYSES

All analyses mentioned under 3.6.1. were carried out at the Analytical Department of BASF Aktiengesellschaft.

3.6.1. Analyses of the test substance preparations

Analytical verifications of the stability of the test substance in doubly distilled water for a period of at least 10 days at room temperature were carried out before the study was initiated.

- Samples of the aqueous test substance solutions were sent to the analytical laboratory twice during the study period (at the beginning and towards the end) for verification of the concentrations.

As the preparations were true solutions, no homogeneity analyses were performed.

3.6.2. Analytical methods

The test substance solutions were analyzed by HPLC.

More details on the methods used for the analytical investigations of the test substance preparations can be found in Volume III (Supplement: 1. Analyses of the solutions of the test substance).

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 August 21, 22, 23 and 27, 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 127.1 – 183.6 g.

The test substance solutions in doubly distilled water were administered to the animals orally (by gavage) once a day from implantation to one day prior to the expected day of parturition (day 6 to day 19 p.c.) always at approx. the same time of day (in the morning). The animals of the control group were treated in the same way with the vehicle (doubly distilled water). The volume administered each day was 10 ml/kg body weight. The calculation of the volume administered was based on the last individual body weight.

On day 20 p.c., blood was taken from the retroorbital venous plexus of 12 randomly selected females/group for trace element analysis. After blood samples had been taken 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 4 sections. Each dose group was represented in each section. For further details, see Table 3.7.1..

Table 3.7.1.: Time schedule

Experimental starting date: August 21, 2002

	Arrival of the animals (day 0 p.c.)	Beginning of treatment (day 6 p.c.)	End of treatment (day 19 p.c.)	Blood sampling* and subsequent sacrifice (day 20 p.c.)
1 st section	August 21, 2002	August 27, 2002	September 09, 2002	September 10, 2002
2 nd section	August 22, 2002	August 28, 2002	September 10, 2002	September 11, 2002
3 rd section	August 23, 2002	August 29, 2002	September 11, 2002	September 12, 2002
4 th section	August 27, 2002	September 02, 2002	September 15, 2002	September 16, 2002

* = from 12 randomized females/group

Experimental completion date: February 14, 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 on Saturdays, Sundays or 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. 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

3.8.2.1. Clinical Pathology

For trace element analysis about 2 ml blood was taken from the retroorbital venous plexus in the morning before sacrifice from non-fasted animals without anesthesia. Serum was prepared and frozen at -80°C till analysis. For trace element examination the sera were delivered to the Central Analytical Department of BASF Aktiengesellschaft.

Trace element analysis was carried out in 12 randomly chosen female animals per test group.

3.8.2.1.1. Trace elements

The samples were measured directly without further sample preparation. Appropriate dilution into measuring range of spectrometer was done by means of deionised water and acidification with nitric acid. The concentrations of total trace elements (ionic and non-ionic) were determined by atomic mass spectrometry with inductively coupled plasma (ICP-MS; Apparatus: Agilent 7500a). Measuring conditions: Hot plasma, external calibration with internal standard RH(103). Isotopes: Cu(65), Mg(26), Mn(55) and Zn(64). These examinations did not correspond the formal GLP-requests, but were performed according "Good Scientific Practice"

The following trace elements were determined:

- copper
- magnesium
- manganese
- zinc

3.8.2.2. Necropsy

On day 20 p.c., after blood samples had been taken from the respective animals, 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 uterus³
- 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)

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

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

$$\frac{\text{number of pregnant animals}}{\text{number of fertilized animals}} \times 100$$

- The **preimplantation loss** (in %) was calculated⁴ according to the following formula:

$$\frac{\text{number of corpora lutea} - \text{number of implantations}}{\text{number of corpora lutea}} \times 100$$

- The **postimplantation loss** (in %) was calculated⁴ from the following formula:

$$\frac{\text{number of implantations} - \text{number of live fetuses}}{\text{number of implantations}} \times 100$$

³ = 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 the treatment group in order to minimize bias.

⁴ = 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 the 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, FRG; 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 ⁵ , 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

⁵ 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.

3.10.2. Statistics of clinical pathology

Statistical analysis was performed with animals pregnant at blood collection. Mean and standard deviation of each test group were calculated (see tables).

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 **N-(2-Aminoethyl)ethanolamine** in tap water from the community of Frankenthal ("Frankenthaler Leitungswasser") for a period up to 10 days at room temperature was demonstrated (for details see Volume III; Tables IIIA-001 - IIIA-004).

4.1.2. Homogeneity analysis of the test substance preparations

Due to the fact, that the test substance preparations were true solutions, it appeared not necessary, to prove the homogeneous distribution of the test substance in the vehicle analytically.

4.1.3. Concentration control analyses of the test substance preparations

The results of the analyses of test substance solutions in doubly distilled water confirmed the correctness of the prepared concentrations. The analytical values of the samples corresponded to the expected values within the limits of the analytical method, i.e. were always above 90% of the nominal concentration. (see Volume III, Tables IIIA-005 - IIIA-014).

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 $5 \cdot 10^5$ /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 and B of Volume II.

4.2.1. Clinical examinations

Only **pregnant dams** were used for the calculations of mean maternal food consumption, body weight and body weight change. 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 excluded from the above mentioned calculations:

Test group 0 (0 mg/kg body weight/day):

- females Nos. 3, 13, 16 and 20 - not pregnant

Test group 1 (0.5 mg/kg body weight/day):

- females Nos. 44 and 47 - not pregnant

Test group 2 (2 mg/kg body weight/day):

- female No. 66 - not pregnant

Test group 3 (10 mg/kg body weight/day):

- females Nos. 88 and 99 - not pregnant

Test group 4 (50 mg/kg body weight/day):

- females Nos. 105, 115 and 123 - 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 disturbances of the general behavior occurred in the dams of test groups 0 - 4 (0; 0.5; 2; 10 and 50 mg/kg body weight/day) during the entire study period (days 0 - 20 p.c.).

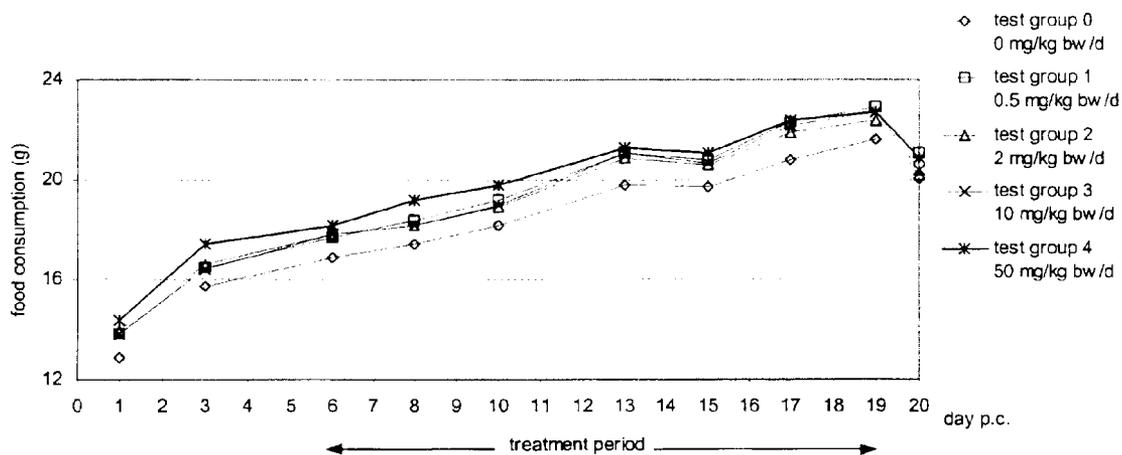
4.2.1.3. Food consumption

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

Food consumption of the rats was not adversely affected by the test substance administration. Food consumption values of the substance-treated females (0.5; 2; 10 and 50 mg/kg body weight/day) were similar to or even exceeded control values.

Actually, the food consumption of the substance-treated dams was slightly higher than the food uptake of the concurrent control group. This was already obvious during the pretreatment phase (days 0 – 6 p.c.). Statistically significantly increased food consumption values, however, were only observed for the high dose dams (50 mg/kg body weight/day) on pretreatment days 0 – 6 p.c. and on treatment days 6 – 13 p.c.. The higher food consumption values in test groups 1 – 4 (0.5; 2; 10 and 50 mg/kg body weight/day) are considered to be spontaneous in nature and do not represent adverse effects. The higher food uptake of the dams may at least partly be related to the incidentally higher number of implants and live fetuses/dam in the substance-treated groups (see 4.2.2.4.), and the correspondingly higher mean gravid uterus weights (see 4.2.2.2.) and mean body weights and/or body weight gains (see 4.2.1.4.).

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.)

The mean body weights of the high mid dose and of the high dose rats (10 and 50 mg/kg body weight/day) were slightly, but statistically significantly above concurrent control values (10 mg/kg: days 13 - 20 p.c.; 50 mg/kg: days 10 - 20 p.c.). On day 20 p.c., the mean body weights of these rats were about 6% above the mean value of the concurrent control females.

The mean body weight gain of the high mid dose dams (10 mg/kg body weight/day) was statistically significantly increased on days 8 – 10 p.c. and that of the high dose dams (50 mg/kg body weight/day) was statistically significantly increased if calculated for the entire treatment phase (days 6 – 19 p.c.).

There were no statistically significant differences on body weight data of the dams of test groups 1 and 2 (0.5 and 2 mg/kg body weight/day).

All observable differences on body weight data of the substance-treated females in comparison to the concurrent controls are without any biological relevance and are considered to be spontaneous in nature.

Fig. 4.2.1.4.1. Mean body weight of pregnant animals

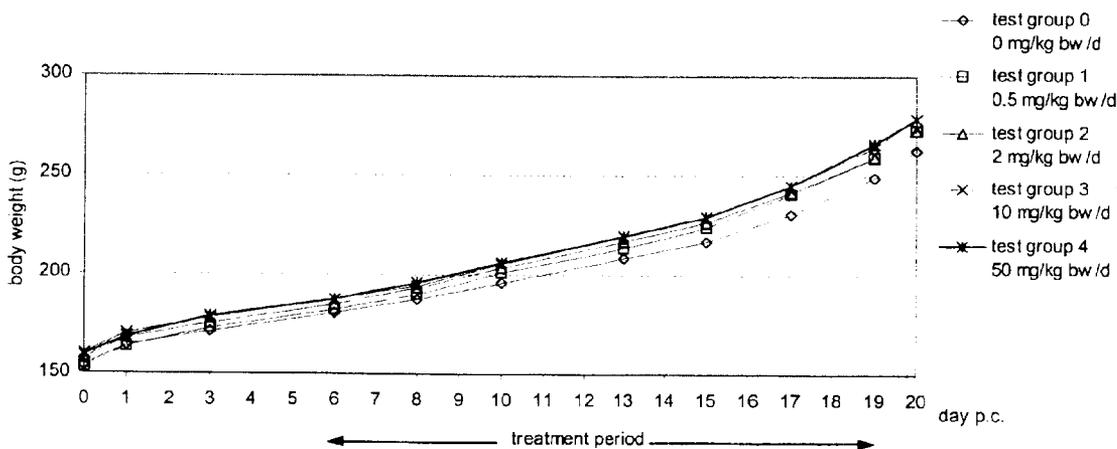
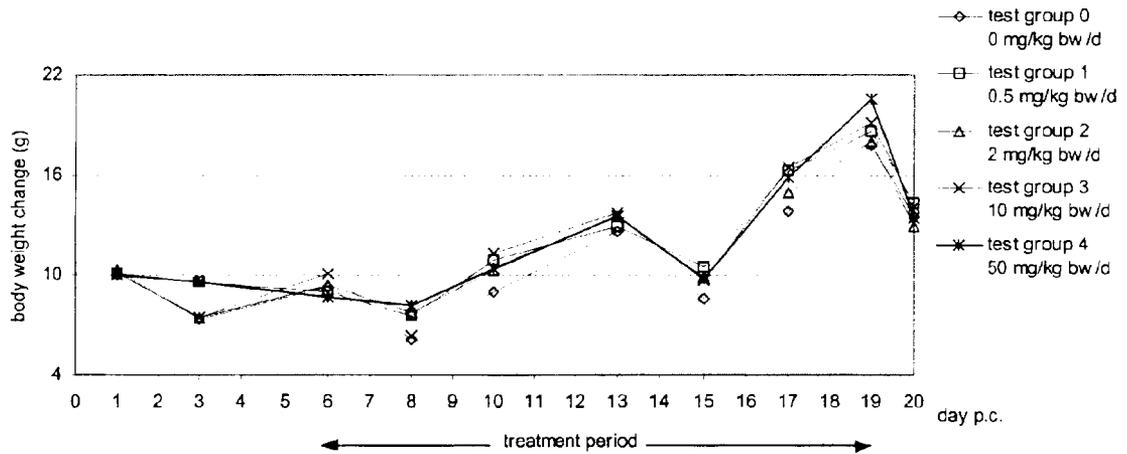


Fig. 4.2.1.4.2. Mean body weight change of pregnant animals

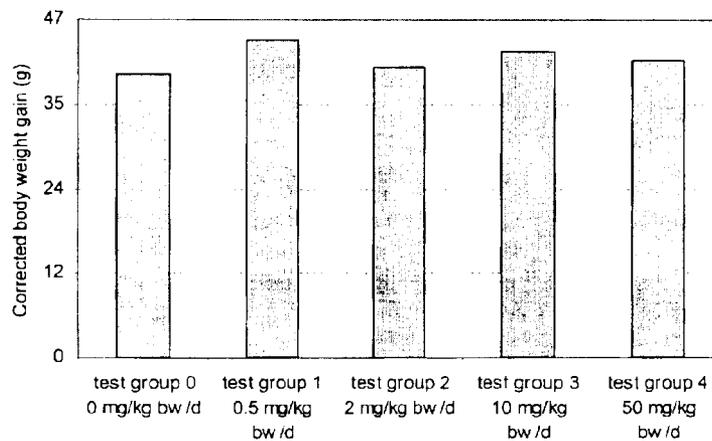


4.2.1.5. Corrected body weight gain (net maternal body weight change)

(Mean values: Tab. IA-007; Fig. 4.2.1.5.1.)

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 - 4 (0.5; 2; 10 and 50 mg/kg body weight/day) revealed no differences of any biological relevance to the corresponding control group.

Fig. 4.2.1.5.1. 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. Clinical Pathology****4.2.2.1.1. Trace elements**

(Mean values: Tab. IB-001)

The determination of total concentrations (ionic and non-ionic) of copper, magnesium, manganese and zinc in the serum of dams revealed no treatment effect in the animals that received the test compound.

4.2.2.2. Uterus weight

(Mean values: Tab. IA-007)

The mean gravid uterus weights of the animals of test groups 1 - 4 (0.5; 2; 10 and 50 mg/kg body weight/day) were not adversely 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. This includes the statistically significantly increased mean gravid uterus weight at the top dose (50 mg/kg body weight/day), which was caused by a distinctly higher number of implants/dam and of live fetuses/dam in this test group (see 4.2.2.4.).

4.2.2.3. Necropsy findings

(Summary of findings: Tab. IA-008)

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

One low dose animal (No. 41) showed a bilateral dilation of the renal pelvis. Moreover, the amniotic fluid was increased in one control female (No. 12). These gross findings are considered to be spontaneous in nature as they occurred without any relation to dosing.

4.2.2.4. Reproduction data of dams

(Mean values: Tabs. IA-009 - IA-011)

The conception rate reached 84% in test group 0 (control group), 88% in test group 4 (50 mg/kg body weight/day), 92% in test groups 1 and 3 (0.5 and 10 mg/kg body weight/day) and 96% in test group 2 (2 mg/kg body weight/day). 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 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. This statement includes:

- the statistically significantly **higher** number of implants/dam in test groups 2, 3 and 4 (2; 10 and 50 mg/kg body weight/day) in comparison to the concurrent control group (8.1/ 8.9/ 9.6*/ 9.5*/ 9.6*; * = $p \leq 0.05$)
- the statistically significantly **lower** number of late resorptions in test groups 2 and 4 (2 and 50 mg/kg body weight/day) in comparison to the concurrent control group (0.3/ 0.1/ 0.0*/ 0.0/ 0.0*; * = $p \leq 0.05$)
- the statistically significantly **higher** number of live fetuses/dam in test group 4 (50 mg/kg body weight/day) in comparison to the concurrent control group (7.9/ 8.5/ 8.7/ 9.0/ 9.7**; ** = $p \leq 0.01$)
- the statistically significantly **higher** number of live female fetuses/dam in test groups 2 and 4 (2 and 50 mg/kg body weight/day) in comparison to the concurrent control group (3.5/ 4.2/ 5.1*/ 4.7/ 5.3**; * = $p \leq 0.05$ and ** = $p \leq 0.01$)
- the statistically significantly **lower** mean percentage of live male fetuses in test group 2 (2 mg/kg body weight/day) in comparison to the concurrent control group (53.6%/ 48.4%/ 37.2%**/ 44.2%/ 44.0%; ** = $p \leq 0.01$)

Moreover, one control dam (No. 12) had 2 late resorptions but no viable fetuses in the uterus, while high dose dam No.121 was only pregnant by stain and had only one very early resorption in the uterus. As these occurred in isolation, it is not an unusual spontaneous finding in the strain of rats used for this study.

All of these findings appeared without a clear relation to dosing and/or are not considered to reflect adverse effects related to treatment.

4.3. EXAMINATIONS OF THE FETUSES

Summary tables of the results are given in the Appendix of Volume I; individual values and findings are given in Part C 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-011)

The sex distribution of the fetuses in test groups 1 - 4 (0.5; 2; 10 and 50 mg/kg body weight/day) was comparable with that of the control fetuses. The differences observed in comparison to the control were without any biological relevance.

4.3.1.2. Weight of placentae

(Mean values: Tab. IC-001)

The mean placental weights in the substance-treated groups (0.5; 2; 10 and 50 mg/kg body weight/day) were similar to the corresponding control values and did not show any dose dependency.

4.3.1.3. Weight of fetuses

(Mean values: Tab. IC-001)

The mean fetal body weights in test groups 1, 2, 3 and 4 (0.5; 2; 10 and 50 mg/kg body weight/day) were not influenced by the test substance administration and were very similar to the corresponding control values.

4.3.1.4. External examination of the fetuses

(Summary of findings: Tabs. IC-002 - IC-005)

External malformations in the form of micrognathia and astomia occurred exclusively in one high dose fetus (female fetus No. 4 from dam No. 119). The affected fetus showed additionally associated malformations, as well as other unassociated malformations (i.e. severely malformed skull bones; absent lumbar vertebra; malpositioned and bipartite sternebra – see 4.3.3.).

Thus in total, none of the 159 control fetuses from 20 litters, none of the 196 low dose fetuses from 23 litters, none of the 208 low mid dose fetuses from 24 litters, none of the 206 high mid dose fetuses from 23 litters and one out of 203 high dose fetuses [= 0.5%] in one out of 21 litters [= 4.8%] showed external malformations. The mean percentages of affected fetuses/litter with external malformations amounted to 0.0, 0.0, 0.0, 0.0 and

0.4%, respectively without attaining statistical significance in any of the test groups (0; 0.5; 2; 10 and 50 mg/kg body weight/day).

The isolated and scattered occurrence of two external head malformations in one high dose fetus does not suggest any treatment relationship. As can be seen from the respective historical control data set (see Volume III (Supplement)) these findings or very similar ones appear also occasionally in control rats of this rat strain and the values from the present study fit to the historical control ranges.

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

There appeared only one kind of **unclassified observation**. Fused placentae occurred in the progeny of one high mid dose dam (No. 100 - 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. IC-006 - IC-009)

Soft tissue malformations in the form of situs inversus and misshapen thymus occurred exclusively in one low mid dose fetus (female fetus No. 12 from dam No. 67). None of the other fetuses showed visceral malformations, in particular no malformations of the pericardial vessels (see also 5. Discussion and Conclusion).

Thus in total, none of the 74 control fetuses from 20 litters, none of the 94 low fetuses from 23 litters, one out of 98 low mid dose fetuses [= 1.0%] in one out of 24 litters [= 4.2%], none of the 98 high mid dose fetuses from 23 litters and none of the 96 high dose fetuses from 21 litters showed soft tissue malformations. The mean percentages of affected fetuses/litter with soft tissue malformations amounted to 0.0, 0.0, 0.8, 0.0 and 0.0%, respectively without attaining statistical significance in any of the test groups (0; 0.5; 2; 10 and 50 mg/kg body weight/day).

The isolated and scattered occurrence of two soft tissue malformations in one low mid dose fetus does not suggest any treatment related origin.

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 uni- or bilaterally dilated ureter(s) - were found in fetuses of all test groups without any statistical 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 (Supplement)) 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 9.6% (control), 9.7% (0.5 mg/kg body weight/day), 13.3% (2 mg/kg body weight/day), 4.1% (10 mg/kg body weight/day) and 7.1% (50 mg/kg body weight/day). All of these

values are lower or within the historical control range (4.4% – 22.2%). 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. IC-010 – IC-026)

As can be seen from Tab. 4.3.3.1. **malformations of the skeletons** were observed in one fetus each of all substance-treated groups (0.5; 2; 10 and 50 mg/kg body weight/day), but not in the control group. The respective malformations affected skull, vertebral column, sternum and humerus.

Table 4.3.3.1. Individual fetal skeletal malformations

Test group	Dam No.-Fetus No., Sex	Malformation
0 (control)		none
1 (0.5 mg/kg bw/day)	30 – 01, F	Cleft sternum (split cartilage)
2 (2 mg/kg bw/day)	56 – 09, F	Misshapen humerus
3 (10 mg/kg bw/day)	89 – 12, M	Malpositioned and bipartite sternebra (unchanged cartilage)
4 (50 mg/kg bw/day)	119 – 04, F	Severely malformed skull bones with changed cartilage (+); absent lumbar vertebra; malpositioned and bipartite sternebra (unchanged cartilage)

mg/kg bw/day = milligram per kilogram body weight per day
M = male
F = female
(+) = fetus with associated external malformations

In total, none of the 85 control fetuses from 20 litters, one out of 102 low dose fetuses [= 1.0%] in one out of 23 litters [= 4.3%], one out of 110 examined low mid dose fetuses [= 0.9%] in one out of 24 litters [= 4.2%], one out of 108 examined high mid dose fetuses [= 0.9%] in one out of 23 litters [= 4.3%] and one out of 107 high dose fetuses [= 0.9%] in one out of 21 litters [= 4.8%] showed skeletal malformations. The mean percentages of affected fetuses/litter with skeletal malformations amounted to 0.0, 0.9, 0.8, 0.7 and 0.7%. All of the noted skeletal malformations appeared without a clear relation to dosing and/or can be found at a comparable frequency in the historical control data (see Volume III (Supplement)). Therefore the observable differences between the groups 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; no or 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; supernumerary thoracic vertebra; fused sacral centrum and arch, misshapen sacral vertebra), the ribs (supernumerary 14th, cervical or wavy ribs), the sternum (misshapen sternebra; unilateral, incomplete or missing ossification of sternebra) and the pelvic girdle (incomplete ossification of pubis). The mean percentages of affected fetuses/litter with skeletal variations amounted to 95.8, 96.8, 95.2, 96.8 and 94.0% at 0; 0.5; 2; 10 or 50 mg/kg body weight/day. 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 between the control and the treated groups were compiled in the following table (Table 4.3.3.2). All incidences were expressed on a fetus per litter basis and 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	0 mg/kg bw/d	0.5 mg/kg bw/d	2 mg/kg bw/d	10 mg/kg bw/d	50 mg/kg bw/d	HCD Mean % (range)
Supraoccipital hole(s)	32.8	32.6	41.3	52.3*	41.2	30.9 (8.6 – 60.5)
Incomplete ossification of parietal; unchanged cartilage	15.0	24.6*	13.7	21.5	18.3	12.4 (3.1 – 17.7)
Fused sacral centrum and arch; unchanged cartilage	0.0	1.1	1.7	0.0	7.3*	2.8 (0.0 – 5.9)
Incomplete ossification of sternebra; unchanged cartilage	46.3	54.4	63.7	66.3*	47.9	53.7 (41.3 – 74.6)
Total fetal skeletal variations	95.8	96.8	95.2	96.8	94.0	93.8 (87.0 – 99.2)

mg/kg bw/d = mg/kg body weight/day

* = p ≤ 0.05, ** = p ≤ 0.01

HCD = Historical control data

The differences concerning skeletal variations of test groups 1 - 4 (0.5; 2; 10 and 50 mg/kg body weight/day) 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 fused sacral centrum and arch

(with unchanged cartilage) in the high dose 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, the overall rate of skeletal variations was actually lowest in this group (95.8% in the control vs. 94.0% at 50 mg/kg body weight/day) and there was no supporting evidence for substance-related delays in skeletal maturation.

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 were related to the skull, the vertebral column, the ribs and the sternum. They appeared without any statistically significant differences between the control and the substance-treated groups. The mean percentages of affected fetuses/litter with unclassified cartilage observations amounted to 60.1, 54.6, 58.3, 61.2 and 47.8% at 0; 0.5; 2; 10 or 50 mg/kg body weight/day. Thus, the lowest rate was noted at the top dose group. The scattered occurrence of these cartilage findings without a clear dose-response relationship 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** (Tabs. IC-002 - IC-003), **soft tissue** (Tabs. IC-006 – IC-007) and **skeletal malformations** (Tabs. IC-010 - IC-012) in single fetuses of all substance-treated groups (0.5; 2; 10 and 50 mg/kg body weight/day) 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 were micrognathia, astomia, situs inversus, cleft sternum and misshapen humerus.

If all different types of **malformations** (Tab. IC-027) are summarized, in total none of the 159 examined control fetuses in 20 litters, one of the 196 examined low dose fetuses [= 0.5%] in one out of 23 litters [= 4.3%], 2 out of 208 low mid dose fetuses [= 1.0%] in 2 out of 24 litters [= 8.3%], one of the 206 examined high low dose fetuses [= 0.5%] in one out of 23 litters [= 4.3%] and one out of 203 high dose fetuses [= 0.5%] in one out of 21 litters [= 4.8%] showed malformations. The mean percentages of affected fetuses/litter with total malformations amounted to 0.0, 0.4, 0.8, 0.4 and 0.4% at 0; 0.5; 2; 10 or 50 mg/kg body weight/day respectively. These very low incidences, and lack of any relationship to dose administered, do not suggest any treatment relationship.

External variations did not occur in any of the fetuses in this study (Tab. IC-004). **Soft tissue variations**, exclusively in the form of dilated renal pelvis and ureter (Tab. IC-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. IC-013 – IC-023), which consisted primarily in transient delays in the ossification process, only one skeletal variation (i.e. fused sacral centrum and arch with unchanged cartilage) showed some relation to dosing and

occurred at a statistically significantly increased rate in the high dose fetuses (50 mg/kg body weight/day) 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: 95.8, 96.8, 95.2, 96.8 and 94.0% at 0; 0.5; 2; 10 or 50 mg/kg body weight/day. 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. IC-027) are summarized, in total 87 of the 159 examined control fetuses [= 55%] in all 20 litters [= 100%], 108 of the 196 examined low dose fetuses [= 55%] in all 23 litters [= 100%], 115 out of 208 low mid dose fetuses [= 55%] in all 24 litters [= 100%], 109 out of 206 high mid dose fetuses [= 53%] in all 23 litters [= 100%] and 107 out of 203 high dose fetuses [= 53%] in all 21 litters [= 100%] showed variations. The mean percentages of affected fetuses/litter with total variations amounted to 56.2, 54.9, 56.6, 52.9 and 52.9% at 0; 0.5; 2; 10 or 50 mg/kg body weight/day respectively without attaining statistical significance.

A spontaneous origin is also assumed for the observed **unclassified external and fetal skeletal observations** (Tabs. IC-005 and IC-024 – IC-026). Distribution and type of these findings do not suggest any relation to treatment.

Thus, the oral administration of **N-(2-Aminoethyl)ethanolamine** up to a dose of 50 mg/kg body weight to pregnant Wistar rats **did not affect fetal morphology** and gave in particular **no indications for teratogenic properties**.

5. DISCUSSION AND CONCLUSION

N-(2-Aminoethyl)ethanolamine was administered to pregnant Wistar rats daily by stomach tube from implantation to one day prior to the expected day of parturition (days 6 - 19 post coitum [p.c.]) in doses of 0.5; 2.0; 10 and 50 mg/kg body weight.

There were no substance-related adverse effects on the dams concerning food consumption, body weight, body weight change, uterine weights, corrected body weight change or clinical and necropsy observations up to and including a dose of 50 mg/kg body weight/day.

There were no differences of toxicological relevance between the control and the substance-treated groups (0.5; 2.0; 10 and 50 mg/kg body weight/day) in 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.

Furthermore, the determination of total concentrations (ionic and non-ionic) of copper, magnesium, manganese and zinc in the serum of dams revealed no treatment effect in the animals that received the test compound.

No substance-related differences were recorded for placental and fetal body weights. 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. In particular, no substance-induced effects on the great vessels of the fetuses occurred.

Such findings in the great vessels were expected to occur based on the results of a preceding reproduction/developmental toxicity screening test (BASF, 2003). In the screening study **N-(2-Aminoethyl)ethanolamine** was administered to groups of 10 male and 10 female healthy young adult rats (F0 parental generation) daily by oral gavage at doses of 50, 250 and 1,000 mg/kg body weight/day throughout the whole study period. No live progeny was delivered at the top dose, but only at 50 and 250 mg/kg body weight/day. At these dose levels pup necropsy observations revealed **dilations and aneurysms** as well as **aberrant (abnormal course) pericardial vessels (aorta, carotids, pulmonary trunk)**. These findings are potentially life-threatening and may well be responsible for the unscheduled death of some mid dose pups. In order to clarify pathogenesis and to assess potential consequences of these malformations for further life of the offspring additional examinations/studies are warranted. Moreover, such additional studies may help to elucidate the reasons, why severe malformations mostly related to the pericardial vessels occurred in the progeny already on the first days after birth, but were not observed in gestation day 20 fetuses, whose mothers were gavaged with 50 mg **N-(2-Aminoethyl)ethanolamine**/kg body weight day.

Thus, under the conditions of the present comprehensive **prenatal** developmental toxicity study, the administration of **N-(2-Aminoethyl)ethanolamine** to pregnant female Wistar rats elicited **no signs of maternal toxicity**, had **no influence on gestational parameters** and induced **no signs of prenatal developmental toxicity** up to and

including the high dose of 50 mg/kg body weight/day; especially, **no indications for teratogenic effects** occurred, which could be causally related to the test substance administration.

Based on the results of this study, the **no observed adverse effect level (NOAEL)** for **maternal and prenatal developmental toxicity** is **50 mg/kg body weight/day**.

6. REFERENCES

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7. APPENDIX (SUMMARY TABLES)**7.1. LIST TO THE ABBREVIATIONS USED IN VOLUMES IA AND IC**

Tables IA-001 – IA-011 (EXAMINATION OF THE DAMS - CLINICS AND GROSS
NECROPSY)

Tables IC-001 – IC-027 (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

All other abbreviations used are explained in the tables.

7.2. LIST TO THE ABBREVIATIONS USED IN VOLUME IB

Table IB-001 (EXAMINATION OF THE DAMS – CLINICAL PATHOLOGY)

Cu	= copper
MEAN	= mean value
Mg	= magnesium
MG/KG BW/D	= milligram per kilogram body weight per day
mg/l	= milligram per liter
Mn	= manganese
N	= number/number of animals or litters
S.D.	= standard deviation
Zn	= zinc

**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 gravid uterine weights and net maternal body weight change	IA-007
Summary of maternal necropsy observations	IA-008
Summary of reproduction data	IA-009 - IA-011
The relevant individual values/findings are to be found in Volume II (Tables IIA-001 - IIA-045).	

**Part B:
EXAMINATIONS OF THE DAMS – CLINICAL PATHOLOGY
(MEAN VALUES)**

Summary of examinations (Cu, Mg, Mn, Zn)	IB-001
The relevant individual values are to be found in Volume II (Tables IIB-001 - IIB-005).	

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

	Tables
Mean placental and fetal body weights (on a litter basis)	IC-001
Summary of all classified fetal external observations	IC-002 - IC-004
Summary of fetal external unclassified observations	IC-005
Summary of all classified fetal soft tissue observations	IC-006 - IC-008
Summary of fetal soft tissue unclassified observations	IC-009
Summary of all classified fetal skeletal observations	IC-010 - IC-023
Summary of fetal skeletal unclassified cartilage observations	IC-024 - IC-026
Summary of all classified fetal external, soft tissue and skeletal observations	IC-027

The relevant individual values or findings are to be found in Volume II (Tables IIC-001 - IIC-0116).

20-FEB-03

01105

TABLE : IA- 001

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 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	
	4	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	
	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	
	4	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	

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01105

TABLE : IA- 002

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- GRAMS/ANIMAL/DAY

DAYS	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D						
						MEAN	S.D.	N	MEAN	S.D.	N
DAYS 0 TO 1	12.9 D 1.75 21	13.8 1.99 23	14.0 1.76 24	13.8 1.47 23	14.4* 1.22 22						
DAYS 1 TO 3	15.7 D 1.15 21	16.5 1.82 23	16.6 1.35 24	16.4 1.13 23	17.4** 1.50 22						
DAYS 3 TO 6	16.9 D 1.03 21	17.7 1.89 23	17.8 1.31 24	17.8 1.42 23	18.2* 1.26 22						
DAYS 6 TO 8	17.4 D 1.10 21	18.4 2.34 23	18.2 1.60 24	18.2 1.50 23	19.2** 1.28 22						
DAYS 8 TO 10	18.2 D 1.21 21	19.2 2.52 23	18.9 1.81 24	19.0 1.48 23	19.8* 1.95 22						
DAYS 10 TO 13	19.8 D 1.24 21	21.1 2.48 23	20.9 1.94 24	21.1 1.59 23	21.3* 1.78 22						
DAYS 13 TO 15	19.7 D 1.46 21	20.8 2.40 23	20.6 1.87 24	20.7 1.71 23	21.1 1.76 22						
DAYS 15 TO 17	20.8 D 1.61 21	22.3 2.61 23	21.9 1.78 24	22.2 2.03 23	22.4 2.51 22						
DAYS 17 TO 19	21.6 D 1.66 21	22.9 2.47 23	22.4 1.86 24	22.7 2.22 23	22.7 2.37 22						
DAYS 19 TO 20	20.1 D 1.71 21	21.1 2.18 23	20.2 1.85 24	20.4 1.58 23	20.9 2.38 22						

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

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TABLE : IA - 003

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- GRAMS/ANIMAL/DAY

DAYS	0 TO 6	MEAN OF MEANS	TEST GROUP				TEST GROUP 4
			0 MG/KG BW/D	0.5 MG/KG BW/D	2 MG/KG BW/D	10 MG/KG BW/D	
DAYS 0 TO 6	MEAN	15.2	16.0	16.1	16.0	16.7	
	S.D.	2.09	2.00	1.96	2.07	2.01	
	N	3	3	3	3	3	
DAYS 6 TO 19	MEAN	19.6	20.8	20.5	20.6	21.1	
	S.D.	1.55	1.75	1.64	1.77	1.38	
	N	6	6	6	6	6	
DAYS 0 TO 20	MEAN	18.3	19.4	19.1	19.2	19.8	
	S.D.	2.65	2.84	2.59	2.77	2.54	
	N	10	10	10	10	10	

PROJECT NO. 30R0019/01105: PREMATURAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

MEAN MATERNAL BODY WEIGHTS DURING GESTATION -- GRAMS

DAY	TEST GROUP 0 0 MG/KG BW/D			TEST GROUP 1 0.5 MG/KG BW/D			TEST GROUP 2 2 MG/KG BW/D			TEST GROUP 3 10 MG/KG BW/D			TEST GROUP 4 50 MG/KG BW/D		
	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N
DAY 0	154.0	8.26	21	153.3	9.50	23	157.8	10.61	24	160.0	10.04	23	158.9	8.79	22
DAY 1	164.2	9.87	21	163.4	9.48	23	168.1	10.53	24	170.1	10.69	23	168.9	8.88	22
DAY 3	171.6	10.22	21	173.0	10.11	23	175.6	10.70	24	177.6	11.65	23	178.6	9.19	22
DAY 6	180.8	10.52	21	182.0	9.88	23	185.0	11.31	24	187.7	12.13	23	187.2	9.96	22
DAY 8	186.9	10.94	21	189.6	11.16	23	192.8	11.81	24	194.0	13.01	23	195.4	10.07	22
DAY 10	195.9	11.92	21	200.5	12.87	23	203.1	12.83	24	205.3	13.91	23	205.8*	11.16	22
DAY 13	208.4	11.88	21	213.5	14.40	23	216.6	13.60	24	219.1*	15.03	23	219.4*	13.23	22
DAY 15	217.0	12.86	21	224.0	15.17	23	226.5	14.97	24	228.8*	15.63	23	229.2*	14.31	22
DAY 17	230.9	14.34	21	240.3	16.95	23	241.5	16.69	24	245.3*	17.44	23	245.1*	18.05	22
DAY 19	248.8	17.20	21	258.9	19.80	23	259.5	18.93	24	264.5*	20.48	23	265.7*	21.51	22
DAY 20	262.4	17.96	21	273.2	20.47	23	272.5	18.77	24	278.7*	21.18	23	279.2*	24.50	22

Statistics: D=Dunnett-test (two-sided)

* : p<=0.05 ** : p<=0.01

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TABLE : IA- 005

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION -- GRAMS

DAYS	TEST GROUP 0 0 MG/KG BW/D		TEST GROUP 1 0.5 MG/KG BW/D		TEST GROUP 2 2 MG/KG BW/D		TEST GROUP 3 10 MG/KG BW/D		TEST GROUP 4 50 MG/KG BW/D	
	MEAN	S.D. N	MEAN	S.D. N	MEAN	S.D. N	MEAN	S.D. N	MEAN	S.D. N
DAYS 0 TO 1	10.2 D	2.70 21	10.1	3.26 23	10.3	3.15 24	10.1	4.05 23	10.0	3.42 22
DAYS 1 TO 3	7.4 D	2.97 21	9.6	3.31 23	7.5	2.55 24	7.5	2.64 23	9.6	2.68 22
DAYS 3 TO 6	9.3 D	2.70 21	9.0	3.05 23	9.4	4.67 24	10.1	3.48 23	8.7	3.13 22
DAYS 6 TO 8	6.1 D	2.92 21	7.6	2.79 23	7.8	4.66 24	6.3	2.33 23	8.2	2.72 22
DAYS 8 TO 10	9.0 D	2.47 21	10.9	3.65 23	10.3	2.28 24	11.3*	3.52 23	10.4	3.11 22
DAYS 10 TO 13	12.6 D	2.89 21	13.0	4.02 23	13.6	2.63 24	13.8	3.12 23	13.6	5.32 22
DAYS 13 TO 15	8.6 D	3.11 21	10.5	2.63 23	9.9	2.48 24	9.7	2.65 23	9.8	2.81 22
DAYS 15 TO 17	13.9 D	3.93 21	16.3	2.78 23	15.0	2.96 24	16.5	3.09 23	15.9	5.71 22
DAYS 17 TO 19	17.8 D	4.94 21	18.6	3.76 23	18.0	4.02 24	19.2	4.04 23	20.6	5.08 22
DAYS 19 TO 20	13.6 D	3.69 21	14.4	2.03 23	13.0	3.91 24	14.2	3.29 23	13.5	5.39 22

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

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01105

TABLE : IA- 006

PROJECT NO. 30R0019/01105; PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAYAGE)
 MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION -- GRAMS

DAYS	0 TO 6	MEAN S.D. N	TEST GROUP				TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
			0 0 MG/KG BW/D	1 0.5 MG/KG BW/D	2 2 MG/KG BW/D	3 10 MG/KG BW/D		
DAYS 0 TO 6	MEAN	26.8 D	28.7	27.2	27.7	28.3		
	S.D.	3.81	4.07	6.01	5.58	5.05		
	N	21	23	24	23	22		
DAYS 6 TO 19	MEAN	67.9 D	76.9	74.5	76.8	78.5*		
	S.D.	9.30	12.65	11.53	10.74	17.08		
	N	21	23	24	23	22		
DAYS 0 TO 20	MEAN	108.4 D	119.9	114.7	118.7	120.3		
	S.D.	11.87	15.96	14.49	15.78	20.66		
	N	21	23	24	23	22		

Statistics: D=Dunnett-test (two-sided)

* : p<=0.05 ** : p<=0.01

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TABLE : IA- 007

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE)

MEAN GRAVID UTERINE WEIGHTS AND NET MATERNAL BODY WEIGHT CHANGE -- GRAMS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
GRAVID UTERUS					
MEAN	42.1	47.1	46.9	48.5	50.6*
S.D.	10.87	7.56	8.92	7.39	13.35
N	21	23	24	23	22
CARCASS					
MEAN	220.2	226.1	225.6	230.2	228.6
S.D.	12.06	15.25	14.32	16.22	14.55
N	21	23	24	23	22
NET WEIGHT CHANGE FROM DAY 6					
MEAN	39.4	44.2	40.5	42.5	41.3
S.D.	5.36	8.78	7.79	7.34	9.48
N	21	23	24	23	22

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

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TABLE : IA- 008

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF MATERNAL NECROPSY OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
DAMS EXAMINED	N 25	25	25	25	25
NOTHING ABNORMAL DETECTED	N 24 %	24 96	25 100	25 100	25 100
DILATED RENAL PELVIS	N 0 %	1 4.0	0 0.0	0 0.0	0 0.0
UTERUS: AMNIOTIC FLUID INCREASED	N 1 %	0 0.0	0 0.0	0 0.0	0 0.0

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01105

TABLE : IA-

009

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF REPRODUCTION DATA

		TEST GROUP 0	TEST GROUP 1	TEST GROUP 2	TEST GROUP 3	TEST GROUP 4
		0 MG/KG BW/D	0.5 MG/KG BW/D	2 MG/KG BW/D	10 MG/KG BW/D	50 MG/KG BW/D
Females Mated	N	25	25	25	25	25
Pregnant	N	21	23	24	23	22
Conception Rate	%	84	92	96	92	88
Aborted	N	0	0	0	0	0
Premature Births	N	0	0	0	0	0
Dams with Viable Fetuses	N	20	23	24	23	21
Dams with all Resorptions	N	1	0	0	0	1
Female Mortality	N	0	0	0	0	0
	%	0.0	0.0	0.0	0.0	0.0
Pregnant at Terminal Sacrifice	N	21	23	24	23	22
	%	84	92	96	92	88
Corpora Lutea	MEAN	9.2	9.6	10.3	9.9	10.3
	S.D.	1.33	1.80	1.42	1.24	1.55
	TOTAL	193	220	246	228	226
Implantation Sites	MEAN	8.1	8.9	9.6*	9.5*	9.6*
	S.D.	2.21	1.41	1.32	1.31	2.44
	TOTAL	170	205	230	219	211
Preimplantation Loss	MEAN%	12.0	6.2	6.0	4.0	7.1
	S.D.	19.56	8.14	9.17	5.17	19.06
Postimplantation Loss	MEAN%	9.5	4.4	9.2	5.8	7.5
	S.D.	22.04	6.81	15.05	9.04	21.31

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

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TABLE : IA-

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF REPRODUCTION DATA

010

	TEST GROUP 0	TEST GROUP 1	TEST GROUP 2	TEST GROUP 3	TEST GROUP 4
	0 MG/KG BW/D	0.5 MG/KG BW/D	2 MG/KG BW/D	10 MG/KG BW/D	50 MG/KG BW/D
Pregnant at Terminal Sacrifice	N	21	23	24	22
Resorptions: Total	MEAN	0.5 D	0.4	0.9	0.6
	S.D.	0.75	0.58	1.50	0.84
	TOTAL	11	9	22	13
Early	MEAN%	9.5 D	4.4	9.2	5.8
	S.D.	22.04	6.81	15.05	9.04
	TOTAL	5	6	21	12
Late	MEAN	0.2 D	0.3	0.9	0.5
	S.D.	0.44	0.54	1.51	0.79
	TOTAL	5	6	21	12
Dead Fetuses	MEAN%	2.7 D	3.1	8.8	5.4
	S.D.	5.08	6.54	15.16	8.56
	TOTAL	6	3	1	1
Resorptions: Total	MEAN	0.3 D	0.1	0.0*	0.0*
	S.D.	0.56	0.34	0.20	0.21
	TOTAL	6	3	1	1
Dead Fetuses	MEAN%	6.8 D	1.3	0.4	0.4
	S.D.	21.78	3.48	1.86	2.09
	TOTAL	0	0	0	0

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

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TABLE : IA- 011

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF REPRODUCTION DATA

	N	TEST GROUP 0		TEST GROUP 1		TEST GROUP 2		TEST GROUP 3		TEST GROUP 4	
		0 MG/KG BW/D	0.5 MG/KG BW/D	0.5 MG/KG BW/D	2 MG/KG BW/D	2 MG/KG BW/D	10 MG/KG BW/D	50 MG/KG BW/D	50 MG/KG BW/D	50 MG/KG BW/D	
Dams with Viable Fetuses		20	23	24	23	21					
Live Fetuses	MEAN	7.9 0	8.5	8.7	9.0	9.7**					
	S.D.	1.70	1.50	1.74	1.43	1.46					
	TOTAL	159	196	208	206	203					
Females	MEAN%	95.0 0	95.6	90.8	94.2	96.9					
	S.D.	7.68	6.81	15.05	9.04	5.38					
	MEAN	3.5 0	4.2	5.1*	4.7	5.3**					
S.D.	1.61	1.62	1.67	1.79	2.03						
TOTAL	69	97	122	108	111						
Males	MEAN%	41.4 0	47.2	53.6	50.0	52.9					
	S.D.	18.52	16.62	17.33	19.51	18.21					
	MEAN	4.5 0	4.3	3.6	4.3	4.4					
S.D.	1.40	1.29	1.93	1.89	1.83						
TOTAL	90	99	86	98	92						
PER CENT LIVE FEMALES	MEAN%	53.6 0	48.4	37.2**	44.2	44.0					
	S.D.	14.48	13.74	19.31	16.99	18.09					
PER CENT LIVE MALES	MEAN%	43.4	49.5	58.7	52.4	54.7					
	S.D.	56.6	50.5	41.3	47.6	45.3					

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

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01105

TABLE : IB - 001

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF EXAMINATIONS (Cu,Mg,Mn,Zn) -- mg/l END OF GESTATION

	TEST GROUP 0	TEST GROUP 1	TEST GROUP 2	TEST GROUP 3	TEST GROUP 4	
	0 MG/KG BW/D	0.5 MG/KG BW/D	2 MG/KG BW/D	10 MG/KG BW/D	50 MG/KG BW/D	
Cu	MEAN	2,2	2,2	2,0	2,0	2,0
	S.D.	0,6	0,2	0,2	0,1	0,1
	N	11	11	11	10	9
Mg	MEAN	21,0	23,4	23,1	23,9	24,2
	S.D.	2,1	2,2	1,7	1,9	1,8
	N	11	11	11	10	9
Mn	MEAN	<0,005	<0,005	<0,005	<0,005	<0,005
	S.D.	0	0	0	0	0
	N	11	11	11	10	9
Zn	MEAN	0,6	0,6	0,6	0,5	0,5
	S.D.	0	0,1	0,2	0,1	0,2
	N	11	11	11	10	9

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TABLE : IC- 001

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 MEAN PLACENTAL AND FETAL BODY WEIGHTS (ON A LITTER BASIS)

TEST GROUP 0 TEST GROUP 1 TEST GROUP 2 TEST GROUP 3 TEST GROUP 4
 0 MG/KG BW/D 0.5 MG/KG BW/D 2 MG/KG BW/D 10 MG/KG BW/D 50 MG/KG BW/D

PLACENTAL WEIGHTS UNITS: GRAMS

	MEAN	0.41 D	0.43	0.42	0.41	0.43
of all Viable Fetuses	S.D.	0.048	0.035	0.046	0.037	0.037
	N	20	23	24	23	21
of Male Fetuses	MEAN	0.42 D	0.44	0.42	0.42	0.44
	S.D.	0.053	0.044	0.043	0.037	0.041
	N	20	23	22	23	21
of Female Fetuses	MEAN	0.40 D	0.43	0.41	0.41	0.42
	S.D.	0.046	0.033	0.047	0.046	0.037
	N	20	22	24	23	21

FETAL WEIGHTS UNITS: GRAMS

	MEAN	3.5 D	3.5	3.5	3.5	3.6
of all Viable Fetuses	S.D.	0.23	0.24	0.23	0.17	0.18
	N	20	23	24	23	21
of Male Fetuses	MEAN	3.6 D	3.6	3.6	3.6	3.7
	S.D.	0.25	0.29	0.25	0.17	0.16
	N	20	23	22	23	21
of Female Fetuses	MEAN	3.4 D	3.5	3.4	3.4	3.5
	S.D.	0.27	0.22	0.25	0.18	0.22
	N	20	22	24	23	21

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

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TABLE : IC- 002

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF ALL CLASSIFIED FETAL EXTERNAL OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	159	196	208	206	203
Live	159	196	208	206	203
Dead	0	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N %	0 0.0	0 0.0	0 0.0	1 0.5
Litter Incidence	N %	0Fi 0.0	0 0.0	0 0.0	1 4.8
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	0.4
	S.D.	0.00	0.00	0.00	1.82
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

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TABLE : IC- 003

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FETAL EXTERNAL MALFORMATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N	23	24	23	21
Fetuses Evaluated	N	159	208	206	203
Live	N	159	208	206	203
Dead	N	0	0	0	0
MICROGNATHIA					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.5
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	MEAN%	0.0	0.0	0.0	0.4
	S.D.	0.00	0.00	0.00	1.82
ASTOMIA					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.5
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	MEAN%	0.0	0.0	0.0	0.4
	S.D.	0.00	0.00	0.00	1.82
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.5
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	MEAN%	0.0	0.0	0.0	0.4
	S.D.	0.00	0.00	0.00	1.82

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

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FETAL EXTERNAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	159	196	208	206	203
Live	159	196	208	206	203
Dead	0	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 %	0 0.0	0 0.0	0 0.0	0 0.0
Affected Fetuses/Litter	MEAN# S.D.	0.0 0.00	0.0 0.00	0.0 0.00	0.0 0.00

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

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TABLE : IC- 005

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL EXTERNAL UNCLASSIFIED OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	23	24	23	21
Fetuses Evaluated	N 159	196	208	206	203
Live	N 159	196	208	206	203
Dead	N 0	0	0	0	0
PLACENTAE FUSED					
Fetal Incidence	N %	0 0.0	0 0.0	1 0.5	0 0.0
Litter Incidence	N %	0 0.0	0 0.0	1 4.3	0 0.0
Affected Fetuses/Litter	MEAN% S.D.	0.0% 0.00	0.0 0.00	0.5 2.32	0.0 0.00
TOTAL FETAL EXTERNAL UNCLASSIFIED OBSERVATIONS					
Fetal Incidence	N %	0 0.0	0.0 0.0	0.5 2.32	0.0 0.00
Litter Incidence	N %	0 0.0	0.0 0.0	1 4.3	0 0.0
Affected Fetuses/Litter	MEAN% S.D.	0.0% 0.00	0.0 0.00	0.5 2.32	0.0 0.00

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

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TABLE : IC-

006

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF ALL CLASSIFIED FETAL SOFT TISSUE OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	N 23	N 24	N 23	N 21
Fetuses Evaluated	N 74	N 94	N 98	N 98	N 96
Live	N 74	N 94	N 98	N 98	N 96
Dead	N 0	N 0	N 0	N 0	N 0
TOTAL MALFORMATIONS					
Fetal Incidence	N 0 0.0	N 0 0.0	N 1 1.0	N 0 0.0	N 0 0.0
Litter Incidence	N 0Fi 0.0	N 0 0.0	N 1 4.2	N 0 0.0	N 0 0.0
Affected Fetuses/Litter	MEAN% 0.0Wi 0.00	MEAN% 0.0 0.00	MEAN% 0.8 4.08	MEAN% 0.0 0.00	MEAN% 0.0 0.00
TOTAL VARIATIONS					
Fetal Incidence	N 6 8.1	N 9 9.6	N 10 10	N 4 4.1	N 6 6.3
Litter Incidence	N 4Fi 20	N 8 35	N 10 42	N 3 13	N 4 19
Affected Fetuses/Litter	MEAN% 9.6Wi 20.82	MEAN% 9.7 14.68	MEAN% 13.3 22.08	MEAN% 4.1 11.93	MEAN% 7.1 15.47

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

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TABLE : IC- 007

PROJECT NO. 30R00019/01105: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SOFT TISSUE MALFORMATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N	23	24	23	21
Fetuses Evaluated	N	94	98	98	96
Live	N	74	98	98	96
Dead	N	0	0	0	0
SITUS INVERSUS					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.2	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.8	0.0	0.0
	S.D.	0.00	4.08	0.00	0.00
MISSHAPEN THYMUS					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.2	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.8	0.0	0.0
	S.D.	0.00	4.08	0.00	0.00
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	0	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0Fi	1	0	0
	%	0.0	4.2	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0Wi	0.8	0.0	0.0
	S.D.	0.00	4.08	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. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF FETAL SOFT TISSUE VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	23	24	23	21
Fetuses Evaluated	N 74	94	98	98	96
Live	N 74	94	98	98	96
Dead	N 0	0	0	0	0
DILATED RENAL PELVIS					
Fetal Incidence	N 6	8	10	4	6
	% 8.1	8.5	10	4.1	6.3
Litter Incidence	N 4Fi 20	7 30	10 42	3 13	4 19
Affected Fetuses/Litter	MEAN% 9.6Wi 20.82	8.6 14.42	13.3 22.08	4.1 11.93	7.1 15.47
DILATED URETER					
Fetal Incidence	N 3	2	3	2	2
	% 4.1	2.1	3.1	2.0	2.1
Litter Incidence	N 3Fi 15	2 8.7	3 13	2 8.7	2 9.5
Affected Fetuses/Litter	MEAN% 5.4Wi 13.86	2.2 7.20	2.7 7.37	2.0 6.53	2.5 8.29
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence	N 6	9	10	4	6
	% 8.1	9.6	10	4.1	6.3
Litter Incidence	N 4Fi 20	8 35	10 42	3 13	4 19
Affected Fetuses/Litter	MEAN% 9.6Wi 20.82	9.7 14.68	13.3 22.08	4.1 11.93	7.1 15.47

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

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TABLE : IC- 009

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SOFT TISSUE UNCLASSIFIED OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	74	94	98	98	96
Live	74	94	98	98	96
Dead	0	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	0	0	0	0
	%	0.0	0.0	0.0	0.0
Affected fetuses/Litter	MEAN%	0.0	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00

Statistics: F1 = Fisher's exact test (one-sided) W1 = Wilcoxon-test (one-sided)

* : p<=0.05 ** : p<=0.01

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01105

TABLE : IC- 010

PROJECT NO. 30R0019/01105; PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF ALL CLASSIFIED FETAL SKELETAL OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	23	24	23	21
Fetuses Evaluated	N 85	102	110	108	107
Live	N 85	102	110	108	107
Dead	N 0	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N 0 0.0	1 1.0	1 0.9	1 0.9	1 0.9
Litter Incidence	N 0 0.0	1 4.3	1 4.2	1 4.3	1 4.8
Affected Fetuses/Litter	MEAN% 0.0% S.D. 0.00	0.9 4.17	0.8 4.08	0.7 3.48	0.7 3.12
TOTAL VARIATIONS					
Fetal Incidence	N 81 95	99 97	105 95	105 97	101 94
Litter Incidence	N 20% 100	23 100	24 100	23 100	21 100
Affected Fetuses/Litter	MEAN% 95.8% S.D. 10.92	96.8 10.61	95.2 12.11	96.8 8.73	94.0 13.19

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

* : p<=0.05 ** : p<=0.01

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SKELETAL MALFORMATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
SEVERELY MALFORMED SKULL BONES: Changed cartilage					
Fetal Incidence	0	0	0	0	1
N	0	0	0	0	0.9
%	0.0	0.0	0.0	0.0	0.9
Litter Incidence	0	0	0	0	1
N	0	0	0	0	4.8
%	0.0	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	0.0	0.0	0.0	0.0	0.7
MEAN%	0.0	0.0	0.0	0.0	3.12
S.D.	0.00	0.00	0.00	0.00	3.12
ABSENT LUMBAR VERTEBRA					
Fetal Incidence	0	0	0	0	1
N	0	0	0	0	0.9
%	0.0	0.0	0.0	0.0	0.9
Litter Incidence	0	0	0	0	1
N	0	0	0	0	4.8
%	0.0	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	0.0	0.0	0.0	0.0	0.7
MEAN%	0.0	0.0	0.0	0.0	3.12
S.D.	0.00	0.00	0.00	0.00	3.12
CLEFT STERNUM: Split cartilage					
Fetal Incidence	0	1	0	0	0
N	0	1.0	0	0	0.0
%	0.0	1.0	0.0	0.0	0.0
Litter Incidence	0	4.3	0	0	0
N	0	4.3	0	0	0.0
%	0.0	4.3	0.0	0.0	0.0
Affected Fetuses/Litter	0.0	0.9	0.0	0.0	0.0
MEAN%	0.0	4.17	0.0	0.0	0.0
S.D.	0.00	4.17	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

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF FETAL SKELETAL MALFORMATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	23	24	23	21
Fetuses Evaluated	N 85	102	110	108	107
Live	N 85	102	110	108	107
Dead	N 0	0	0	0	0
MALPOSITIONED AND BIPARTITE STERNEBRA: Unchanged cartilage					
Fetal Incidence	N 0	0	0	1	1
	% 0.0	0.0	0.0	0.9	0.9
Litter Incidence	N 0	0	0	1	1
	% 0.0	0.0	0.0	4.3	4.8
Affected Fetuses/Litter	MEAN± 0.0W±	0.0	0.0	0.7	0.7
	S.D. 0.00	0.00	0.00	3.48	3.12
MISSHAPEN HUMERUS					
Fetal Incidence	N 0	0	1	0	0
	% 0.0	0.0	0.9	0.0	0.0
Litter Incidence	N 0	0	1	0	0
	% 0.0	0.0	4.2	0.0	0.0
Affected Fetuses/Litter	MEAN± 0.0W±	0.0	0.8	0.0	0.0
	S.D. 0.00	0.00	4.08	0.00	0.00
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N 0	1	1	1	1
	% 0.0	1.0	0.9	0.9	0.9
Litter Incidence	N 0	1	1	1	1
	% 0.0	4.3	4.2	4.3	4.8
Affected Fetuses/Litter	MEAN± 0.0W±	0.9	0.8	0.7	0.7
	S.D. 0.00	4.17	4.08	3.48	3.12

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

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01105

TABLE : IC- 013

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	23	24	23	21
Fetuses Evaluated	N 85	102	110	108	107
Live	N 85	102	110	108	107
Dead	N 0	0	0	0	0
SUPRACCCIPITAL HOLE(S)					
Fetal Incidence	N 28	34	45	59	42
	% 33	33	41	55	39
Litter Incidence	N 17Fi	18	19	20	17
	% 85	78	79	87	81
Affected Fetuses/Litter	MEAN% 32.8Wi	32.6	41.3	52.3*	41.2
	S.D. 20.82	25.74	30.66	32.72	32.76
INCOMPLETE OSSIFICATION OF BASISPHENOID					
Fetal Incidence	N 5	9	8	7	9
	% 5.9	8.8	7.3	6.5	8.4
Litter Incidence	N 3Fi	7	5	6	6
	% 15	30	21	26	29
Affected Fetuses/Litter	MEAN% 5.5Wi	8.7	6.9	6.2	8.4
	S.D. 14.32	14.79	14.28	11.34	14.82
INCOMPLETE OSSIFICATION OF INTERPARIETAL: Unchanged cartilage					
Fetal Incidence	N 16	25	24	36	26
	% 19	25	22	33	24
Litter Incidence	N 11Fi	14	15	15	10
	% 55	61	63	65	48
Affected Fetuses/Litter	MEAN% 18.0Wi	24.0	21.1	33.5	23.4
	S.D. 21.48	25.28	21.50	32.31	32.74

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

* : p<=0.05 ** : p<=0.01

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	23	24	23	21
Fetuses Evaluated	N 85	102	110	108	107
Live	N 85	102	110	108	107
Dead	N 0	0	0	0	0
INCOMPLETE OSSIFICATION OF PARIETAL: Unchanged cartilage					
Fetal Incidence	N 13	25	14	24	19
	% 15	25	13	22	18
Litter Incidence	N 10Fi	17	11	11	11
	% 50	74	46	48	52
Affected Fetuses/Litter	MEAN% 15.0Wi	24.6*	13.7	21.5	18.3
	S.D. 17.47	19.16	18.42	26.05	20.40
INCOMPLETE OSSIFICATION OF SUPRAOCCIPITAL: Unchanged cartilage					
Fetal Incidence	N 16	18	14	15	12
	% 19	18	13	14	11
Litter Incidence	N 12Fi	12	9	11	9
	% 60	52	38	48	43
Affected Fetuses/Litter	MEAN% 18.7Wi	17.2	11.7	13.8	10.6
	S.D. 17.83	20.98	17.41	17.53	13.83
INCOMPLETE OSSIFICATION OF SKULL: Unchanged cartilage					
Fetal Incidence	N 5	9	4	2	0
	% 5.9	8.8	3.6	1.9	0.0
Litter Incidence	N 4Fi	7	3	2	0
	% 20	30	13	8.7	0.0
Affected Fetuses/Litter	MEAN% 6.0Wi	8.5	3.5	1.7	0.0
	S.D. 13.44	15.32	10.05	5.76	0.00

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

* : p<=0.05 ** : p<=0.01

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TABLE : IC- 015

PROJECT NO. 30R00019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
UNOSSIFIED HYOID: Cartilage present					
Fetal Incidence	N %	1 1.0	1 0.9	0 0.0	0 0.0
Litter Incidence	N %	1 4.3	1 4.2	0 0.0	0 0.0
Affected Fetuses/Litter	MEAN% S.D.	1.1 5.21	0.8 4.08	0.0 0.00	0.0 0.00
INCOMPLETE OSSIFICATION OF HYOID: Cartilage present					
Fetal Incidence	N %	1 1.0	0 0.0	0 0.0	0 0.0
Litter Incidence	N %	1 4.3	0 0.0	0 0.0	0 0.0
Affected Fetuses/Litter	MEAN% S.D.	0.9 4.17	0.0 0.00	0.0 0.00	0.0 0.00
UNOSSIFIED CERVICAL CENTRUM: Unchanged cartilage					
Fetal Incidence	N %	1 1.0	0 0.0	3 2.8	0 0.0
Litter Incidence	N %	1 4.3	0 0.0	1 4.3	0 0.0
Affected Fetuses/Litter	MEAN% S.D.	1.4 6.95	0.0 0.00	2.6 12.51	0.0 0.00

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

* : p<=0.05 ** : p<=0.01

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
INCOMPLETE OSSIFICATION OF CERVICAL ARCH: Cartilage present					
Fetal Incidence	N %	0 0.0	0 0.0	0 0.0	1 0.9
Litter Incidence	N %	0Fi 0.0	0 0.0	0 0.0	1 4.8
Affected Fetuses/Litter	MEAN% S.D.	0.0Wi 0.00	0.0 0.00	0.0 0.00	1.0 4.36
INCOMPLETE OSSIFICATION OF THORACIC CENTRUM: Unchanged cartilage					
Fetal Incidence	N %	3 3.5	1 1.0	3 2.7	4 3.7
Litter Incidence	N %	3Fi 15	1 4.3	3 13	4 17
Affected Fetuses/Litter	MEAN% S.D.	3.5Wi 8.60	0.9 4.17	2.4 6.41	3.9 8.78
INCOMPLETE OSSIFICATION OF THORACIC CENTRUM: Dumbbell-shaped cartilage of centrum					
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	1 4.8
Affected Fetuses/Litter	MEAN% S.D.	0.0Wi 0.00	0.0 0.00	0.0 0.00	1.0 4.36

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

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
DUMBBELL OSSIFICATION OF THORACIC CENTRUM; Unchanged cartilage					
Fetal Incidence	3	6	9	4	3
	3.5	5.9	8.2	3.7	2.8
Litter Incidence	3Fi	6	7	4	3
	15	26	29	17	14
Affected Fetuses/Litter	3.3Wi	5.4	8.1	3.7	2.7
MEAN%	7.99	9.40	14.36	8.29	6.80
S.D.					
DUMBBELL OSSIFICATION OF THORACIC CENTRUM; Dumbbell-shaped cartilage of centrum					
Fetal Incidence	9	12	12	14	5
	11	12	11	13	4.7
Litter Incidence	8Fi	10	9	10	4
	40	43	38	43	19
Affected Fetuses/Litter	10.0Wi	12.7	9.8	12.8	4.8
MEAN%	14.05	18.34	14.01	17.36	10.78
S.D.					
BIPARTITE OSSIFICATION OF THORACIC CENTRUM; Dumbbell-shaped cartilage of centrum					
Fetal Incidence	0	0	2	0	0
	0.0	0.0	1.8	0.0	0.0
Litter Incidence	0Fi	0	2	0	0
	0.0	0.0	8.3	0.0	0.0
Affected Fetuses/Litter	0.0Wi	0.0	1.7	0.0	0.0
MEAN%	0.00	0.00	5.65	0.00	0.00
S.D.					

Statistics: Fi - Fisher's exact test (one-sided) Wi - Wilcoxon-test (one-sided)

* : p<=0.05 ** : p<=0.01

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TABLE : IC-

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

018

ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N	20	23	23	21
Fetuses Evaluated	N	85	102	110	107
Live	N	85	102	108	107
Dead	N	0	0	0	0
SUPERNUMERARY THORACIC VERTEBRA					
Fetal Incidence	N	1	3	0	1
	%	1.2	2.9	0.0	0.9
Litter Incidence	N	IFi	3	0	1
	%	5.0	13	0.0	4.8
Affected Fetuses/Litter	MEAN±	1.0Wi	1.9	0.0	1.0
	S.D.	4.47	8.73	0.00	4.36
DUMBBELL OSSIFICATION OF LUMBAR CENTRUM: Unchanged cartilage					
Fetal Incidence	N	0	0	2	0
	%	0.0	0.0	1.8	0.0
Litter Incidence	N	0Fi	0	2	0
	%	0.0	0.0	8.3	0.0
Affected Fetuses/Litter	MEAN±	0.0Wi	0.0	1.9	0.0
	S.D.	0.00	0.00	6.40	0.00
DUMBBELL OSSIFICATION OF LUMBAR CENTRUM: Dumbbell-shaped cartilage of centrum					
Fetal Incidence	N	0	2	0	0
	%	0.0	2.0	0.0	0.0
Litter Incidence	N	0Fi	2	0	0
	%	0.0	8.7	0.0	0.0
Affected Fetuses/Litter	MEAN±	0.0Wi	1.7	0.0	0.0
	S.D.	0.00	5.76	0.00	0.00

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

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01105

TABLE : IC- 019

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
MISSHAPE SACRAL VERTEBRA					
Fetal Incidence	2 2.4	1 1.0	2 1.8	4 3.7	0 0.0
Litter Incidence	2Fi 10	1 4.3	2 8.3	3 13	0 0.0
Affected Fetuses/Litter	MEAN% S.D.	1.1 5.21	1.9 6.40	3.7 10.25	0.0 0.00
FUSED SACRAL CENTRUM AND ARCH; Unchanged cartilage					
Fetal Incidence	0 0.0	1 1.0	2 1.8	0 0.0	7 6.5
Litter Incidence	0Fi 0.0	1 4.3	1 4.2	0 0.0	4 19
Affected Fetuses/Litter	MEAN% S.D.	0.0Wi 0.00	1.1 5.21	1.7 8.16	0.0 0.00
DUMBBELL OSSIFICATION OF SACRAL CENTRUM; Dumbbell-shaped cartilage of centrum					
Fetal Incidence	0 0.0	2 2.0	0 0.0	0 0.0	1 0.9
Litter Incidence	0Fi 0.0	2 8.7	0 0.0	0 0.0	1 4.8
Affected Fetuses/Litter	MEAN% S.D.	0.0Wi 0.00	1.7 5.76	0.0 0.00	0.8 3.64

Statistics: Fi - Fisher's exact test (one-sided) Wi - Wilcoxon-test (one-sided)

* : p<=0.05 ** : p<=0.01

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0	TEST GROUP 1	TEST GROUP 2	TEST GROUP 3	TEST GROUP 4
	0 MG/KG BW/D	0.5 MG/KG BW/D	2 MG/KG BW/D	10 MG/KG BW/D	50 MG/KG BW/D
Litters Evaluated	N	23	24	23	21
Fetuses Evaluated	N	102	110	108	107
Live	N	102	110	108	107
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF SACRAL ARCH: Cartilage present					
Fetal Incidence	N	6	11	7	4
	%	5.9	10	6.5	3.7
Litter Incidence	N	4	7	4	2
	%	17	29	17	9.5
Affected Fetuses/Litter	MEAN	5.2	9.1	7.9	2.8
	S.D.	20.37	16.95	18.75	9.86
UNOSSIFIED STERNEBRA: Unchanged cartilage					
Fetal Incidence	N	10	9	12	5
	%	9.8	8.2	11	4.7
Litter Incidence	N	5	6	8	3
	%	22	25	35	14
Affected Fetuses/Litter	MEAN	9.5	7.4	10.4	3.9
	S.D.	23.03	14.11	16.02	10.75
INCOMPLETE OSSIFICATION OF STERNEBRA: Unchanged cartilage					
Fetal Incidence	N	56	71	73	52
	%	45	65	68	49
Litter Incidence	N	19	23*	23**	18
	%	83	96	100	86
Affected Fetuses/Litter	MEAN	54.4	63.7	66.3*	47.9
	S.D.	38.02	27.93	26.17	32.03

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

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PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FETAL SKELETAL VARIATIONS

TABLE : IC-

021

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
MISSHAPEN STERNEBRA: Unchanged cartilage					
Fetal Incidence	27 32	26 25	22 20	28 26	28 26
Litter Incidence	17Fi 85	18 78	14 58	14 61	15 71
Affected Fetuses/Litter	MEAN% S.D.	30.8Wi 17.91	19.3 20.92	25.4 23.54	25.4 20.32
UNILATERAL OSSIFICATION OF STERNEBRA: Unchanged cartilage					
Fetal Incidence	3 3.5	2 2.0	0 0.0	4 3.7	2 1.9
Litter Incidence	3Fi 15	2 8.7	0 0.0	3 13	2 9.5
Affected Fetuses/Litter	MEAN% S.D.	3.5Wi 8.60	0.0 0.00	3.2 8.73	1.7 5.54
SUPERNUMERARY RIB (14TH): Cartilage present					
Fetal Incidence	4 4.7	3 2.9	0 0.0	3 2.8	4 3.7
Litter Incidence	4Fi 20	3 13	0 0.0	3 13	4 19
Affected Fetuses/Litter	MEAN% S.D.	4.9Wi 10.39	0.0 0.00	2.8 7.51	3.5 7.41

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

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N 20	23	24	23	21
Fetuses Evaluated	N 85	102	110	108	107
Live	N 85	102	110	108	107
Dead	N 0	0	0	0	0
SUPERNUMERARY RIB (14TH): Cartilage not present					
Fetal Incidence	N 41	52	42	49	45
	% 48	51	38	45	42
Litter Incidence	N 19Fi	20	18	21	17
	% 95	87	75	91	81
Affected Fetuses/Litter	MEAN% 48.5Wi	53.6	37.1	45.0	41.1
	S.D. 28.74	36.25	28.17	29.15	27.89
CERVICAL RIB: Cartilage present					
Fetal Incidence	N 0	0	0	0	1
	% 0.0	0.0	0.0	0.0	0.9
Litter Incidence	N 0Fi	0	0	0	1
	% 0.0	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	MEAN% 0.0Wi	0.0	0.0	0.0	0.7
	S.D. 0.00	0.00	0.00	0.00	3.12
CERVICAL RIB: Cartilage not present					
Fetal Incidence	N 4	4	6	9	0
	% 4.7	3.9	5.5	8.3	0.0
Litter Incidence	N 4Fi	2	5	8	0
	% 20	8.7	21	35	0.0
Affected Fetuses/Litter	MEAN% 5.3Wi	3.5	6.5	8.3	0.0
	S.D. 11.36	13.01	14.02	12.21	0.00

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

* : p<=0.05 ** : p<=0.01

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SKELETAL VARIATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N	23	24	23	21
Fetuses Evaluated	N	85	110	108	107
Live	N	85	110	108	107
Dead	N	0	0	0	0
WAVY RIB					
Fetal Incidence	N	11	5	0	1
	x	5.9	4.5	0.0	0.9
Litter Incidence	N	7	2	0	1
	x	3Fi	8.3	0.0	4.8
Affected Fetuses/Litter	MEAN%	5.8Wi	5.8	0.0	1.2
	S.D.	17.42	21.65	0.00	5.46
INCOMPLETE OSSIFICATION OF PUBIS; Cartilage present					
Fetal Incidence	N	0	0	0	1
	x	0.0	0.0	0.0	0.9
Litter Incidence	N	0	0	0	1
	x	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	MEAN%	0.0Wi	0.0	0.0	1.0
	S.D.	0.00	0.00	0.00	4.36
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence	N	81	105	105	101
	x	95	95	97	94
Litter Incidence	N	20Fi	24	23	21
	x	100	100	100	100
Affected Fetuses/Litter	MEAN%	95.8Wi	95.2	96.8	94.0
	S.D.	10.92	12.11	8.73	13.19

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

* : p<=0.05 ** : p<=0.01

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF FETAL SKELETAL UNCLASS. CARTILAGE OBS.

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
	MEAN% S.D.	MEAN% S.D.	MEAN% S.D.	MEAN% S.D.	MEAN% S.D.
NOTCHED CARTILAGE BETWEEN BASISPHENOID AND BASIOCCIPITAL					
Fetal Incidence	1	2	2	4	0
	1.2	2.0	1.8	3.7	0.0
Litter Incidence	1Fi	2	2	3	0
	5.0	8.7	8.3	13	0.0
Affected Fetuses/Litter	1.0Mi	2.2	1.7	3.2	0.0
	4.47	7.20	5.65	9.35	0.00
FUSED CERVICAL ARCH CARTILAGE					
Fetal Incidence	0	0	1	1	0
	0.0	0.0	0.9	0.9	0.0
Litter Incidence	0Fi	0	1	1	0
	0.0	0.0	4.2	4.3	0.0
Affected Fetuses/Litter	0.0Mi	0.0	2.1	1.1	0.0
	0.00	0.00	10.21	5.21	0.00
BIPARTITE PROCESSUS XIPHOIDEUS					
Fetal Incidence	50	53	63	65	45
	59	52	57	60	42
Litter Incidence	18Fi	20	21	22	18
	90	87	88	96	86
Affected Fetuses/Litter	59.1Mi	51.3	56.3	58.4	42.3
	33.13	32.84	32.37	29.92	31.52

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

10-FEB-03

01105

TABLE : IC- 025

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF FETAL SKELETAL UNCLASS. CARTILAGE OBS.

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	20	23	24	23	21
Fetuses Evaluated	85	102	110	108	107
Live	85	102	110	108	107
Dead	0	0	0	0	0
NOTCHED MANUBRIUM					
Fetal Incidence	6	11	4	13	11
	7.1	11	3.6	12	10
Litter Incidence	4Fi	6	4	8	6
	20	26	17	35	29
Affected Fetuses/Litter	6.8Hi	11.7	4.6	11.4	10.5
MEAN%	14.89	21.96	11.79	18.98	20.61
S.D.					
CARTILAGINOUS PARTS OF RIBS DISPLACED					
Fetal Incidence	0	0	0	1	1
	0.0	0.0	0.0	0.9	0.9
Litter Incidence	0Fi	0	0	1	1
	0.0	0.0	0.0	4.3	4.8
Affected Fetuses/Litter	0.0Wi	0.0	0.0	0.7	0.7
MEAN%	0.00	0.00	0.00	3.48	3.12
S.D.					
BIPARTITE RIB CARTILAGE					
Fetal Incidence	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.9
Litter Incidence	0Fi	0	0	0	1
	0.0	0.0	0.0	0.0	4.8
Affected Fetuses/Litter	0.0Wi	0.0	0.0	0.0	0.7
MEAN%	0.00	0.00	0.00	0.00	3.12
S.D.					

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

10-FEB-03

01105

TABLE : IC- 026

PROJECT NO. 30R0019/01105: PRENATAL TOXICITY STUDY IN RATS
 ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FETAL SKELETAL UNCLASS. CARTILAGE OBS.

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
TOTAL FETAL SKELETAL UNCLASS. CARTILAGE OBS.					
Fetal Incidence	N 60	56 55	64 58	68 63	51 48
Litter Incidence	N 90	18Fi 87	21 88	22 96	18 86
Affected Fetuses/Litter	MEAN% S.D.	60.1Wi 33.46	54.6 32.96	58.3 33.54	61.2 31.54
					47.8 33.00

Statistics: Fi - Fisher's exact test (one-sided) Wi - Wilcoxon-test (one-sided)

* : p<=0.05 ** : p<=0.01

7-FEB-03

01105

PROJECT NO. 30R0019/01105; PRENATAL TOXICITY STUDY IN RATS

ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF ALL CLASSIFIED FETAL EXTERNAL, SOFT TISSUE, AND SKELETAL OBSERVATIONS

TABLE : IC-

027

SUMMARY OF ALL CLASSIFIED FETAL EXTERNAL, SOFT TISSUE, AND SKELETAL OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 0.5 MG/KG BW/D	TEST GROUP 2 2 MG/KG BW/D	TEST GROUP 3 10 MG/KG BW/D	TEST GROUP 4 50 MG/KG BW/D
Litters Evaluated	N	20	24	23	21
Fetuses Evaluated	N	159	208	206	203
Live	N	159	208	206	203
Dead	N	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N	0	1	1	1
	%	0.0	0.5	0.5	0.5
Litter Incidence	N	0Fi	2	1	1
	%	0.0	8.3	4.3	4.8
Affected Fetuses/Litter	MEAN%	0.0Wi	0.8	0.4	0.4
	S.D.	0.00	2.82	1.74	1.82
TOTAL VARIATIONS					
Fetal Incidence	N	87	115	109	107
	%	55	55	53	53
Litter Incidence	N	20Fi	24	23	21
	%	100	100	100	100
Affected Fetuses/Litter	MEAN%	56.2Wi	56.6	52.9	52.9
	S.D.	11.95	11.80	7.64	8.54

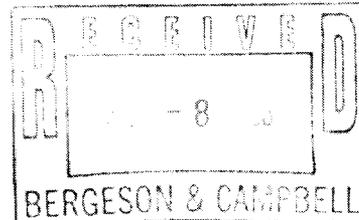
Statistics: Fi = Fisher's exact test (one-sided) Wi = Wilcoxon-test (one-sided)
 * : p<=0.05 ** : p<=0.01

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AEEA

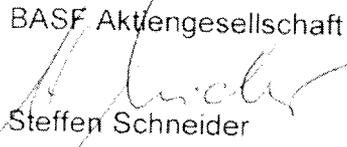
Dear Mrs. Burchi,

please find attached the following report:

Report: **AEEA** Additional histopathological examination of pups
From an OECD 421 screening study
(Project No.: 90R0019/01075) (August 11, 2003).

With kind regards

BASF Aktiengesellschaft


Steffen Schneider

STUDY TITLE

Report

AEEA

Additional histopathological examination
of pups from an OECD 421 screening study
(Project No.: 90R0019/01075)

AUTHORS

Dr. S. Burkhardt
Dr. S. Schneider
Dr. B. van Ravenzwaay

STUDY COMPLETED ON

11. August 2003

PERFORMING LABORATORY

Experimental Toxicology and Ecology
BASF Aktiengesellschaft
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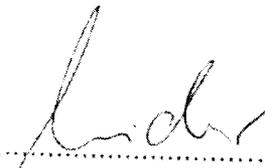
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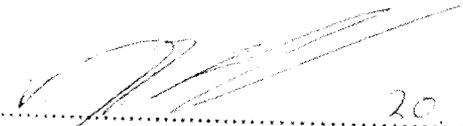
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SIGNATURE PAGE

 14.08.2003
.....
Dr. med. vet. S. Burkhardt

 14.08.2003
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Dr. med. vet. S. Schneider

 20.08.2003
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1. SUMMARY

This is a histopathological follow up investigation of two pups from a Reproduction/ Developmental Toxicity Screening Study (OECD 421). In this study the test compound was administered to groups of 10 male and 10 female healthy young adult rats (F0 parental generation). Fourteen days after the beginning of treatment, F0 animals were mated to produce a litter. The F1 pups were killed on day 4 post partum (p.p.). All pups were examined macroscopically for external and visceral findings at necropsy. Histopathological examinations in pups were not provided in this screening study. A total of 4 pups which died between days 1 and 3 post partum were routinely assessed macroscopically. Two of these pups (Nos. 1 and 2) were dedicated for further histopathological investigations. This additional histopathological examination was not conducted in accordance with the principles of Good Laboratory Practice (GLP). Cause of death in both treated pups was a dissecting aneurysm of aorta and pulmonary trunk. Furthermore, both animals revealed focal necroses of the aortic wall, and showed a focal dilation of the right carotid.

2. SYNOPSIS OF THE INLIFE STUDY

This follow up investigation was performed to gain more detailed information on macroscopical findings that were observed in preterminally deceased pups from a Reproduction/Developmental Toxicity Screening Test (SIDS, according to OECD Guideline 421) with **N-(2-Aminoethyl)ethanolamine** in Wistar Rats (BASF Project No. 90R0019/01075).

In this Reproduction/Developmental Toxicity Screening Study the test compound was administered to groups of 10 male and 10 female healthy young adult rats (F0 parental generation) by oral gavage at doses of 50, 250 and 1,000 mg/kg body weight/day throughout the whole study period. The test substance was administered as an aqueous solution at a standard dose volume of 10 ml/kg body weight. The control groups were dosed with the vehicle only (doubly distilled water). Fourteen days after the beginning of treatment, F0 animals were mated to produce a litter. Mating pairs were from the same dose group. The F0 adult males were sacrificed about 32 days after the first test substance administration, the F1 pups were killed on day 4 post partum (p.p.) and the F0 females on one of the following days.

Daily observations were done on the parents and pups and parental animals were examined for their mating and reproductive performances; this included determinations of the number of implantations and the calculation of the postimplantation loss.

Food consumption of the F0 parents was determined regularly during premating, after the mating period and - in dams - during gestation and lactation periods.

In general, body weights of F0 parents were determined once weekly. However, during gestation and lactation F0 females were weighed on days 0, 7, 14 and 20 of gestation, on the day of parturition and on days 4 and 7 after birth.

All F0 parental animals were assessed by gross pathology (including weight determinations of several organs). Histopathological examination in F0 parental animals was limited to all gross lesions, as well as ovaries, testes and epididymides of control and top dose animals.

The pups were sexed and were weighed on the day after birth and on day 4 post partum. Their viability was recorded. All pups were examined macroscopically for external and visceral findings at necropsy. Histopathological examinations in pups were not provided in this screening study.

3. PATHOLOGY

3.1. MATERIAL AND METHODS

A total of 4 pups which died between days 1 and 3 post partum were routinely assessed macroscopically and the findings were reported within the frame of the above mentioned Reproduction/Developmental Toxicity Screening Test. Afterwards they were preserved in toto in Harrison's fluid. Two of these pups (Nos. 1 and 2) were dedicated for further histopathological investigations.

As no control animals of this study were available, one rat of the same age (4 days) was sacrificed by CO₂ inhalation and fixed in Bouin's fluid. This additional histopathologic examination was not conducted in accordance with the principles of Good Laboratory Practice (GLP).

3.1.1. Paraffin embedding, sectioning and staining

After fixation, the proximal part of the heart (beginning in the region of the sulcus coronarius) with leaving vessels (aortic arch, descending aorta, pulmonary trunk with pulmonary arteries, ductus arteriosus botalli, carotid arteries and subclavian arteries) were embedded in paraffin, in pup No. 1 including the connective tissue, in pup No. 2 without the connective tissue. Of pup No. 1 and the control animal, cross sections through the entire thorax and neck (skin, skeletal muscle, spine, trachea, esophagus, parts of the lungs) were taken to avoid destruction of areas of interest. In pup No. 2, the heart and vessels were dissected and cross sections were taken without adjacent connective tissue.

Thereafter, serial sections of all animals were taken every 50 µm and stained with Hematoxylin-Eosin (H&E). Every 100 µm, additional sections were taken from all animals and left unstained for further investigations. From all animals observed one to two of these unstained sections of aorta, pulmonary trunk, and carotid arteries respectively were stained with Elastica van Giesson for demonstration of elastic fibers.

3.2. RESULTS

3.2.1. Gross lesions

Pups No. 1 and 2 revealed both a severe dilatation of the pulmonal trunk, starting directly after leaving the heart and extending to the ductus arteriosus botalli (DAB). In addition, the aorta was also severely dilated, starting at the aortic arch/DAB throughout the descending aorta until the region of the kidneys. Finally in both animals, a focal dilatation of about 0.4 cm in diameter of the right carotid artery was observed (s. figure 1).

3.2.2. Histopathology

3.2.2.1. Hematoxylin-Eosin stain

Pup No. 1:

The **aorta** showed a severe **dissecting aneurysm**, beginning in the region where the ductus arteriosus botalli (DAB) opens into the aorta throughout the descending aorta until the region of the kidneys (s. figure 2). The **aortic wall** revealed a **focal necrosis** directly distal where the DAB opens into the aorta (s. figure 3). The **pulmonary trunk** showed a severe **dissecting aneurysm** from the exit of the heart up to the DAB (s. figure 4). The lumina of both vessels were consequently extremely compressed. In the wall of the **right pulmonary artery** a **focal necrosis** with beginning dissection was observed (s. figure 5). The **right carotid** was in the region of the first rib on a stretch of about 4 mm severely **dilated**. Consequently, the vessel wall extremely stretched and thin (s. figure 6).

Pup No. 2:

The **aorta** showed a severe **dissecting aneurysm** beginning in the region where the ductus arteriosus botalli (DAB) opens into the aorta throughout the descending aorta until the region of the kidneys. The **aortic wall** revealed an extensive **focal necrosis** in the region of the aortic arch (s. figure 7). The **pulmonary trunk** showed a severe **dissecting aneurysm** from the exit of the heart to the DAB. The lumina of both vessels are in consequence extremely compressed. The **right carotid** was in the region of the first rib on a stretch of ca. 2 mm severely **dilated**. Consequently, the vessel wall extremely stretched and thin. The left **subclavian artery** revealed a small **dissecting aneurysm** (on a stretch of ca. 1 mm) directly after leaving the aorta. The vessel lumen was severely compressed (s. figure 8).

3.2.2.2. Elastica van Giesson stain (E.v.G.)

The untreated animal of the same age revealed in the **aortic** wall and in the wall of the **pulmonary trunk** up to 12 layers and in the **carotid** wall up to 5 layers of elastic fibers which stained darkly brown to black and were arranged circularly and in parallel (s. figures 9 and 10).

Both treated pups revealed in the regions of the aneurysms in **aorta** and **pulmonary trunk** moderately thinner elastic fibers as compared to the control animal, with some discontinuity and slight fragmentation (s. figure 11). In the dilated region of the **carotid** wall of both treated pups, only fragmented and very thin elastic fibers were observed. In some areas, almost no elastic fibers were detectable (s. figure 12).

3.3. CONCLUSIONS

Cause of death in both treated pups was the dissecting aneurysm of aorta and pulmonary trunk. Furthermore, both animals revealed focal necroses of the aortic wall, and showed a focal dilation of the right carotid. It was morphologically not possible to define a cause of the aneurysms. The necroses as well as the fragmented elastic fibers could have both been cause or consequence. The fragmented elastic fibers of the carotids without aneurysm formation may indicate the pathologic pathway, but further investigations will be necessary, especially as the control animal could not be taken from the same study.

3.4. APPENDIX

3.4.1. Fotodocumentation



Fig. 1: white arrow = severely dilated pulmonary trunk; black arrow = dilated right carotid

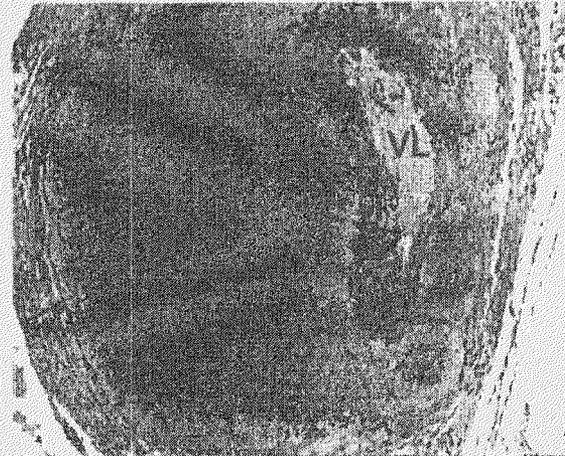


Fig. 2: descending aorta with dissecting aneurysm, VL = vessel lumen, H&E



Fig. 3: aorta: focal necrosis of vessel wall, H&E



Fig. 4: TP = pulmonary trunk with dissecting aneurysm, A = aorta with focal necrosis of vessel wall, H&E



Fig 5: right pulmonary artery with focal necrosis of vessel wall and dissecting aneurysm, H&E

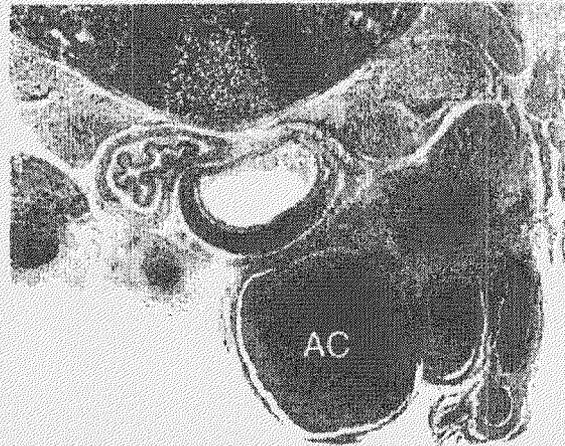


Fig. 6: AC = right carotid, severely dilated, H&E



Fig. 7: aortic arch with focally extensive necrosis of the vessel wall, H&E

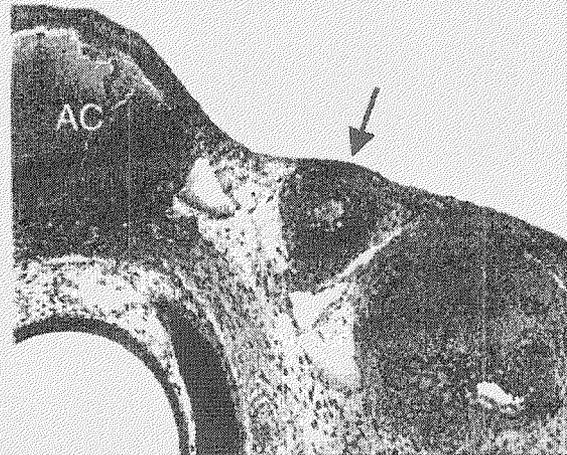


Fig. 8: AC = right carotid, dilated; AS = subclavian artery with dissecting aneurysm; arrow = "normal" appearing left carotid, H&E

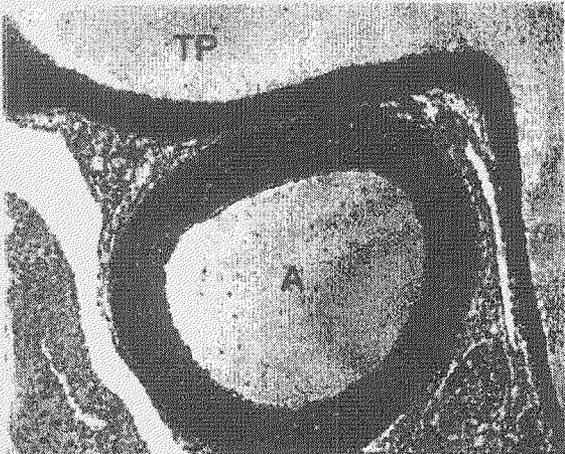


Fig. 9: A = aorta, TP = pulmonal trunk of untreated pup, E.v.G.

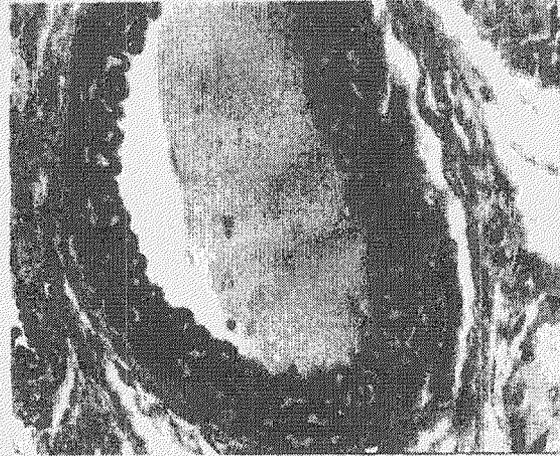


Fig. 10: carotid of untreated pup, E.v.G.

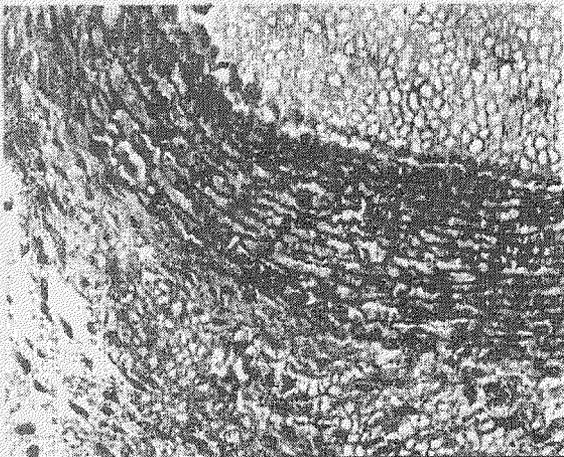


Fig. 11: aorta of pup No. 2, elastic fibers showing fragmentation and discontinuity, E.v.G.

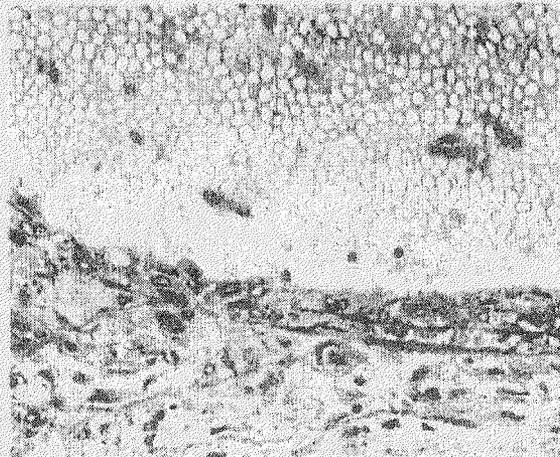


Fig. 12: right carotid of pup No. 2; only remnants of elastic fibers, E.v.G.

ETHYLENEAMINES PRODUCT STEWARDSHIP DISCUSSION GROUP

AEEA Testing Consortium

N-(2-Aminoethyl)ethanolamine (AEEA) CAS No. 111-41-1 Developmental Toxicity and Fertility Testing Status of studies: June 17, 2003-06-17

Summary

Developmental toxicity was observed with N-(2-Aminoethyl)ethanolamine after oral (gavage) treatment following the OECD 421 screening protocol. Effects were only observed in the aorta and associated major arteries such as carotids and pulmonary arteries. The effects consisted of dilated arteries which probably resulted in aneurysms and abnormal courses which was most noted in the carotid arteries. In a follow up developmental toxicity study (OECD 414) no such findings were obtained at a dose level which caused 50% malformations in 100% of the litters of the OECD 421 study. Limited histological investigations with respect to the macroscopic lesions from 2 pups (OECD 421 study) revealed dissecting aneurysms and/or focal necrosis in greater vessels such as aorta, pulmonary trunk, and arteria subclavia. Fragmented elastic fibers could be shown using special staining technique. These differences in findings observed indicated a need for further investigation to understand the marked differences observed in the OECD 421 and 414 studies and elucidate a possible mode of action and its relevance for man.

Introduction

N-(2-Aminoethyl)ethanolamine in the following text called AEEA is an ICCA^a compound sponsored by the EPSDG^b consortium and has been assigned to Japan as the sponsor country. AEEA is almost exclusively used as a chemical intermediate for the industrial production of lube oil additives, fuel additives, chelating agent, coatings and urethanes. AEEA is used in the production of cationic and amphoteric surfactants. Cationic surfactants are used to impart softness and reduce static build-up in the production of textiles from fiber. AEEA has also been used to make fabric softeners for use in machine washed laundry to improve the feel of washed clothing. Thus end users could only be exposed potentially to AEEA from its residues in chemicals made with the help of AEEA.

To fulfil its obligations under the ICCA Initiative, using information from a 28 day (gavage) study in rats (OECD 407), the consortium initiated an OECD 421 screening test in Wistar rats performed by BASF's department of toxicology. This was followed by an OECD 414 study. Appended is a flowchart comparing the exposure periods from the OECD 407, 421, and 414 studies.

ETHYLENEAMINES PRODUCT STEWARDSHIP DISCUSSION GROUP

AEEA Testing Consortium

Page 2

1) OECD 421 Screening Study in Rats Via Gavage (Proj. No. 90R0019/01075)

Material and methods: The study followed exactly the OECD guideline 421. AEEA was administered to groups of 10 male and 10 female healthy young adult Wistar rats (F0 parental generation) daily by oral gavage at doses of 50, 250 and 1,000 mg/kg body weight/day throughout the whole study period of this reproduction/developmental toxicity test (SIDS). The doses were selected from an OECD 407 study with 4 week oral gavage exposure at similar dose levels in another rat strain. The test substance was administered as an aqueous solution at a standard dose volume of 10 ml/kg body weight. The control groups were dosed with the vehicle only (doubly distilled water). Fourteen days after the beginning of treatment, F0 animals were mated to produce a litter. Mating pairs were from the same dose group. The F0 adult males were sacrificed about 32 days after the first test substance administration, the F1 pups were killed on day 4 post partum (p.p.) and the F0 females on one of the following days.

Daily observations were conducted on the parents and pups and parental animals were examined for their mating and reproductive performances; this included determinations of the number of implantations and the calculation of the postimplantation loss. Food consumption of the F0 parents was determined regularly during premating, after the mating period and - in dams - during gestation and lactation periods. In general, body weights of F0 parents were determined once weekly. However, during gestation and lactation F0 females were weighed on days 0, 7, 14 and 20 of gestation, on the day of parturition and on days 4 and 7 after birth.

The pups were sexed and were weighed on the day after birth and on day 4 post partum. Their viability was recorded. All pups were examined macroscopically for external and visceral findings at necropsy.

All F0 parental animals were assessed by gross pathology (including weight determinations of several organs). Histopathological examination in F0 parental animals was limited to all gross lesions, as well as ovaries, testes and epididymides of control and top dose animals.

Results: The following adverse findings were obtained and assessed as being test substance-related:

Test group 3 (1,000 mg/kg body weight/day)

F0 parental animals:

The following clinical signs were observed:

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- salivation after treatment in all males from study day 4 onwards as well as in all female rats from study week 0 onwards and through gestation, daily incidences ranged between 1 and 10 out of 10 animals;
- the regular care on fur appeared to be impaired in individual male and female rats (urine-smearred fur around its anogenital region) on several occasions during the study;
- prior to mating, male and female rats gained weight albeit at a much slower rate than controls. After mating, body weights of female rats continued to increase but since the number of implantations was much lower than controls and number of live pups was zero, at a much slower rate than controls. Thus there does not appear to be severe toxicity associated with oral administration of 1000 mg/kg/day. The difference in body weight was also associated with decreased feed consumption.

Effects on fertility and reproductive performance noted were:

- male and female fertility indices 60%, though mating index unaffected (100%);
- female gestation index 0%, none of the females had live pups; and
- less implantations per dam (3.2 vs 10.8 control), 100% post implantation loss.

No test substance-related effects in F0 males and F0 females with respect to organ weights and macroscopic pathology were obtained. No F1 pups were delivered (100% post implantation loss).

Test group 2 (250 mg/kg body weight/day)

F0 parental animals:

- no test substance-related effects in F0 males and F0 females were observed with respect to clinical findings, fertility, reproductive performance, organ weights and gross respectively histopathological findings.

F1 pups:

The following clinical signs were observed:

- slightly higher number of stillborn pups (5 against 1 in the control);
- more pups died or were cannibalized (18 against 1 in the control), lower viability index (80%);
- significantly higher incidence of macroscopic changes mostly related to the pericardial vessels (dilations and aneurysms as well as aberrant (abnormal course) vessels, e.g. aorta, carotids, pulmonary trunk); and

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- incidence of adverse pup necropsy observations (such as changes of pericardial vessels) was 89% pups in 100% of the litters, ratio of affected pups per litter was 87.8% (against 0.0% in the controls).

Test group 1 (50 mg/kg body weight/day)

F0 parental animals:

- no test substance-related effects in F0 males and F0 females were observed with respect to clinical findings, fertility, reproductive performance, organ weights and gross respectively histopathological findings.

F1 pups:

The following clinical signs were observed:

- significantly higher incidence of macroscopic changes mostly related to the pericardial vessels (dilations and aneurysms as well as aberrant (abnormal course) vessels, e.g. aorta, carotids, pulmonary trunk); and
- incidence of adverse pup necropsy observations (such as changes of pericardial vessels) was 48% pups in 100% of the litters, ratio of affected pups per litter was 48.4% (against 0.0% in the controls).

A typical macroscopic finding of cardiac vessel effects is shown in the appendix (fig. 1).

Conclusion: Thus, under the conditions of this reproduction/developmental toxicity screening test (SIDS) the NOAEL (no observed adverse effect level) for reproductive performance and fertility is 250 mg/kg body weight/day for the F0 parental rats. At the top dose level of 1,000 mg/kg body weight/day the presumed severe developmental toxicity (based on the pup findings at 50 and 250 mg/kg body weight/day) may be responsible for the “apparent infertility”. Histopathologic examination of reproductive organs of male and female rats (testes, epididymides and ovaries) in the 1,000 mg/kg body weight/day group did not identify any treatment-related effects.

The NOAEL for general, systemic toxicity of the test substance is 250 mg/kg body weight/day for the F0 parental rats of both genders.

No NOAEL for developmental toxicity in the F1 progeny could be determined under the conditions of this screening test. The most salient dose-dependent adverse findings were dilations and aneurysms as well as aberrant (abnormal course) pericardial vessels (aorta, carotids, pulmonary trunk).

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2) OECD 414 Developmental Toxicity Study in Rats (Project No.: 30R0019/01105)

Rational for testing: Based on the results of the OECD 421 screening study, an OECD 414 test was initiated in the same rat strain and the same laboratory to define the potential developmental toxicity of AEEA. The dose levels were derived from the OECD 421 results assuming that a NOAEL for these effects would be below the highest dose tested (50 mg/kg body weight) which caused malformation in 50% of the pups and 100% of the litters which would be regarded as a clear effect dose.

Methods: AEEA was tested for its prenatal developmental toxicity in Wistar rats. The test substance was administered as an aqueous solution to 25 time-mated female Wistar rats/group by stomach tube at doses of 0.5, 2, 10 and 50 mg/kg body weight on day 6 through day 19 post coitum (p.c.). A standard dose volume of 10 ml/kg body weight was used for each group. The control group, consisting of 25 females, was dosed with the vehicle only (doubly distilled water). Between 21 - 24 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 blood samples were taken for trace element analysis from 12 randomly selected females/group. Thereafter, 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. Subsequently, nearly one half of the fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal (incl. cartilage) findings.

Results: There were no substance-related adverse effects on the dams concerning food consumption, body weight, body weight change, uterine weights, corrected body weight change or clinical and necropsy observations up to and including a dose of 50 mg/kg body weight/day.

There were no differences of toxicological relevance between the control and the substance-treated groups (0.5, 2, 10 and 50 mg/kg body weight/day) in 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.

Furthermore, the determination of copper, magnesium, manganese and zinc in the serum of dams revealed no treatment effects in the animals which received the test compound.

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No substance-related differences were recorded for placental and fetal body weights. 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. In particular, no substance-induced effects on fetal cardio-vascular system occurred.

Thus, under the conditions of the full-scale toxicity study, the administration of AEEA to pregnant female Wistar rats elicited no signs of maternal toxicity, had no influence on gestational parameters and induced no signs of prenatal developmental toxicity up to and including the high dose of 50 mg/kg body weight/day; especially, no indications of teratogenic effects occurred, which could be causally related to the test substance administration.

Conclusion: Based on the results of this prenatal developmental toxicity study, the no observed adverse effect level (NOAEL) for maternal and prenatal developmental toxicity is 50 mg/kg body weight/day. There were no indications for teratogenicity up to and including the top dose of 50 mg/kg body weight/day. In particular, no substance-induced effects on fetal great vessels occurred, which were observed in the preceding reproduction/developmental toxicity screening test with AEEA at doses of 50 and 250 mg/kg/day (BASF, 2003).

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3) Limited Histopathology Examination OECD 421 Screening Study in Rats (Project No.: 90R0019/01075)

Based on the differences of the test results from both OECD 421 and 414 studies, a few pups which had been preserved and fixed from the OECD 421 study were examined histopathology.

Material and methods: A total of 4 pups which died between days 1 and 3 post partum were routinely assessed macroscopically and the findings were reported within the framework of the above mentioned Reproduction/Developmental Toxicity Screening Test. Afterwards they were preserved in toto in Harrison's fluid. Two of these pups (Nos. 1 and 2) were dedicated for further histopathological investigations.

As no control animals of this study were available, one rat of the same age (4 days) was sacrificed by CO₂ inhalation and fixed in Bouin's fluid. This additional histopathologic examination was not conducted in accordance with the principles of Good Laboratory Practice (GLP) however this does not impair the quality.

Paraffin embedding, sectioning and staining: after fixation, the proximal part of the heart (beginning in the region of the sulcus coronarius) with leaving vessels (aortic arch, descending aorta, pulmonary trunk with pulmonary arteries, ductus arteriosus botalli, carotid arteries and subclavian arteries) were embedded in paraffin, in pup No. 1 including the connective tissue, in pup No. 2 without the connective tissue. Of pup No. 1 and the control animal, cross sections through the entire thorax and neck (skin, skeletal muscle, spine, trachea, esophagus, parts of the lungs) were taken to avoid destruction of areas of interest. In pup No. 2, the heart and vessels were dissected and cross sections were taken without adjacent connective tissue. Thereafter, serial sections of all animals were taken every 50 µm and stained with Hematoxylin-Eosin (H&E). Every 100 µm, additional sections were taken from all animals and left unstained for further investigations. From all animals observed one to two of these unstained sections of aorta, pulmonary trunk, and carotid arteries respectively were stained with Elastica van Giesson for demonstration of elastic fibers.

Results: Macroscopically pups No. 1 and 2 revealed both a severe dilatation of the pulmonal trunk, starting directly after leaving the heart and extending to the ductus arteriosus botalli (DAB). In addition, the aorta was also severely dilated, starting at the aortic arch/DAB throughout the descending aorta until the region of the kidneys. Finally in both animals, a focal dilatation of about 0.4 cm in diameter of the right carotid artery was observed (s. figure 1).

Histopathological examinations (Hematoxylin-Eosin staining) of pup No. 1 revealed that the aorta showed a severe dissecting aneurysm, beginning in the region where the DAB opens into the aorta throughout the descending aorta until the region of the kidneys (s.

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figure 2). The aortic wall revealed a focal necrosis directly distal where the DAB opens into the aorta (s. figure 3). The pulmonary trunk showed a severe dissecting aneurysm from the exit of the heart up to the DAB (s. figure 4). The lumina of both vessels were consequently extremely compressed. In the wall of the right pulmonary artery a focal necrosis with beginning dissection was observed (s. figure 5).

The right carotid was in the region of the first rib on a stretch of about 4 mm severely dilated. Consequently, the vessel wall extremely stretched and thin (s. figure 6).

In pup No. 2, the aorta showed a severe dissecting aneurysm beginning in the region where the DAB opens into the aorta throughout the descending aorta until the region of the kidneys. The aortic wall revealed an extensive focal necrosis in the region of the aortic arch (s. figure 7). The pulmonary trunk showed a severe dissecting aneurysm from the exit of the heart to the DAB. The lumina of both vessels are in consequence extremely compressed.

The right carotid was in the region of the first rib on a stretch of ca. 2 mm severely dilated. Consequently, the vessel wall extremely stretched and thin.

The left subclavian artery revealed a small dissecting aneurysm (on a stretch of ca. 1 mm) directly after leaving the aorta. The vessel lumen was severely compressed (s. figure 8).

The following results have been obtained with Elastica van Giesson stain (E.v.G.): the untreated animal of the same age revealed in the aortic wall and in the wall of the pulmonary trunk up to 12 layers and in the carotid wall up to 5 layers of elastic fibers which stained darkly brown to black and were arranged circularly and in parallel (s. figures 9 and 10).

Both treated pups revealed in the regions of the aneurysms in aorta and pulmonary trunk moderately thinner elastic fibers as compared to the control animal, with some discontinuity and slight fragmentation (s. figure 11). In the dilated region of the carotid wall of both treated pups, only fragmented and very thin elastic fibers were observed. In some areas, almost no elastic fibers were detectable (s. figure 12).

Conclusion: The cause of death in both treated pups was the dissecting aneurysm of aorta and pulmonary trunk. Furthermore, both animals revealed focal necroses of the aortic wall, and showed a focal dilation of the right carotid. It was not possible morphologically to define a cause of the aneurysms. The necroses as well as the fragmented elastic fibers could have both been cause or consequence. The fragmented elastic fibers of the carotids without aneurysm formation may indicate the pathologic pathway, but further investigations will be necessary, especially as the control animal could not be taken from the same study.

APPENDIX: Table 1

Comparison of treatment OECD 407 / 421 / 414 study in rats
 (start of treatment, treatment period, examination with respect to vessels)

OECD 407 – 28-day study CRJ-CD rat with 14 day recovery of satellite animals

Untreated period (birth to first treatment) Appr. > 10 weeks (no study info - guideline says young healthy animals)	Treatment period: 28 d	Treatment time: 28 d
Examination of vessels: macroscopic inspection, no indication of gross lesions		

OECD 421 reproduction toxicity screening study in Wistar rats

Untreated period (birth to first treatment) Appr. 11-12 weeks	Prenating 14 d	Mating up to 14 d	Gestation 21 d	Lactation 4 d	Treatment time: 65 d (parents) 25d (progeny) Examination of vessels: parents and pups by macroscopic inspection (only pups affected)
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OECD 414 developmental toxicity study in Wistar rats

Untreated period (birth to mating) Appr. 10-15 weeks	G1 6 d	G2 14 d	Treatment time: 14 d (dams/fetuses)
Examination of vessels: dams and fetuses by macroscopic inspection, no indication of gross lesions			

Agenda

Green field: periods **without** treatment

Red field: periods **with** treatment

G1 = gestation period **without** treatment (day 0-5)

G2 = gestation period **with** treatment (day 6-19)

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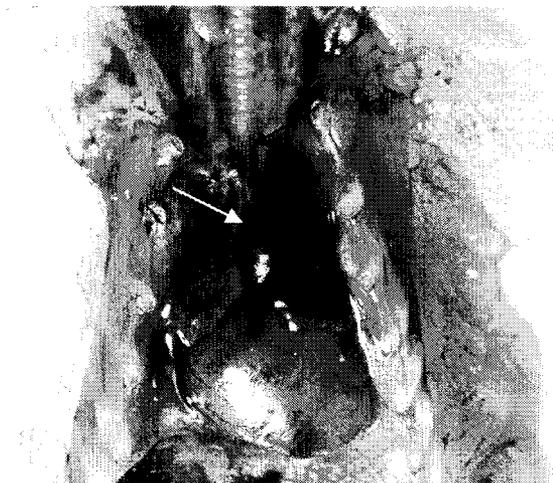


Fig. 1: white arrow = severely dilated pulmonary trunk; black arrow = dilated right carotid



Fig. 2: descending aorta with dissecting aneurysm, VL = vessel lumen, H&E



Fig. 3: aorta: focal necrosis of vessel wall, H&E

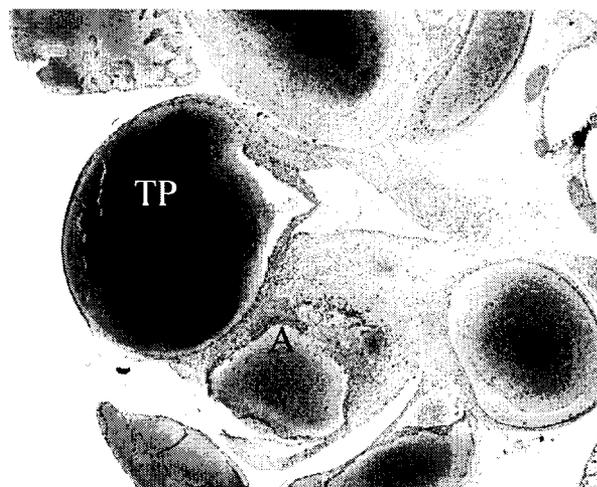


Fig. 4: TP = pulmonary trunk with dissecting aneurysm, A = aorta with focal necrosis of vessel wall, H&E

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Fig 5: right pulmonary artery with focal necrosis of vessel wall and dissecting aneurysm, H&E

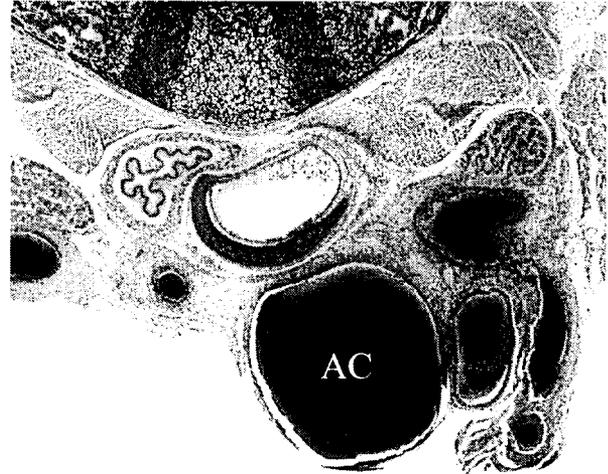


Fig. 6: AC = right carotid, severely dilated, H&E

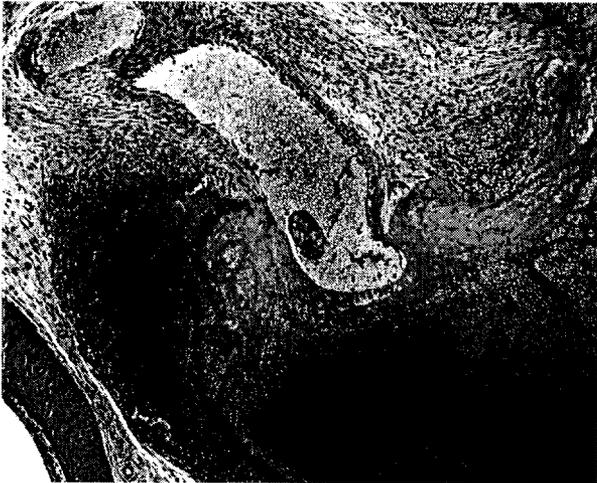


Fig. 7: aortic arch with focally extensive necrosis of the vessel wall, H&E

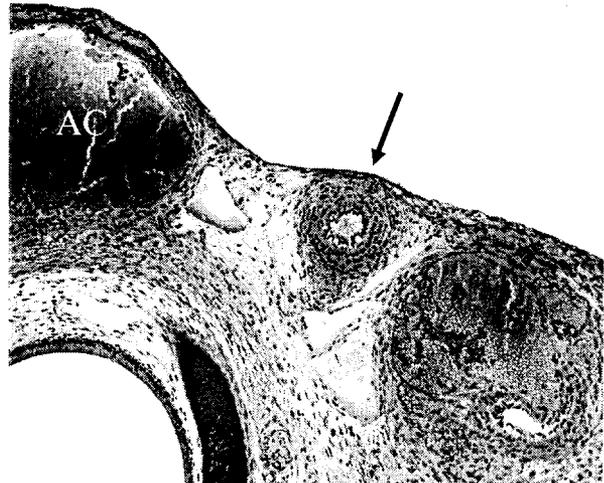


Fig. 8: AC = right carotid, dilated; AS = subclavian artery with dissecting aneurysm; arrow = "normal" appearing left carotid, H&E.

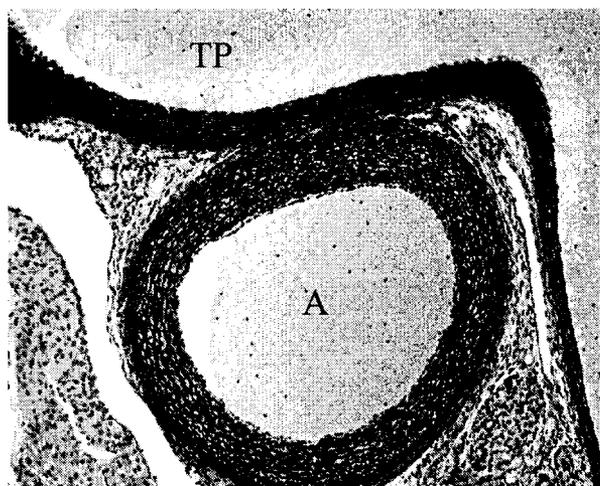


Fig. 9: A = aorta, TP = pulmonal trunk of untreated pup, E.v.G.



Fig. 10: carotid of untreated pup, E.v.G.

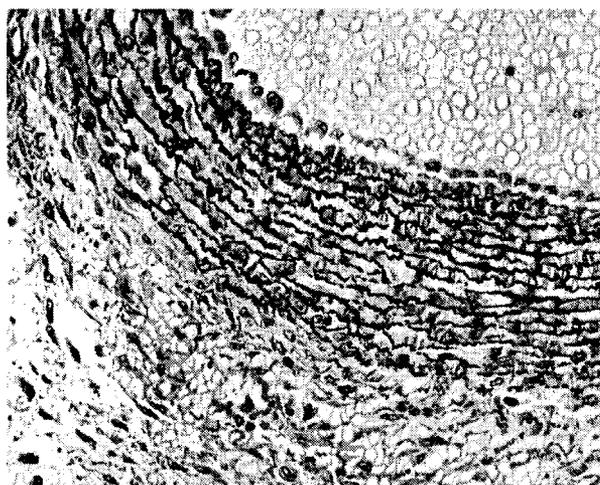


Fig. 11: aorta of pup No. 2, elastic fibers showing fragmentation and discontinuity, E.v.G.

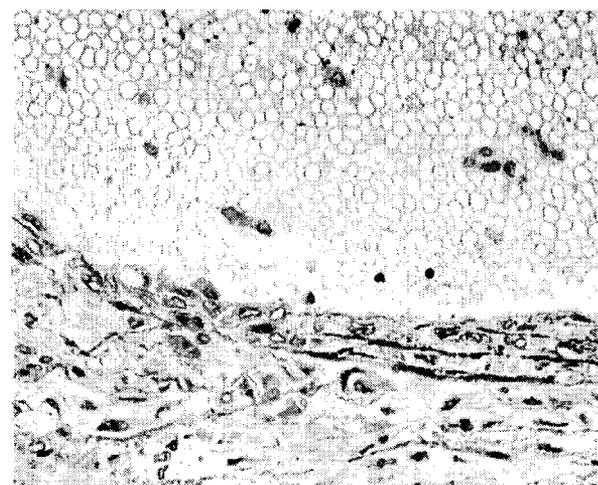


Fig. 12: right carotid of pup No. 2; only remnants of elastic fibers, E.v.G.

STUDY TITLE

Report

**N-(2-Aminoethyl)ethanolamine – Reproduction/Developmental Toxicity
Screening Test (SIDS) in Wistar Rats
Oral Administration (Gavage)**

DATA REQUIREMENT

OECD Guideline No. 421
OPPTS 870.3550

AUTHORS

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STUDY COMPLETED ON

May 26, 2003

PERFORMING LABORATORY

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LABORATORY PROJECT IDENTIFICATION

90R0019/01075

SPONSOR

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VOLUME I OF III
(REPORT SECTION AND SUMMARY TABLES)

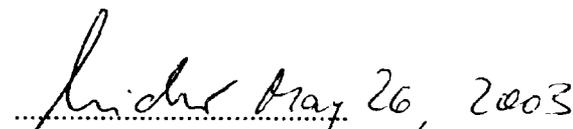
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GLP COMPLIANCE STATEMENT

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


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

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SIGNATURE PAGE

Study Director:

Schneider May 26 2003
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Dr. med. vet. S. Schneider

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.....
Dr. med. vet. Chr. Gembardt

Management:

B. van Ravenzwaay 23. May, 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:	04-02-2002	04-02-2002
Conduct of study:	04-15-2002	04-15-2002
	05-24-2002	05-24-2002
	06-03-2002	06-03-2002
Report:	02-06-2003	02-06-2003
	05-19-2003	05-19-2003

Ludwigshafen,

May 23, 2003

Hajok

Rheinland-Pfalz



**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:

Assesment 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.

Schiering 24. Sept. 2001

Unterschrift, Datum / Signature, Date
(Name und Funktion der verantwortlichen Person /
Name and function of responsible person)



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

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**Part A:
CLINICAL EXAMINATIONS AND EXAMINATION OF REPRODUCTIVE
PERFORMANCE
(MEAN VALUES AND SUMMARY TABLES)**

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The tables with individual values/findings (excluding tables for "pup body weight change") are to be found in VOLUME II. Further information (detailed analytical results and historical control data) is included in VOLUME III (Supplement).

* none of the F1 pups/litter showed any abnormal clinical observation

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* none of the F1 pups/litter showed any abnormal clinical observation

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THIS REPORT CONSISTS OF VOLUMES I, II AND III.

1. SUMMARY

1.1. METHODS

N-(2-Aminoethyl)ethanolamine was administered to groups of 10 male and 10 female healthy young adult rats (F0 parental generation) daily by oral gavage at doses of 50, 250 and 1,000 mg/kg body weight/day throughout the whole study period of this reproduction/developmental toxicity test (SIDS). The test substance was administered as an aqueous solution at a standard dose volume of 10 ml/kg body weight. The control groups were dosed with the vehicle only (doubly distilled water). Fourteen days after the beginning of treatment, F0 animals were mated to produce a litter. Mating pairs were from the same dose group. The F0 adult males were sacrificed about 32 days after the first test substance administration, the F1 pups were killed on day 4 post partum (p.p.) and the F0 females on one of the following days.

Daily observations were done on the parents and pups and parental animals were examined for their mating and reproductive performances; this included determinations of the number of implantations and the calculation of the postimplantation loss.

Food consumption of the F0 parents was determined regularly during pre-mating, after the mating period and - in dams - during gestation and lactation periods.

In general, body weights of F0 parents were determined once weekly. However, during gestation and lactation F0 females were weighed on days 0, 7, 14 and 20 of gestation, on the day of parturition and on days 4 and 7 after birth.

The pups were sexed and were weighed on the day after birth and on day 4 post partum. Their viability was recorded. All pups were examined macroscopically for external and visceral findings at necropsy.

All F0 parental animals were assessed by gross pathology (including weight determinations of several organs). Histopathological examination in F0 parental animals was limited to all gross lesions, as well as ovaries, testes and epididymides of control and top dose animals.

1.2. RESULTS

The following adverse findings were obtained and assessed as being test substance-related:

Test group 3 (1,000 mg/kg body weight/day)**F0 parental animals****CLINICAL EXAMINATIONS**

- salivation after treatment in all males from study day 4 onwards as well as in all female rats from study week 0 onwards and through gestation, daily incidences ranged between 1 and 10 out of 10 animals
- the regular care on fur appeared to be impaired in individual male and female rats (urine-smearred fur around its anogenital region) on several occasions during the study
- statistically significantly reduced (by approx. 21%) food consumption of F0 male animals during first study week, 9% lower than the corresponding control if calculated for the whole study period (weeks 0-4), food consumption of females about 12% lower during pre-mating period (weeks 0-2) and about 10% lower during gestation days 0-20 p.c.
- at study week 4 body weight of males about 6% lower, if calculated for the entire study period (weeks 0-4) about 30% less body weight gain than controls, females gained 72% less weight during gestation, on gestation day 20 mean body weight about 24% lower than the control value

FERTILITY/ REPRODUCTIVE PERFORMANCE

- male and female fertility indices 60%, though mating index unaffected (100%)
- female gestation index 0%, none of the females had live pups
- less implantations per dam (3.2 vs 10.8 control), 100% post implantation loss

ORGAN WEIGHTS/ GROSS AND HISTOPATHOLOGICAL FINDINGS

- no test substance-related effects in F0 males and F0 females

F1 pups

- no live pups delivered

Test group 2 (250 mg/kg body weight/day)**F0 parental animals****CLINICAL EXAMINATIONS/ FERTILITY/ REPRODUCTIVE PERFORMANCE/ ORGAN WEIGHTS/ GROSS AND HISTOPATHOLOGICAL FINDINGS**

- no test substance-related effects in F0 males and F0 females

F1 pups

- slightly higher number of stillborn pups (5 against 1 in the control)
- more pups died or were cannibalized (18 against 1 in the control), lower viability index (80%)
- significantly higher incidence of macroscopic changes mostly related to the pericardial vessels (dilations and aneurysms as well as aberrant (abnormal course) vessels, e.g. aorta, carotids, pulmonary trunk)
- incidence of adverse pup necropsy observations (such as changes of pericardial vessels) was 89% pups in 100% of the litters, ratio of affected pups per litter was 87.8% (against 0.0% in the controls)

Test group 1 (50 mg/kg body weight/day)**F0 parental animals****CLINICAL EXAMINATIONS/ FERTILITY/ REPRODUCTIVE PERFORMANCE/ ORGAN WEIGHTS/ GROSS AND HISTOPATHOLOGICAL FINDINGS**

- no test substance-related effects in F0 males and F0 females

F1 pups

- significantly higher incidence of macroscopic changes mostly related to the pericardial vessels (dilations and aneurysms as well as aberrant (abnormal course) vessels, e.g. aorta, carotids, pulmonary trunk)
- incidence of adverse pup necropsy observations (such as changes of pericardial vessels) was 48% pups in 100% of the litters, ratio of affected pups per litter was 48.4% (against 0.0% in the controls)

1.3. CONCLUSION

Thus, under the conditions of this reproduction/developmental toxicity screening test (SIDS) the **NOAEL** (no observed adverse effect level) for **reproductive performance and fertility** is **250 mg/kg body weight/day** for the **F0 parental rats**. At the top dose level of 1,000 mg/kg body weight/day the presumed severe developmental toxicity (based on the pup findings at 50 and 250 mg/kg body weight/day) may be responsible for the "apparent infertility". Histopathologic examination of reproductive organs of male and female rats in the 1,000 mg/kg body weight/day group did not identify any treatment-related effects.

The **NOAEL** for **general, systemic toxicity** of the test substance is **250 mg/kg body weight/day** for the **F0 parental rats of both genders**.

No **NOAEL** for **developmental toxicity** in the **F1 progeny** could be determined under the conditions of this screening test. The most salient dose-dependent adverse findings were dilations and aneurysms as well as aberrant (abnormal course) pericardial vessels (aorta, carotids, pulmonary trunk), some typical photographs are appended (Supplement IIC-001). In order to clarify pathogenesis, to assess potential consequences of these malformations for further life of the offspring, and to define a **NOAEL** for these findings additional examinations/studies are warranted.

2. INTRODUCTION AND DOSE SELECTION

2.1. AIM OF THE STUDY

It was the aim of this reproduction/developmental toxicity screening test, to provide initial information on possible effects of **N-(2-Aminoethyl)ethanolamine** on male and female reproductive performance such as gonadal function, mating behavior, conception, development of the conceptus and parturition.

2.2. SELECTION OF DOSES/CONCENTRATIONS

The dose levels (see table) for the present study with **N-(2-Aminoethyl)ethanolamine** were chosen on the basis of the outcome of a Japanese 4-week oral toxicity study. In this repeated dose study slight signs of systemic toxicity were observed at 1000 mg/kg body weight/day (i.e. slightly changed hematology and clinical chemistry parameters, histopathological findings in kidneys and stomach) and similar, even less severe findings were seen at a dose level of 250 mg/kg body weight/day. A NOAEL of 60 mg/kg body weight/ was determined. Thus, the following dose levels (see table) were found to be suitable for purpose of the present reproduction/ developmental toxicity screening test with **N-(2-Aminoethyl)ethanolamine**:

50 mg/kg body weight/day:	as the expected no observed adverse effect level
250 mg/kg body weight/day:	as the intermediate dose level and eventually as higher no observed adverse effect level
1,000 mg/kg body weight/day:	as the dose level where toxic effects were expected

The oral route was selected since this has been proven to be suitable for the detection of a toxicological hazard.

2.3. TEST GUIDELINES

The study was carried out in accordance with or exceeding the requirements of the following test guidelines:

- OECD Guideline for Testing of Chemicals; Method No. 421 (SIDS): Reproduction/Developmental Toxicity Screening Test, July 1995
- EPA, Health Effects Test Guidelines; OPPTS 870.3550: Reproduction/Developmental Toxicity Screening Test, July 2000

3. MATERIAL AND METHODS**3.1. TEST SUBSTANCE**

Name of test substance: N-(2-Aminoethyl)ethanolamine

Test substance No.: 01/0019-2

CAS No.: 111-41-1

Batch No.: Continuous production

Date of production: September 19, 2001

Supplier: BASF Aktiengesellschaft, Antwerpen

Physical state/color: Liquid/yellowish-clear

Purity: 99.8 area% (analytical report 01L00492)

Homogeneity: Homogeneous (analytical report 01L00492)

Stability: The stability under storage conditions was confirmed by reanalysis

Storage conditions: Room temperature

Analytical laboratory: Analytical Department, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany

3.2. TEST ANIMALS

3.2.1. Species and strain

Male and female Wistar rats (CrIGlxBrHan:WI) supplied by Charles River Laboratories, Germany, which were free from any clinical signs of disease, were used for the investigations. The females were nulliparous and non-pregnant at the beginning of the study. According to a written statement from the breeder, male and female animals were derived from different litters. This was necessary to rule out the possibility of sibling mating.

3.2.2. Animal identification

The rats of the parental generation (F0 generation) were uniquely identified by ear tattoo. The unit digit of the animal number was tattooed on the outside of a rat's left ear, the ten digit on the inside of the left ear and the hundred digit was tattooed on the inside of the right ear.

All live pups were identified by skin tattoo on day 1 post partum (p.p.).

3.2.3. Reason for species selection

This strain was selected since extensive historical control data were available on Wistar rats and the rat is the preferred animal species for reproduction studies according to the various test guidelines.

3.3. HOUSING AND DIET

During the study period, the rats were housed individually in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, GERMANY (floor area of about 800 cm²), with the following exceptions: for the overnight mating the females were put into the cages of the males; from day 18 p.c. until sacrifice, the pregnant animals and their litters were housed in Makrolon type M III cages (floor area about 800 cm²). The M III cages were also supplied by BECKER & CO. Pregnant females were provided with nesting material (cellulose wadding) toward the end of pregnancy.

The cages with the test animals were arranged on the racks in such a way that uniform experimental conditions (ventilation and light) were ensured.

The animals were accommodated in fully air-conditioned rooms (floor area about 22 m²) 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 during the entire study.

The light cycle rhythm was 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 use, each room was completely disinfected using a disinfectant ("AUTEX" fully automatic, formalin-ammonia-based terminal disinfectant). 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 laboratory diet rat/mouse/hamster, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland, which was available to the animals ad libitum throughout the study (from the day of supply to the day before necropsy). Drinking water was supplied from water bottles (ad libitum).

The bedding used throughout the study was SSNIFF (type 3/4) supplied by SSNIFF SPEZIALDIÄTEN GmbH, Soest, GERMANY.

3.4. TEST GROUPS AND DOSES

F0 generation parental animals

Test group	Dose (mg/kg body weight/day)	Concentration (mg/100ml)	Dose volume (ml/kg body weight/day)	Number of animals		Animal No.	
				Male	Female	Male	Female
0	0	0	10 ¹⁾	10	10	1-10	101-110
1	50	500	10 ²⁾	10	10	11-20	111-120
2	250	2,500	10 ²⁾	10	10	21-30	121-130
3	1,000	10,000	10 ²⁾	10	10	31-40	131-140

1) doubly distilled water

2) test substance solutions in doubly distilled water

3.5. TEST SUBSTANCE PREPARATION AND ANALYSES

3.5.1. Test substance preparations and preparation frequency

For the preparation of the solutions the test substance was weighed in a graduated measuring flask depending on the dose group, topped up with doubly distilled water and subsequently thoroughly mixed using a magnetic stirrer.

The test substance solutions were prepared at the beginning of the administration period and thereafter at 10 day intervals.

3.5.2. Analyses

All analyses of the test substance preparations were carried out as separate studies at the Analytical Department, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany, under the responsibility of the study director of this test facility. The studies were carried out in compliance with the Principles of Good Laboratory Practice.

Analytical verifications of the stability of the test substance in "Frankenthaler Leitungswasser" for a period of 10 days at room temperature were carried out before the study was initiated.

As the preparations were true solutions, no homogeneity analyses were performed.

Samples of the aqueous test substance solutions were sent to the analytical laboratory twice during the study period for verification of the concentrations.

3.5.3. Analytical methods

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

3.5.4. Food analyses

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

3.5.5. Drinking water analyses

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

3.5.6. Bedding analyses

The bedding is regularly assayed for contaminants (chlorinated hydrocarbons and heavy metals).

3.6. EXPERIMENTAL PROCEDURE

3.6.1. F0 generation parental animals and their progeny

The 45 male and 45 female rats were supplied at an age of 70 - 77 days. During an acclimatization period of 6 days the animals with the lowest and highest body weights were sorted out and used for other purposes. The 40 male and 40 female animals used in the study were 76 - 83 days old at the beginning of treatment, and their mean weights and weight ranges were:

- male animals: 287.8 (268.5 – 307.5) g
- female animals: 191.6 (179.4 – 206.7) g

The assignment of the animals to the test groups was carried out using a randomization program (NIJENHUIS, A. and WILF, H.S.; 1978), according to their weight before the beginning of the administration period.

The test substance was administered to the parental animals once daily by oral gavage at approximately the same time of day (in the morning). The treatment lasted up to one day prior to sacrifice. The animals of the control group were treated in the same way with the vehicle only (doubly distilled water). The volume administered each day was 10 ml/kg body weight. The calculation of the volume administered was based on the most recent individual body weight.

At least 14 days after the beginning of treatment, males and females from the same dose group were mated at a ratio of 1 : 1 (for details of pairing see 3.6.2.).

Approx. 32 days after the beginning of the administration period, the male animals were sacrificed.

The females were allowed to litter and rear their pups until day 4 after parturition (p.p.).

Thereafter, the pups and the F0 generation female parental animals were sacrificed.

3.6.2. Pairing of F0 generation parental animals

In general, each male and female animal was paired overnight at a 1 : 1 ratio for a maximum of 2 weeks. Each male animal was paired with a preselected female animal from the same dose group throughout the whole pairing period (generally male No. 1 with female No. 101, male No. 2 with female No. 102 etc.).

Pairing occurred by placing the female in the cage of the male pairing partner from about 4.00 p.m. until 7.00 - 9.00 a.m. of the following morning. Deviations from the specified times were possible on weekends and public holidays and were reported in the raw data.

A vaginal smear was prepared after each mating and examined for sperm. If sperm was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted "day 0" and the following day "day 1" p.c. (post coitum).

3.6.3. Determination of implantation sites

After sacrifice of the female animals in the pathology laboratory, the uteri and ovaries were removed (including the uteri of apparently non-pregnant animals) and transferred to the reproduction laboratory, for further investigation. To determine the number of implantation sites, the uteri were stained in 10% ammonium sulfide solution for about 5 minutes according to the method of SALEWSKI (Salewski, E.; 1964). Then, the uteri were rinsed carefully with fresh tap water. The implantation sites were recorded for calculation of the postimplantation loss.

After these examinations, the uteri were transferred back to the pathology laboratory for further investigations.

3.6.4. Pups on day 4 p.p.

All surviving pups were sacrificed (by means of CO₂) on day 4 p.p.

These pups, all stillborn pups and those that died before schedule, were examined externally, eviscerated and their organs were assessed macroscopically. If there were notable findings or if abnormalities were found in the daily clinical observation of the animals after their delivery, the affected animals were, if it deemed necessary, examined additionally using appropriate methods (e.g., skeletal staining according to a modified method of KIMMEL and TRAMMELL (Kimmel, C.A. and Trammell C., 1981) or pups' fixation in Harrison's Fluid for examination for any visceral findings according to the method of BARROW and TAYLOR (Barrow and Taylor, 1969)).

The stained skeleton was evaluated under a stereomicroscope or a magnifying glass.

All pups were discarded after their evaluation except of the stained skeleton.

3.6.5. Time schedule

In the following table, the relevant intervals for certain study phases are given:

Table 3.6.5.1.: Time schedule

Phase of study/examination	F0 generation parental animals and progeny
Arrival of the animals / Experimental starting date	April 9, 2002
Acclimatization period	April 9 – April 14, 2002
Administration period	April 15 – May 16, 2002 (male animals) April 15 – June 2, 2002 (female animals)
Mating period for litter	April 28 – May 11, 2002
Gestation period	April 29 – May 23, 2002
Birth of litter	May 20 – May 24, 2002
Lactation period (including period after sacrifice of the litters)	May 20 – May 28, 2002
Sacrifice of litter (on day 4 p.p.)	May 24 – May 28, 2002
Sacrifice of parental animals*	May 17, 2002 (male animals) June 3, 2002 (female animals)

Experimental completion date: January 27, 2003

* before necropsy, food was withdrawn for about 16 hours

3.7. CLINICAL EXAMINATIONS AND EXAMINATION OF REPRODUCTIVE PERFORMANCE

3.7.1. Parental animals

3.7.1.1. Mortality

A check was made twice daily on working days or once daily on Saturdays, Sundays and public holidays. If animals were in a moribund state, they were sacrificed and necropsied. The terminal examinations of the animals which died or had to be sacrificed before schedule were carried out in the laboratory of pathology.

3.7.1.2. Clinical observations

All parental animals were checked daily for clinically evident signs of toxicity.

The **nesting, littering, and lactation behavior** of the dams was generally evaluated in the mornings in connection with the daily clinical inspection of the dams. Only special findings (e.g., animal could not litter, umbilical cord not cut) were documented on an individual dam basis.

The **littering behavior** of the dams was also inspected on weekdays (except public holidays) in the afternoons in addition to the evaluations in the mornings.

The day of littering was considered the 24-hour period from about 3.00 p.m. of one day until about 3.00 p.m. of the following day. Deviations from this procedure were possible on Saturdays, Sundays and on public holidays.

3.7.1.3. Food consumption

Generally, food consumption was determined once a week (in general for a period of 7 days) for male and female animals.

The following exceptions were notable:

- Food consumption was **not** determined during the pairing period (e.g., between week 2 and 3 for F0 parental males).
- Food consumption of the **females with evidence of sperm** was determined for days 0-7, 7-14 and 14-20 p.c.
- Food consumption of **females, which gave birth to a litter** was determined for days 0-4 and 4-7 p.p.

3.7.1.4. Body weight data

In general, the body weight of the **male and female parental animals** was determined once a week at the same time of the day (in the morning).

The body weight change of the animals was calculated from these results.

The following exceptions are notable for the **female animals**:

- During the mating period the parental females were weighed on the day of positive evidence of sperm (day 0 p.c.) and on days 7, 14 and 20 post coitum.
- Females **without positive evidence of sperm** were weighed weekly. These body weight data were solely used for the calculations of the dose volume; therefore these values are not reported in the Summary Tables (Appendix), but can be found in the tables with the individual body weight data (Volume II).
- Females **with litter** were weighed on the day of parturition (day 0 p.p.) and on days 4 and 7 post partum.
- Females **without litter** were weighed weekly. These body weight data were solely used for the calculations of the dose volume; therefore these values are not reported in the Summary or Individual Tables (Appendix/Volume II).

3.7.1.5. Male reproduction data

The pairing partners, the number of mating days until vaginal sperm could be detected in the female animals, and the gestational status of the female were noted for F0 breeding pairs.

For the **males**, mating and fertility indices were calculated according to the following formulas:

$$\text{Male mating index (\%)} = \frac{\text{number of males with confirmed mating}^*}{\text{number of males placed with females}} \times 100$$

* defined by a female with vaginal sperm or that gave birth to a litter or with pups/implantations in utero

$$\text{Male fertility index (\%)} = \frac{\text{number of males proving their fertility}^*}{\text{number of males placed with females}} \times 100$$

* defined by a female giving birth to a litter or with pups/implantations in utero

3.7.1.6. Female reproduction and delivery data

The pairing partners, the number of mating days until vaginal sperm could be detected, and gestational status were recorded.

For the **females**, mating, fertility and gestation indices were calculated according to the following formulas:

$$\text{Female mating index (\%)} = \frac{\text{number of females mated}^*}{\text{number of females placed with males}} \times 100$$

- * defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/implantations in utero

$$\text{Female fertility index (\%)} = \frac{\text{number of females pregnant}^*}{\text{number of females mated}^{**}} \times 100$$

- * defined as the number of females that gave birth to a litter or with pups/implantations in utero
- ** defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/implantations in utero

$$\text{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant}^*} \times 100$$

- * defined as the number of females that gave birth to a litter or with pups/implantations in utero

The total number of pups delivered and the number of liveborn and stillborn pups were noted and the live birth index was calculated according to the following formulas:

$$\text{Live birth index (\%)} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$$

Moreover, after sacrifice of the female animals, the implantation sites were counted and the postimplantation loss was calculated for each individual pregnant animal according to the following formula:

$$\text{Post implantation loss (\%)} = \frac{\text{number of implantations} - \text{number of pups delivered}}{\text{number of implantations}} \times 100$$

3.7.2. Litters/pups

3.7.2.1. Litter data

3.7.2.1.1. Pup number and status at delivery

All pups derived from the F0 parents were examined as soon as possible on the day of birth to determine the total number of pups and the number of liveborn and stillborn members of each litter. Pups, which died before the first determination of their status on the day of birth, were designated as stillborn pups.

3.7.2.1.2. Pup viability/mortality

In general, a check was made for any dead or moribund pups twice daily on workdays (once in the morning and once in the afternoon) or as a rule, only in the morning on Saturdays, Sundays or public holidays. Dead pups were evaluated by the methods which will be described in detail in section 3.7.2.4.

The number and percentage of dead pups on the day of birth (day 0) and of pups dying between days 1-4 of the lactation period were determined; however, pups which died accidentally and pups which were sacrificed due to maternal death were not included in these calculations. The number of live pups/litter was calculated on the day of birth, and on lactation day 4. Furthermore the viability index was calculated according to the following formula:

$$\text{Viability index (\%)} = \frac{\text{number of live pups on day 4 after birth}}{\text{number of liveborn pups on the day of birth}} \times 100$$

3.7.2.1.3. Sex ratio

On the day of birth (day 0) the sex of the pups was determined by observing the distance between the anus and the base of the genital tubercle; normally, the anogenital distance is considerably greater in male than in female pups. The sex of the pups finally confirmed at necropsy.

The sex ratio was calculated at day 0 and day 4 after birth according to the following formula:

$$\text{Sex ratio} = \frac{\text{number of live male or female pups on day 0/4}}{\text{number of live male and female pups on day 0/4}} \times 100$$

3.7.2.2. Pup body weight data

The pups were weighed on the day after birth (day 1 p.p.) and on day 4 after birth.

Pups' body weight change was calculated from these results.

Furthermore the body weights on day 1 p.p. were used for the calculation of "runts" (pups, which weighed more than 25% less than the mean weight of the respective control pups).

The individual weights were always determined at about the same time of the day (in the morning).

In the relevant summary tables pup body weights (including "runts") and pup body weight gains are listed for males, females and males + females.

3.7.2.3. Pup clinical observations

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

3.7.2.4. Pup necropsy observations

All surviving pups (after sacrifice on day 4 p.p. by means of CO₂), all stillborn pups and those pups that died before schedule, were examined externally, eviscerated and their organs were assessed macroscopically.

If there were notable findings or if abnormalities were found in the daily clinical observation of the pups after their delivery or at necropsy, the affected pups were, if it was deemed necessary, additionally examined for skeletal findings according to a modified method of KIMMEL and TRAMMELL (Kimmel, C.A. and Trammell C., 1981) and/or for any visceral findings fixed in Harrison's Fluid and examined according to the method of BARROW and TAYLOR (Barrow and Taylor, 1969).

The stained skeleton was evaluated under a stereomicroscope or a magnifying glass.

All pups were discarded after their evaluation except of the stained skeleton.

3.7.3. Statistics of the clinical examinations

Statistical analyses were performed according to following tables:

Parameter	Statistical test	Markers in the tables	References
Food consumption* (parental animals), body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), number of mating days, duration of gestation, number of pups delivered per litter	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
Male and female mating Index, male and female fertility index, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, number of litters with affected pups at necropsy	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions	* for $p \leq 0.05$ ** for $p \leq 0.01$	Siegel S. (1956): Non-parametric statistics for behavioural sciences. McGraw-Hill New York
Proportions of affected pups per litter with necropsy observations	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

* Note: 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 the different time intervals (pre mating, gestation and/or lactation); they are not exactly precise values, because the size of the intervals taken for calculation may differ (especially during gestation and lactation periods). For the "mean of means" values no statistical analysis was performed.

3.8. PATHOLOGY

3.8.1. Necropsy

The animals were sacrificed by decapitation under CO₂ anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Animals that died intercurrently were necropsied and assessed grossly as soon as possible after death to avoid post mortem autolysis.

3.8.2. Organ weights

The following weight parameters of all parental animals sacrificed at scheduled dates were determined:

1. anesthetized animals
2. testes
3. epididymides
4. ovaries

3.8.3. Histopathology

The following organs or tissues were fixed in 4% formaldehyde solution (Fo) or in BOUIN's solution (B), respectively:

1. vagina (Fo)
2. cervix uteri (Fo)
3. uterus (Fo)
4. ovaries (B)
5. oviducts (Fo)
6. testes (B)
7. epididymides (B)
8. seminal vesicles (Fo)
9. coagulating glands (Fo)
10. prostate gland (Fo)
11. pituitary gland (Fo)
12. all gross lesions (Fo)

Testes, epididymides and/or ovaries of animals that died intercurrently were fixed in 4% formaldehyde solution (Fo).

After fixation, testes, epididymides and both ovaries of all animals were embedded in paraplast.

After fixation, processing, the examination by light microscopy and the evaluation was performed according to the following tables:

organs F0 generation parental animals	test groups			
	0	1	2	3
all gross lesions	A2	A2	A2	A2
ovaries	A1			A1
testes	A1			A1
epididymides	A1			A1

Methods and scope of examination:

- A = hematoxylin and eosin stain
- 1 = all animals per group
- 2 = all animals affected per group

An attempt was made to correlate the gross lesions with a meaningful microscopic finding.

Differential Ovarian Follicle Count (DOFC):

After appropriate fixation, both ovaries of each animal were embedded in the same way one upon another in one paraplast block. As an association with right or left ovary was not possible at that time due to technical reasons, the ovaries were indicated as "Ovary 1" and "Ovary 2" in the tables. "Ovary 1" refers to the lower ovary on the slide when the label of it showed to the right side under the microscope, and "ovary 2" indicates the upper ovary on the same slide, respectively. Hematoxylin and eosin (H.E.) stained serial sections of about 3 microns thickness were prepared, with a distance of about 50 microns of dismissed ovarian tissue between each consecutive slide.

The first cut was taken, when both ovaries showed a reasonable amount of tissue, i. e. both organs revealed parts of cortex and medulla. This slide was labeled "No. 1". The last slide was taken, when the medulla faded away in one or both organs at gross appearance. Depending on the size of the ovaries, 10 to 13 serial sections were obtained, which were numbered from No. 1 to No. 10 or No. 13, respectively on the slide label. Evaluation was performed on 10 slides, both showing cortex and medulla. The inner part of the medulla (located in the mid part of the slide series - comprising a distance of about 200 microns and consisting of about three to four slides) was excluded from the assessment where possible (from more than 10 slides onwards). Depending on the number of serial sections obtained at the microtome, the 10 slides selected for assessment were chosen as follows:

No. of serial ovarian sections per animal	Slide Nos. assessed dorsal of the ovarian core	Slide Nos. assessed ventral of the ovarian core
10	1-5	6-10
11	1-5	7-11
12	1-5	8-12
13	1-5	9-13
14	2-6	10-14
15	2-6	10-14
16	3-7	11-15
17	3-7	11-15
18	4-8	12-16
19	4-8	13-17
20	4-8	13-17

On each of the ten selected slides, follicle count was performed on "primordial follicles" (comprising Types 1, 2, 3a, and 3b), "growing follicles" (comprising Types 4, 5a, and 5b), "primordial plus growing follicles" (Types 1 to 5b combined) and antral follicles according to the definitions given by Plowchalk et al. (PLOWCHALK, D. R., B. J. SMITH, and D. R. MATTISON: Assessment of Toxicity to the Ovary Using Follicle Quantitation and Morphometrics. In: Methods in Toxicology, Vol. 3, Part B: Female Reproductive Toxicology (J. J. HEINDEL and R. E. CHAPIN, Editors), p. 57-68, 1993, Academic Press). In addition, on the 5th slide of every series, the number of corpora lutea (CL) was determined.

To prevent multiple counting especially of the growing follicles and the antral follicles, only follicles which had an oocyte with visible chromatin on the slide were counted. In general, on the 5th slide, the other microscopic findings associated with the ovaries were assessed. However, if a specific finding was noted in any of the other slide levels investigated that could not be detected on the 5th slide, this finding was also recorded. Using EXCEL tables for the reporting of the results, the incidence of each type of follicle was recorded individually for ovary 1 and ovary 2 of every animal on any of the slide levels, giving in summary the incidence of each type of the follicles counted in ten levels. Finally, the results of all types of follicles were summarized for all 10 animals per group in dose groups 0 and 3 (F0 generation female parental animals). The results for the number of corpora lutea was obtained accordingly.

As primordial follicles continuously develop into growing follicles, the assessment of the follicles was extended to the combined incidence of primordial plus growing follicles. The Differential Ovarian Follicle Count (DOFC) was performed in the F0 generation parental females of the control group (group 0) and of the high dose group (group 3). The DOFC was also performed on all F0 generation parental females of groups 1 and 2 that were indicated "animal not pregnant".

3.8.3.1. Statistics of pathology

Means and standard deviations of each test group were calculated for the variables of terminal body weight and of absolute and relative organ weights (related to terminal body weight) of the animals in each test group. Further statistical analyses were performed according to following tables:

Parameters	Statistical test	Markers in the table	References
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANSPERGER, T. P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-142. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3. MILLER, R. G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167. NIJENHUIS, A. and S. W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33.
Follicles: primordial, growing, primordial + growing, antral and corpora lutea	Pairwise comparison of the high dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians.	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for behavioural sciences. Mc.Graw-Hill, New York.

3.9. 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 OF THE TEST SUBSTANCE PREPARATIONS

4.1.1. Stability analyses

The stability of N-(2-Aminoethyl)ethanolamin in tap water "Frankenthaler Leitungswasser" for a period up to 10 days at room temperature was demonstrated (for details see Volume III; Analytical Report IIIA-002 - IIIA-005).

4.1.2. Homogeneity analyses

Due to the fact, that the test substance preparations were true solutions, it appeared not necessary, to prove the homogeneous distribution of the test substance in the vehicle analytically.

4.1.3. Concentration control analyses

The concentration control analyses of the aqueous test substance solutions revealed that the values were in the expected range of the target concentrations (Mean values: 98.0% - 104.0%). Thus, the correctness of the prepared concentrations was confirmed (for details see Volume III; Analytical Report IIIA-006 - IIIA-013).

4.1.4. Food analyses¹

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 09, 1979, p. 27354 (EPA), served as a guideline for maximum tolerable chemical contaminants. The number of microorganisms did not exceed $5 \cdot 10^5$ /g feed.

4.1.5. Drinking water analyses¹

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

4.1.6. Bedding analyses¹

On the basis of the findings of analysis the bedding was found to be suitable. Levels given in Lab Animal, Nov. - Dec. 1979, pp. 24 - 33, served as a guideline for maximum tolerable contaminants.

¹ Individual results are to be found at the archives of the Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany.

4.2. CLINICAL EXAMINATIONS AND EXAMINATION OF REPRODUCTIVE PERFORMANCE

Summary Tables of the results are given in the Appendix of Volume I, Part A; individual values and findings are given in Part A of Volume II (with the exception of tables concerning "pup body weight gain").

4.2.1. F0 generation parental animals

4.2.1.1. Mortality

(Summary of mortalities: Tabs. IA-001 – IA-007)

There were no substance-related mortalities in any of the male and female F0 parental animals in any of the groups.

One control female (No. 105) was unable to deliver and finally found dead on gestation day 24. Pathological examinations of this rat revealed no findings (see 4.3. Pathology), so this death is considered to be spontaneous in nature.

4.2.1.2. Clinical observations

(Summary of findings: Tabs. IA-001 – IA-007)

4.2.1.2.1. Clinical observations for males and females (except gestation and lactation periods)

(Tabs. IA-001 – IA-004)

Salivation after treatment was noted in all high dose males (test group 3 – 1,000 mg/kg body weight/day) from study day 4 onwards, in one mid dose male (test group 2 – 250 mg/kg body weight/day) between days 11 to 14 as well as in all high dose female rats from study week 0 onwards. In the high dose group daily incidences ranged between 4 and 10 out of 10 animals.

In individual male and female rats of test group 3 (Nos. 32, 131, 132 and 136 – 1,000 mg/kg body weight/day) the regular fur hygiene appeared to be impaired (urine-smearred fur around its anogenital region) on several occasions during the study.

Furthermore, dark yellow discolored urine was recorded in all substance-treated male and female F0 parental animals of test groups 1-3 (50, 250 and 1,000 mg/kg body weight/day) during the whole treatment period. This is most likely due to excreted test compound or its metabolites.

4.2.1.2.2. Clinical observations for females during gestation

(Tabs. IA-005 – IA-006)

With the exception of discolored urine in all substance-treated females (test groups 1, 2, and 3 – 50, 250 and 1,000 mg/kg body weight/day) and salivation after treatment in all high dose females (test group 3 – 1,000 mg/kg body weight/day), there were no particular substance-related clinical findings in F0 females during the gestation period for F1 litter.

In test group 3 in five females urine-smear on fur was noted during the whole gestation period for F1 litter. Furthermore, female No. 105 (control) was not able to deliver and, as mentioned before, was found dead on gestation day 24 p.c.

After pairing, one mid dose F0 female (No. 127 – 250 mg/kg body weight/day) had no sperm in vaginal smear (no day 0 p.c.). Furthermore, one sperm positive female of the control group (No. 107), one female of test group 1 (No. 112, 50 mg/kg body weight/day) and all females of test group 3 (1,000 mg/kg body weight/day) did not deliver any F1 pups (for discussion of female fertility see 4.2.1.6).

4.2.1.2.3. Clinical observations for females during lactation

(Tab. IA-007)

Discolored urine was still noticed in all substance-treated dams of test groups 1-2 (50 and 250 mg/kg body weight/day).

One female of the low dose group (No. 115) did not nurse her F1 pups properly on day 3 p.p.; some of the affected pups died/were cannibalized on day 4 p.p. This is considered to be spontaneous in nature due to the isolated occurrence.

4.2.1.3. Food consumption

(Mean values: Tabs. IA-008 – IA-011; Figs. 4.2.1.3.1. – 4.2.1.3.3.)

The food consumption of the high dose F0 male animals was statistically significantly reduced (by approx. 21%) during the first study week but recovered afterwards. Food consumption of high dose females was also below control during pre-mating and gestation being statistically significant during pre-mating weeks 0-2 and gestation days 14-20. No food consumption data of test group 3 (1,000 mg/kg body weight/day) were recorded during lactation, because none of the high dose females delivered F1 pups.

If calculated for the whole study period of the males (weeks 0-4), the food consumption of the high dose group was about 9% lower than the corresponding control value. Furthermore, the food consumption of the high dose females was about 12% lower during pre-mating period (weeks 0-2) and about 10% lower during gestation days 0-20 p.c.

No changes of food consumption were noted at low (50 mg/kg body weight/day) and mid (250 mg/kg body weight/day) dose level.

Fig. 4.2.1.3.1.: Mean food consumption of F0 males

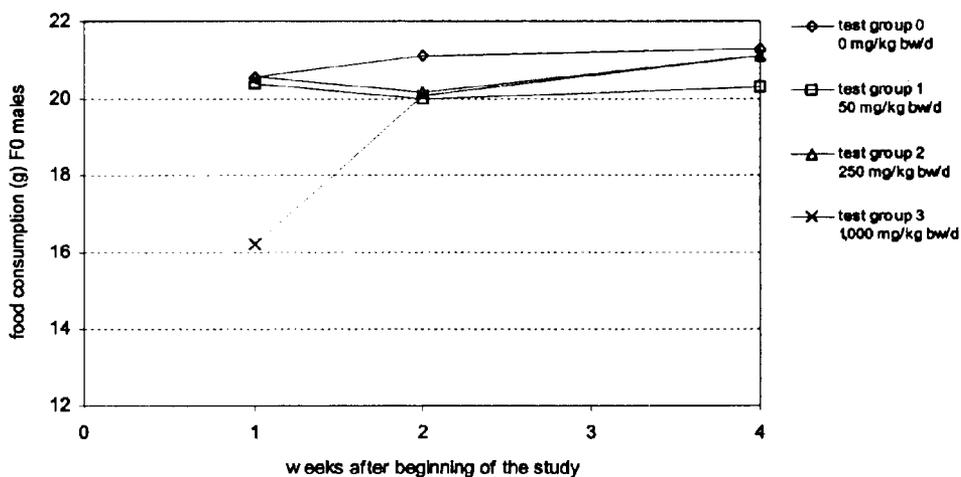


Fig. 4.2.1.3.2.: Mean food consumption of F0 females during pre-mating

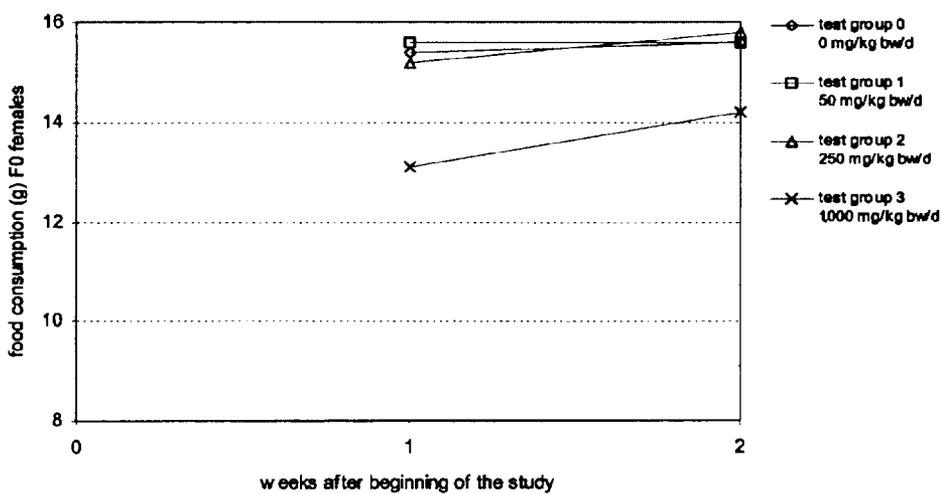
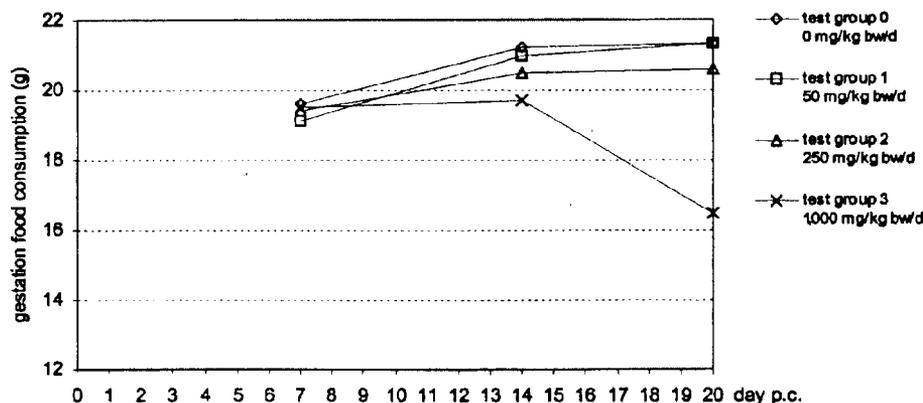


Fig. 4.2.1.3.3.: Mean food consumption of F0 females during gestation



4.2.1.4. Body weight data

(Mean values: Tabs. IA-012 – IA-019; Figs. 4.2.1.4.1 – 4.2.1.4.4.)

Mean body weights (BW) and mean body weight gains (BWC) of the high dose F0 males (1,000 mg/kg body weight/day) were slightly lower during pre-mating period, being statistically significant only during study week 1 (BW) and weeks 0-1 (BWC). At study week 4 the body weight of the high dose males was about 6% lower than in the controls. The body weight gain for the high dose animals for weeks 0-4 was 30% less than for the control group.

Body weights/body weight gains of the F0 parental males of the 50 and 250 mg/kg groups were not impaired by the test substance.

Mean body weights and mean body weight gains of the F0 females were generally comparable to that of the control group during pre-mating, but from gestation day 7 onwards, the mean body weights of the high dose females were markedly lower compared to concurrent control (they lost weight between days 14-20). If calculated for the entire gestation period (days 0-20), the high dose animals gained statistically significantly 72% less weight and on gestation day 20 the mean body weight of these females was about 24% lower than the concurrent control value reaching also statistical significance.

No data concerning body weights or body weight changes of animals in test group 3 (1,000 mg/kg body weight/day) could be collected during lactation, because none of the high dose females delivered any F1 pups.

Body weights/body weight gains of F0 parental females in test groups 1 and 2 (50 and 250 mg/kg body weight/day) were not influenced by the test substance administration neither during pre-mating nor during gestation and lactation. All differences in body weights and body weight gains observed for these rats were without any biological relevance.

Fig. 4.2.1.4.1.: Mean body weight of F0 males during the study

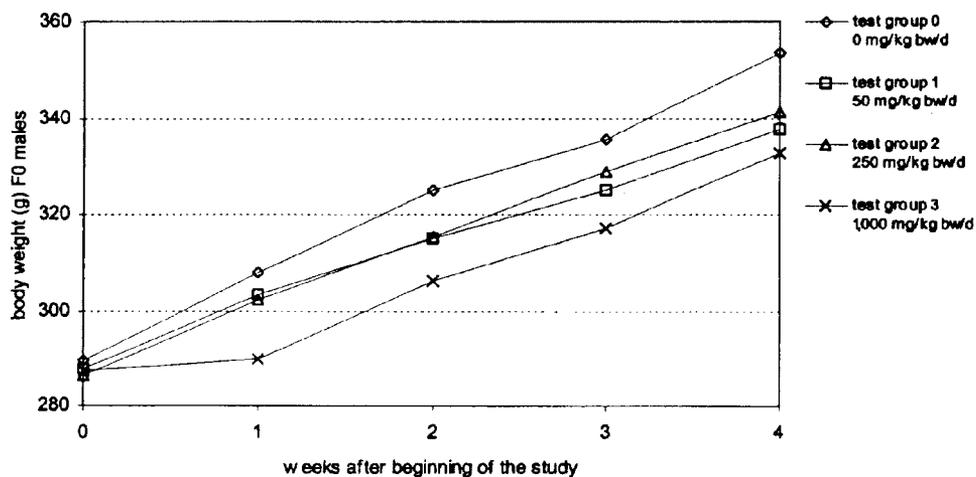


Fig. 4.2.1.4.2.: Mean body weight of F0 females during pre-mating

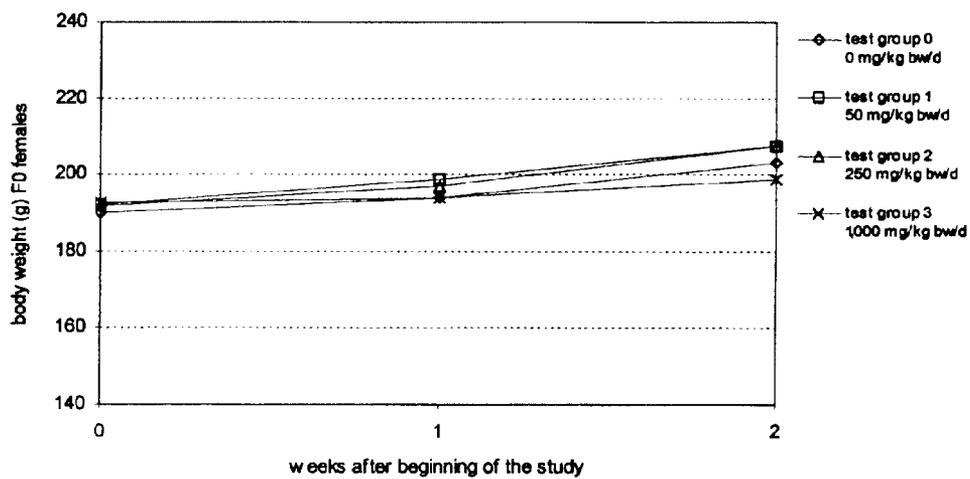


Fig. 4.2.1.4.3.: Mean body weight of F0 females during gestation

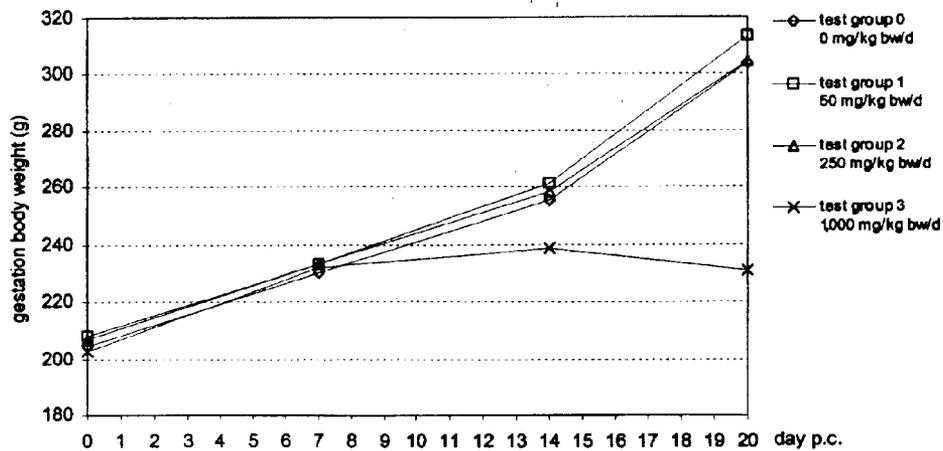
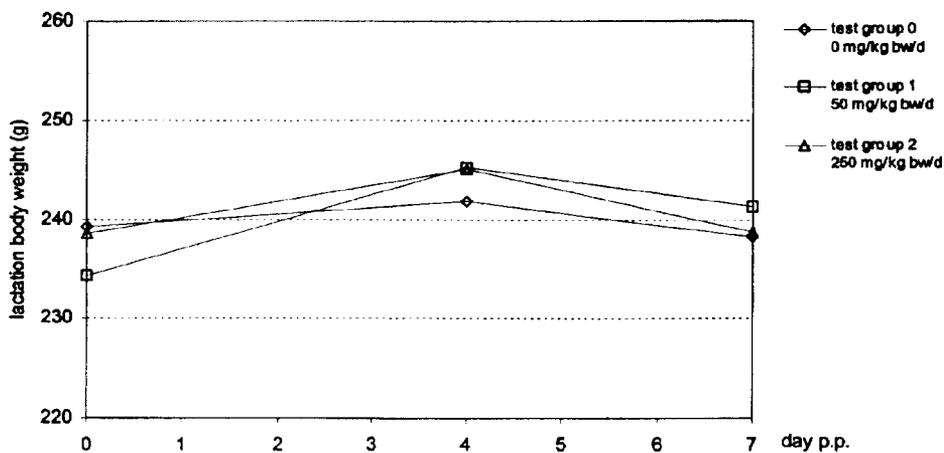


Fig. 4.2.1.4.4.: Mean body weight of F0 females during lactation



4.2.1.5. Male cohabitation data
(Mean values: Tab. IA-020)

For nearly all F0 parental males of test groups 0-3 (0, 50, 250 and 1,000 mg/kg body weight/day) which were placed with females to generate F1 pups, mating was confirmed within the scheduled mating interval, except for mid dose male No. 27. Thus, the **male mating index** was 90% in test group 2 (250 mg/kg body weight/day) and 100% for all remaining groups (0, 50 and 1,000 mg/kg body weight/day)

The **male fertility index** was 90% in groups 0, 1 and 2 and 60% in group 3 (see Fig. 4.2.1.5.1.). Female mating partners of one control male (male No. 7 mated with female No. 107), one male of test group 1 (male No. 12 mated with female No. 112 – 50 mg/kg body weight/day), one mid dose male (male No. 27 mated with female No. 127 – 250 mg/kg body weight/day) and four high dose males (males Nos. 32, 38, 39 and 40 mated with females Nos. 132, 138, 139 and 140 – 1,000 mg/kg body weight/day) had neither pups nor implantation sites.

Except for low dose male No. 12 and mid dose male No. 27, where diffuse tubular atrophy and aspermia was found, histopathological examination of testes/epididymides revealed no correlate for the non-pregnancies of female mating partners.

Fig. 4.2.1.5.1.: Fertility indices for F0 parental males (in %)

Test group 0 (0 mg/kg body weight/day)	Test group 1 (50 mg/kg body weight/day)	Test group 2 (250 mg/kg body weight/day)	Test group 3 (1,000 mg/kg body weight/day)
90	90	90	60

4.2.1.6. Female reproduction and delivery data
(Mean values: Tabs. IA-021 – IA-022)

The **female mating index** calculated after the mating was 90% in test group 2 (250 mg/kg body weight/day) and 100% for the remaining groups (0, 50 and 1,000 mg/kg body weight/day).

The mean cohabitation time (duration until sperm was detected (i.e. day 0 p.c.)) amounted to 1.8 days/2.9 days/2.1 days/2.9 days (0, 50, 250 and 1,000 mg/kg body weight/day). These values reflect the normal range of biological variation inherent in the strain used in this study. Consequently, the differences between the groups are assessed as spontaneous in nature and without any biological relevance.

One control F0 parental female (No. 107), one low dose female (No. 112 – 50 mg/kg body weight/day), one mid dose female (No. 127 – 250 mg/kg body weight/day) and four high dose females (Nos. 132, 138, 139 and 140 – 1,000 mg/kg body weight/day) had neither pups nor implantation sites. Therefore, the **fertility indices** ranged between 60% and 100% (see Fig. 4.2.1.6.1.).

The mean duration of gestation was very similar in test groups 0-2 (between 21.8 and 22.0 days).

The **gestation index** was 89% in the control group and 100% in test groups 1 and 2 (50 and 250 mg/kg body weight/day).

None of the 10 successfully paired high dose females (1,000 mg/kg body weight/day) delivered live F1 pups though 6 of them had implantation sites. However, the mean number of implantation sites per dam was statistically significantly decreased in this test group (3.2 implantations against 10.8 in the control group, see Tab. IA-021). Furthermore, postimplantation loss was 100% in this dose group, indicating total intrauterine embryo-/fetoletality.

The mean post implantation loss values amounted to 4.7/5.5/6.4/100.0% (test groups 0-3; 0, 50, 250, 1,000 mg/kg body weight/day).

Except the high dose group, the mean number of F1 pups delivered/dam was not affected by the test substance administered. The number of liveborn and stillborn pups was comparable between the control and test groups 1 and 2, and the **live birth index** varied between 95% and 99%.

Fig. 4.2.1.6.1.: Fertility indices for F0 parental females (in %)

Test group 0 (0 mg/kg body weight/day)	Test group 1 (50 mg/kg body weight/day)	Test group 2 (250 mg/kg body weight/day)	Test group 3 (1,000 mg/kg body weight/day)
90	90	100	60

4.2.2. F1 generation pups/litters

Since none of the high dose females (1,000 mg/kg body weight/day) had pups no reference is made to this dose group in this section of the report.

4.2.2.1. Litter data

(Mean values: Tabs. IA-023 – IA-025)

4.2.2.1.1. Pup number and status at delivery

Though slightly more pups were stillborn by mid dosed dams (5 pups from 3 litters against 1 pup from 1 litter in the control) the mean number of delivered F1 pups/dam and the percentage of liveborn pups were not affected by the administration of the test substance up to the mid dose level. The differences observed are without any biological relevance and do not show a clear dose-response relationship (10.3, 11.9 and 10.3 pups/dam in test groups 0, 1 and 2).

4.2.2.1.2. Pup viability/mortality

More pups died or were cannibalized in the mid dose litters than control. That is, 9 died and 9 were cannibalized in the mid dose group (250 mg/kg body weight/day), compared to 1 dead and none cannibalized in the control group. Consequently, the viability index as indicator for pup mortality between days 0-4 p.p. was significantly lower in test group 2 (80%) than in the control and test group 1 (i.e. 99% and 96% at 0 and 50 mg/kg body weight/day, respectively).

4.2.2.1.3. Sex ratio

The sex distribution and sex ratios of live F1 pups on the day of birth and on day 4 p.p. did not show any substantial differences between controls and test groups 1 and 2; all observable differences are regarded to be spontaneous in nature.

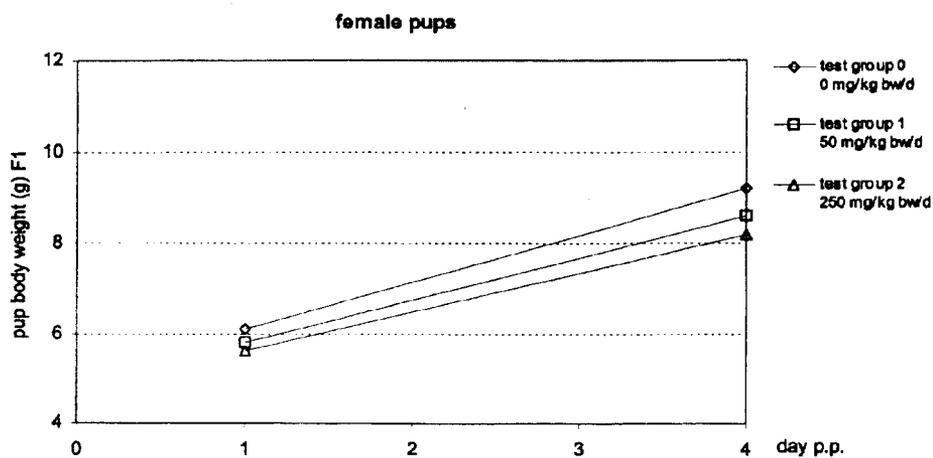
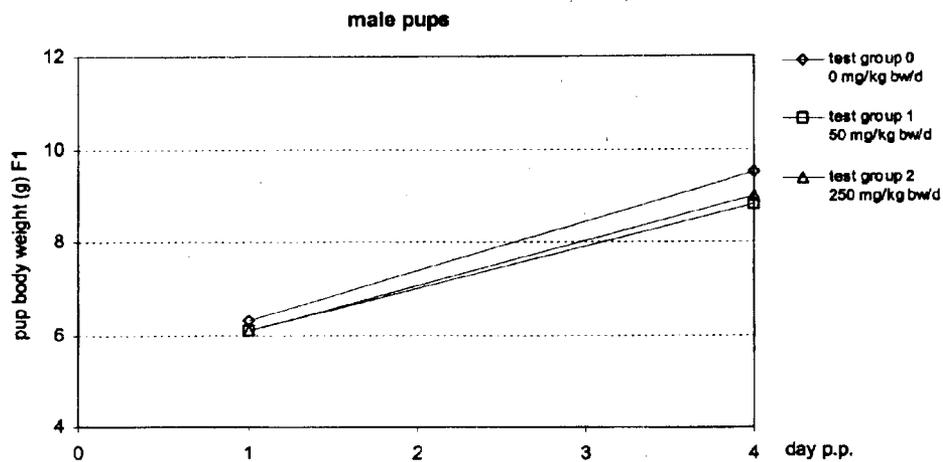
4.2.2.2. Pup body weight data

(Mean values: Tabs. IA-026 – IA-027; Fig. 4.2.2.2.1.)

Mean pup body weights/pup body weight gains did not show any statistically significant differences between groups 1 and 2 (50 and 250 mg/kg body weight/day) and the concurrent control group. The observable fluctuations between the groups are without any biological relevance and are related to the differences in litter size.

Furthermore, the number of "runts" (i.e. stunted pups) did not show differences between the groups for which a substance-induced origin could be assumed.

Fig. 4.2.2.2.1.: Mean body weight of F1 pups



4.2.2.3. Pup clinical observations
(Summary of findings: Tab. IA-007)

None of the F1 pups showed any clinical signs.

4.2.2.4. Pup necropsy observations
(Summary of findings: Tabs. IA-028 – IA-033)

The macroscopic examination of all pups at necropsy revealed a number of findings concerning the pericardial vessels, which are considered to be substance-induced:

Occurrence of findings of the pericardial vessels[#]:
(expressed as mean percentage of affected pups/litter)

	Test group 1 50 mg/kg body weight/day	Test group 2 250 mg/kg body weight/day
Aneurysm of aorta	17.7**	55.2**
Aneurysm of pulmonary trunk	16.3**	44.5**
Aneurysm of carotid	0.0	5.0
Aneurysm of ductus arteriosus	1.0	1.0
Dilated carotid	18.8**	35.8**
Dilated descending aorta	21.1**	55.2**
Dilated pulmonary trunk	4.6	0.0
Abnormal course of carotids	10.3	21.9**
High aortic arch	0.0	2.8
Pericardium filled with blood	0.0	1.1

The incidence of all these findings was 0 in the control group

* for $p \leq 0.05$ ** for $p \leq 0.01$

Additionally, a few of the examined F1 pups showed some spontaneous findings at necropsy (e.g. post mortem autolysis, situs inversus, dilated renal pelvis, hemorrhagic testis, agnathia and astomia) scattered throughout the substance-treated test groups. These findings occurred without a clear relation to dosing and/or most of it can be found in the historical control data at comparable or even higher incidences.

The head of one F1 pup (No. 10 from dam No. 113, test group 1, 50 mg/kg body weight/day) was fixed in ethylalcohol and further investigated for skeletal findings according to a modified method of KIMMEL and TRAMMELL (Kimmel, C.A. and Trammell C., 1981). The finding agnathia (indicated by severely malformed skull bones, i.e. small mandible, absent hyoid, misshapen basisphenoid, fused auditory ossicles) was confirmed.

The cardiopulmonary parts of some F1 pups, that were stillborn or died before schedule, (No. 7 from dam No. 128, No. 5 from dam No. 130, Nos. 7 and 12 from dam No. 121 and No. 10 from dam No. 124) and the remaining body of pup No. 10 from dam No. 113 were fixed in Harrison's Fluid and further examined according to the method of BARROW and TAYLOR (Barrow and Taylor, 1969). The findings were related to the pericardial vessels similar to the term pups. They were included in the indices reported above.

4.3. PATHOLOGY

Throughout the sections of the pathology report, when intergroup differences were referred to as "significant" it implied that the differences had attained statistical significance ($p \leq 0.05$) when compared with the respective control groups.

4.3.1. Weight parameters

(Tables IB 1 - IB 4)

4.3.2. Absolute weights

Absolute weights of the epididymides and ovaries from the high dose group F0 parental animals were significantly (or = p-level of significance ≤ 0.05 or ≤ 0.01 , respectively) reduced by 14.0%** and 16.3%* respectively compared to the control group. No other mean absolute weight parameters showed any significant differences to control.

4.3.3. Relative organ weights (related to terminal body weight)

The mean relative weight parameters of the parental animals of the F0 generation did not show significant differences when compared with control group.

4.3.4. Gross lesions

(Tables IB 5)

In the following section, four numbers separated by slashes give the incidence of the described gross lesion in animals of the control group, low, mid and high dose groups, respectively.

Gross lesions of the animals killed at termination were recorded from the **epididymides** (*organ size reduced or abscess*), **testes** (*organ size reduced*) and **uterus** (*inflammation*). All gross lesions occurred only once per group, with no indication of a relationship to treatment.

One female rat of the control group (animal No. 105) died intercurrently 39 days after start of treatment. The animal was pregnant and did not show any further gross lesion. As a control animal, a relationship of the premature death to treatment was excluded although the cause of death was not obvious.

Seven animals (1/1/1/4) were not pregnant:

No. 107 (control group): no gross lesion noted;
No. 112 (low dose group): no gross lesion noted;
No. 127 (mid dose group): no gross lesion noted;
No. 132 (high dose group): no gross lesion noted;
No. 138 (high dose group): no gross lesion noted;
No. 139 (high dose group): no gross lesion noted;
No. 140 (high dose group): no gross lesion noted.

Their male mating partners were:

No. 7 (control group): no gross lesion noted;
No. 12 (low dose group): gross lesions noted were moderately reduced organ sizes of testes and epididymides;
No. 27 (mid dose group): gross lesions noted were severely reduced organ sizes of testes and epididymides;
No. 32 (high dose group): no gross lesion noted;
No. 38 (high dose group): no gross lesion noted;
No. 39 (high dose group): no gross lesion noted;
No. 40 (high dose group): no gross lesion noted.

The gross lesion noted in the testes and epididymides of male rats No. 12 (low dose group) and 27 (mid dose group) were most likely responsible for the animals' status "impaired fertility?". As no such gross lesions were noted in the high dose animals, their relationship to treatment was, however, unsupported.

4.3.5. Histopathology (Tables IB 6)

In the following section, four numbers separated by slashes give the incidence of the described microscopic findings in animals of the control, low, mid and high dose groups, respectively.

The few gross lesions noted were all correlated with a meaningful histopathologic finding.

Histopathology failed to correlate the significantly decreased mean absolute weights of epididymides (high dose males) and ovaries (high dose females) with a meaningful microscopic finding.

In the reproductive organs of the female rats, no treatment-related microscopic findings were noted. The following incidental findings were recorded, microscopically:

Moderate *inflammation* in the **uterus** was diagnosed in a mid dose female (animal No. 129). A relationship of this finding to treatment was regarded unlikely, as it was noted in only one animal, whereas no such finding was reported from the high dose group. However, as the uterus was not a protocol organ for histopathology, this assumption was not supported microscopically.

No other microscopic findings were recorded from treated or control females, as "status of pregnancy" does not refer to a pathologic situation but to a physiologic one.

The results of the differential ovarian follicle count (Tables IB 7 – IB 10) in the ovaries – comprising the number of primordial, growing and antral follicles, as well as the combined incidence of primordial plus growing follicles and corpora lutea – is summarized in the following table:

Generation	Group	Number of animals	Primordial Follicles	Growing Follicles	Primordial + Growing Follicles	Antral Follicles	Corpora lutea	
F0	Absolute values							
	0	10	2557	445	3002	63	184	
	3	10	2808	671**	3479	79	266**	
	% of control group			110%	151%	116%	125%	145%
	Mean values							
	0	10	256	45	300	6.3	18.4	
	3	10	281	67	348	7.9	26.8	

* for $p \leq 0.05$ ** for $p \leq 0.01$

Histopathological examination of the ovaries could not detect qualitative depletion of the primordial, growing or antral follicle populations. Over all, the mean numbers of primordial follicles, growing follicles, the combined incidence of primordial plus growing follicles, antral follicles and corpora lutea were all higher in the high dose group than in control animals, showing statistical significance in the high dose group for the number of growing follicles and corpora lutea (indicated by asterix). These deviations, however, were not regarded to be responsible for the recorded reproduction deficits and are not adverse in nature.

The differential ovarian follicle count (DOFC) of the two animals of the low (animal No: 112) and mid dose group (animal No. 127) also did not indicate alterations in the follicle and corpora lutea parameters that might be made responsible for their status "animal not pregnant". This was most probably related to the recorded testicular atrophy in their male mating partners that had developed unrelated to treatment.

In the reproductive organs of the male rats, no treatment-related microscopic finding were noted. The following incidental findings were recorded, microscopically:

Total (extreme) bilateral *diffuse tubular atrophy* was diagnosed in the **testes** of a low dose (No. 12) and a mid dose animal (No. 27), which coincided with bilateral *aspermia* in their **epididymides**. As no such findings were noted in any of the high dose animals, a relationship of this finding to treatment was regarded unlikely. The recorded infertility of these animals was, however, confirmed by these findings.

In addition, minimal *focal tubular atrophy* was noted in both testes of a control male (No. 10). In fact, in both testes, only one tubulus was affected.

In the left epididymis of control male No. 8, a spermatogenic granuloma of spontaneous origin was reported.

No other histopathologic findings were recorded from testes or epididymides of treated male rats. In particular, in the testes of treated males, treatment-related findings such as retained spermatids, missing germ cell layer or types, multinucleated giant cells or sloughing of spermatogenic cells into the lumen were not observed.

In addition, in the epididymides of treated male rats, histopathologic findings like leukocyte infiltration, change in prevalence of cell types, aberrant cell types, and phagocytosis of sperm were also not observed in longitudinal sections through caput, corpus, and cauda of the intact epididymides.

One female rat of the control group (animal No. 105) died intercurrently 39 days after start of treatment. The animal was pregnant. However it did not show any further microscopic finding. As a control animal, a relationship of the premature death to treatment was excluded although morphology failed to identify the cause of animals' premature death.

Seven animals (1/1/1/4) were not pregnant:

No. 107 (control group): no histopathologic findings noted;
No. 112 (low dose group): no histopathologic findings noted;
No. 127 (mid dose group): no histopathologic findings noted;
No. 132 (high dose group): no histopathologic findings noted;
No. 138 (high dose group): no histopathologic findings noted;
No. 139 (high dose group): no histopathologic findings noted;
No. 140 (high dose group): no histopathologic findings noted.

Their male mating partners were:

No. 7 (control group): no histopathologic findings noted;
No. 12 (low dose group): histopathology revealed extreme diffuse tubular atrophy in the testes and concurrent aspermia in the epididymides;
No. 27 (mid dose group): histopathology revealed extreme diffuse tubular atrophy in the testes and concurrent aspermia in the epididymides;
No. 32 (high dose group): no histopathologic findings noted;
No. 38 (high dose group): no histopathologic findings noted;
No. 39 (high dose group): no histopathologic findings noted;
No. 40 (high dose group): no histopathologic findings noted.

None of the microscopic findings recorded in the two male animals were regarded to be treatment-related. They had, however, contributed to the animals' status of "impaired fertility?".

The few other microscopic findings noted were single observations, giving no indication of a relationship to treatment.

4.3.6. Assessment of parental organ weights, gross and histopathological findings

There was no morphologic indication that the administration of the test article had negatively influenced the male (spermatogenesis) or the female (oogenesis) reproduction cycles. By contrast, in ovaries of female rats of the high dose group, the number of growing follicles and the number of corpora lutea was significantly higher as compared to the control group. This was, however, not regarded to be responsible for the reproductive deficits recorded in this study. It was even not clear, whether the increased number of all follicle parameters and corpora were related to the administration of the test article or whether they were simply a by chance observation.

Morphology failed to identify an alteration that may account for the significantly decreased mean absolute weights of the epididymides in high dose males or the significantly decreased mean absolute weight of the ovaries in females of the high dose group. As the mean terminal body weight was decreased in males (- 6.8%) and in females (- 3.2%) in the high dose group coincidentally and as the mean relative weights for both organs were no longer significantly decreased, the recorded weight changes were not regarded to be treatment-related. They were interpreted to be rather related to the decreased mean terminal body weight.

5. DISCUSSION AND CONCLUSION

N-(2-Aminoethyl)ethanolamine was administered daily by oral gavage to F0 male and female parental Wistar rats at doses of 50, 250 and 1,000 mg/kg body weight/day in this **reproduction/developmental toxicity screening test**.

The markedly lower fertility (60%) and gestation (0%, no live progeny) indices of high dose (1,000 mg/kg body weight/day) rats apparently **indicated a significantly impaired fertility / reproductive performance** in these animals. However, the animals of both genders showed an unchanged mating behavior that resulted in a 100% rate of sperm positive parental females. Furthermore, thorough histopathological investigations of testes/epididymides and ovaries (inclusive differential ovarian follicle count) did not reveal any microscopic alterations in the primary sexual organs that may account for the recorded deficits in reproduction. Therefore, it cannot be ruled out that the test substance affected the conceptuses around or after fecundation and, thus, lead to non-pregnancies and lower implantation rates at the top dose level.

There were no indications from the clinical and pathological examinations, that the test material had adverse effects on **reproductive performance or fertility** of the F0 parental animals up to a dose level of 250 mg/kg body weight/day. Mating behavior, conception, gestation, parturition and lactation as well as the determined sexual organ weights, gross and histopathological findings of these organs were similar between the substance-treated rats and the corresponding controls. With the exception of one mating pair in each of test groups 0-2 (male No. 7 mated with female No. 107, male No. 12 mated with female No. 112, male No. 27 mated with female No. 127) all F0 parental rats in these dose groups proved to be fertile. The scattered cases of infertility is regarded to be incidental in nature and not of toxicological or biological concern. Furthermore, no treatment-related findings in the examined reproductive organs (testes, epididymides, ovaries) of either sex were noted by gross and histopathological examinations.

Signs of **general, systemic toxicity** in the F0 parental animals were only noted in rats of both genders of the 1,000 mg/kg body weight/day group. Toxicity was minimal and characterized by salivation after treatment as well as a slightly decreased food consumption, lower mean body weights and impaired body weight gain. The dark yellow discoloration of the urine of all treated animals may most probably attributed to the excretion of the compound or its metabolites rather than to an adverse effect in the urinary tract.

There were **clear indications of developmental toxicity** in the progeny of the F0 parents at all dose levels.

No live fetuses at all and a 100% resorption of the few implantations were recorded at the top dose (1,000 mg/kg body weight/day). This complete postimplantation loss also indicates that there is ought to be a secondary mechanism of severe developmental toxicity with a very early onset during or after fecundation which is responsible for the "apparent infertility" of the high dose females.

At the mid dose (250 mg/kg body weight/day) the viability index was lower (80%) apart from a non-significantly higher number of stillborn pups compared to control. Other

parameters such as the mean number of delivered F1 pups/dam, the percentage of liveborn pups, the mean number of live pups/litter on day 0, the sex ratio, pup body weight/body weight gain as well as clinical observations were not influenced by the test compound.

The mean number of delivered F1 pups/dam, the percentage of liveborn pups, the viability index, the mean number of live pups/litter on days 0 and 4 p.p., the sex ratio, pup body weight/body weight gain as well as clinical observations did not show any difference of biological relevance between the low dose (50 mg/kg body weight/day) and the control group.

Pup necropsy revealed a **significant incidence of test substance induced macroscopic changes** in low and mid dose pups which were **mostly related to the pericardial vessels**. All pup necropsy observations together occurred at an incidence of 48% pups in 100% of the low dose (50 mg/kg body weight/day) litters, and in 89% pups in 100% of the mid dose (250 mg/kg body weight/day) litters. The ratio of affected pups per litter was 48.4% in the low dose group and 87.8% in the mid dose group.

The most salient findings were dilations and aneurysms as well as aberrant (abnormal course) pericardial vessels (aorta, carotids, pulmonary trunk), some example photographs are appended (Supplement III C-001). This type of findings is potentially life-threatening and may well be responsible for the preterminal deaths of some mid dose pups. In order to clarify pathogenesis, to assess potential consequences of these malformations for further life of the offspring, and to define a NOAEL for these findings additional examinations/studies are warranted.

Any other necropsy observations in a few of the examined F1 pups (e.g. post mortem autolysis, situs inversus, dilated renal pelvis, hemorrhagic testis, agnathia and astomia) were regarded as spontaneous findings and not related to the test material. These findings occurred without a clear relation to dosing and/or most of it can be found in the historical control data at comparable or even higher incidences.

Thus, under the conditions of this reproduction/developmental toxicity screening test the **NOAEL (no observed adverse effect level) for reproductive performance and fertility is 250 mg/kg body weight/day for the F0 parental rats**, though at the top dose level of 1,000 mg/kg body weight/day severe developmental toxicity may be responsible for the "apparent infertility". Histopathologic examination of reproductive organs of male and female rats in the 1,000 mg/kg body weight/day group did not identify any treatment-related effects.

The **NOAEL for general, systemic toxicity of the test substance is 250 mg/kg body weight/day for the F0 parental rats of both genders**.

No **NOAEL for developmental toxicity in the F1 progeny** could be determined under the conditions of this screening test.

6. REFERENCES

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APPENDIX
SUMMARY TABLES

7. APPENDIX**7.1. LIST OF ABBREVIATIONS USED IN TABLES IA (CLINICAL EXAMINATIONS)**

DAY 0 P.C.	= day when sperm was detected in the vaginal smear of a mated female for the first time within a mating period
MEAN	= mean value
MG/KG BW/D	= milligram per kilogram body weight/day
N/#/No.	= number/number of animals or litters
P	= significance level
S.D.	= standard deviation
TOTAL	= total number
-	= no animals examined
0	= no such finding/observation
%	= per cent

All other abbreviations used are explained in the tables.

7.2. LIST OF ABBREVIATIONS USED IN TABLES IB (PATHOLOGY)

F	= female animals
F1	= final sacrifice groups
g	= weight determination in gram
M	= male animals (under sex); mean value (on weight level)
mg	= weight determination in milligram
mg/kg	= dose in milligram per kilogram body weight
n	= number of values measured for the determination of mean value and standard deviation
NAD	= number of animals without gross lesions
SD	= standard deviation
%	= percentage related to the reference weight in relative organ weight calculations

Codes for the status at necropsy:

1	= planned sacrifice
2	= killed moribund
3	= intercurrent death

Codes used at finding level:

The codes are used for a grading system which takes into consideration either the severity or the number or the size of a microscopic finding.

	severity	number	size
grade 1	minimal	very few	very small
grade 2	slight	few	small
grade 3	moderate	moderate number; several	moderate size
grade 4	marked; severe	many	large
grade 5	massive; extreme	extensive number	extensive size

Whenever a grading was not used, the microscopic finding was indicated to be present (P).

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TABLE : IA- 001

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF MALE CLINICAL OBSERVATIONS

MALES

	GROUP#	DAY OF STUDY																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
# OF ANIMALS EXAMINED	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	1	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
NORMAL		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
NOTHING ABNORMAL DETECTED	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	1	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DEAD		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SCHEDULED SACRIFICE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ORAL-BUCCAL		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SALIVATION (AFTER TREATMENT)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	4	5	6	8	7	8	9	9	6	9	8	8	8	8	8	8	8	9
SKIN/FUR		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FUR SMEARED WITH URINE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
STOOL/URINE		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URINE. DISCOLORED	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	2	0	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	3	0	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

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TABLE : IA- 002

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 MALES
 SUMMARY OF MALE CLINICAL OBSERVATIONS

	GROUP#	DAY OF STUDY											32	TOTAL		
		22	23	24	25	26	27	28	29	30	31					
# OF ANIMALS EXAMINED	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	1	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
NORMAL																
NOTHING ABNORMAL DETECTED	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DEAD																
SCHEDULED SACRIFICE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ORAL-BUCCAL																
SALIVATION (AFTER TREATMENT)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	10	9	10	6	7	7	6	9	9	8	0	10			
SKIN/FUR																
FUR SMEARED WITH URINE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
STOOL/URINE																
URINE, DISCOLORED	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

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TABLE : IA- 003

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FEMALE CLINICAL OBSERVATIONS (EXCEPT GESTATION AND LACTATION PERIODS)

	GROUP#	WEEK OF STUDY							7 TOTAL		
		0	1	2	3	4	5	6			
# OF ANIMALS EXAMINED	0	10	10	10	0	0	1	1	9		
	1	10	10	10	0	0	0	1	10		
	2	10	10	10	1	1	1	1	10		
	3	10	10	10	0	0	0	0	10		
NORMAL											
NOTHING ABNORMAL DETECTED	0	10	10	5	-	-	-	0	1	1	10
	1	10	0	0	-	-	-	-	0	0	10
	2	10	0	0	0	0	0	0	0	0	10
	3	10	0	0	-	-	-	-	0	0	10
DEAD											
FOUND DEAD/UNABLE TO DELIVER	0	0	0	0	-	-	-	1	0	0	1
	1	0	0	0	-	-	-	-	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	-	-	-	-	0	0	0
SCHEDULED SACRIFICE											
	0	0	0	0	-	-	-	0	0	9	9
	1	0	0	0	-	-	-	-	0	10	10
	2	0	0	0	0	0	0	0	0	10	10
	3	0	0	0	-	-	-	-	0	10	10
MISCELLANEOUS											
SPERM IN VAGINAL SMEAR (DAY 0 P.C. FOR FI)	0	0	0	10	-	-	-	0	0	0	10
	1	0	0	10	-	-	-	-	0	0	10
	2	0	0	9	0	0	0	0	0	0	9
	3	0	0	10	-	-	-	-	0	0	10
NO SPERM IN VAGINAL SMEAR (NO DAY 0 P.C. FOR FI)	0	0	0	0	-	-	-	0	0	0	0
	1	0	0	0	-	-	-	-	0	0	0
	2	0	0	0	1	0	0	0	0	0	1
	3	0	0	0	-	-	-	-	0	0	0
NO PUPS DELIVERED (FI)	0	0	0	0	-	-	-	0	1	0	1
	1	0	0	0	-	-	-	-	1	0	1
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	-	-	-	-	9	1	10

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TABLE : IA- 004

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)

SUMMARY OF FEMALE CLINICAL OBSERVATIONS (EXCEPT GESTATION AND LACTATION PERIODS)

	GROUP#	WEEK OF STUDY							TOTAL
		0	1	2	3	4	5	6	
# OF ANIMALS EXAMINED	0	10	10	10	0	0	1	1	9
	1	10	10	10	0	0	0	1	10
	2	10	10	10	1	1	1	1	10
	3	10	10	10	0	0	0	9	10
ORAL-BUCCAL									
SALIVATION (AFTER TREATMENT)	0	0	0	0	-	-	0	0	0
	1	0	0	0	-	-	0	0	0
	2	0	0	0	0	0	0	0	0
	3	9	10	7	-	-	-	8	0
SKIN/FUR									
FUR SMEARED WITH URINE	0	0	0	0	-	-	0	0	0
	1	0	0	0	-	-	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	2	2	-	-	-	1	3
STOOL/URINE									
URINE, DISCOLORED	0	0	0	0	-	-	0	0	0
	1	10	10	10	-	-	1	1	10
	2	10	10	7	1	1	1	1	10
	3	10	10	7	-	-	-	9	10

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01075F1

TABLE : IA- 007

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF MATERNAL/PUP CLINICAL OBSERVATIONS DURING LACTATION

	GROUP#	DAY OF LACTATION															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	TOTAL
# OF FEMALES EXAMINED	0	8	8	8	8	8	8	8	8	8	8	8	7	6	4	2	
	1	9	9	9	9	9	9	9	9	9	9	9	5	3	1	0	
	2	9	9	9	9	9	9	9	9	9	9	9	8	6	3	0	
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
NORMAL																	
NOTHING ABNORMAL DETECTED	0	8	8	8	8	8	8	8	8	8	8	8	7	6	4	2	8
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
DEAD																	
SCHEDULED SACRIFICE	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2	8
	1	0	0	0	0	0	0	0	0	0	4	2	2	1	-	9	
	2	0	0	0	0	0	0	0	0	0	1	2	3	3	-	9	
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
STOOL/URINE																	
URINE, DISCOLORED	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	9	9	9	9	9	9	9	9	9	9	9	5	3	1	-	9
	2	9	9	9	9	9	9	9	9	9	9	9	8	6	3	-	9
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
DAM / LITTER																	
INSUFFICIENT MATERNAL CARE OF PUP(S)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

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01075F0M

TABLE : IA- 008

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)

MEAN PARENTAL FOOD CONSUMPTION -- GRAMS/ANIMAL/DAY

MALES

WEEK	0 TO 1	MEAN S.D. N	TEST GROUP 0 0 MG/KG BW/D			TEST GROUP 1 50 MG/KG BW/D			TEST GROUP 2 250 MG/KG BW/D			TEST GROUP 3 1,000 MG/KG BW/D		
			MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N
WEEK	0 TO 1	20.6 D 1.86 10	20.4 1.26 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	20.6 2.57 10	16.2** 2.58 10
WEEK	1 TO 2	21.1 D 1.74 10	20.0 1.43 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.2 2.16 10	20.1 1.87 10
WEEK	3 TO 4	21.3 D 1.71 10	20.3 1.58 10	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 1.73 9	21.1 2.11 10
WEEK	0 TO 4	MEAN OF MEANS S.D. N	21.0 0.35 3	20.2 0.23 3	20.6 0.45 3	20.6 0.45 3	20.6 0.45 3	20.6 0.45 3	20.6 0.45 3	20.6 0.45 3	20.6 0.45 3	20.6 0.45 3	20.6 0.45 3	19.1 2.60 3

Statistics: 0=Dunnett-test (two-sided)
* : p<0.05 ** : p<0.01

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01075F0F

TABLE : IA- 009

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)

IN RATS -- ORAL ADMINISTRATION (GAVAGE)

MEAN PARENTAL FOOD CONSUMPTION DURING PREGNANCY -- GRAMS/ANIMAL/DAY

WEEK	0 TO 1	0 TO 2	0 TO 2	TEST GROUP 0			TEST GROUP 1			TEST GROUP 2			TEST GROUP 3					
				MEAN	S.D.	N	MEAN	S.D.	N									
				15.4 D	1.02	10	15.6	0.90	10	15.2	0.80	10	13.1**	0.97	10			
				15.6 D	1.29	10	15.6	0.91	10	15.8	0.88	10	14.2*	0.96	10			
				15.5	0.10	2	15.6	0.01	2	15.5	0.37	2	13.7	0.79	2			

Statistics: D=Dunnett-test (two-sided)

* : p<=0.05 ** : p<=0.01

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01075F1

TABLE : IA- 010

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAYAGE)

MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- GRAMS/ANIMAL/DAY

DAYS		TEST GROUP 0 0 MG/KG BW/D			TEST GROUP 1 50 MG/KG BW/D			TEST GROUP 2 250 MG/KG BW/D			TEST GROUP 3 1,000 MG/KG BW/D		
		MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N
0 TO 7		19.6	1.01	9	19.1	1.12	9	19.4	0.89	9	19.5	1.22	6
7 TO 14		21.2	1.36	9	21.0	1.62	9	20.5	0.98	9	19.7	1.72	6
14 TO 20		21.3	1.15	9	21.3	1.70	9	20.6	1.16	9	16.5**	1.86	6
0 TO 20	MEAN OF MEANS	20.7	0.97	3	20.5	1.18	3	20.2	0.66	3	18.6	1.78	3

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

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01075F0M

TABLE : IA- 012

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)

IN RATS -- ORAL ADMINISTRATION (GAVAGE)
MEAN PARENTAL BODY WEIGHTS -- GRAMS

WEEK		TEST GROUP 0		TEST GROUP 1		TEST GROUP 2		TEST GROUP 3	
		0 MG/KG BW/D	50 MG/KG BW/D	50 MG/KG BW/D	250 MG/KG BW/D	1,000 MG/KG BW/D	1,000 MG/KG BW/D		
WEEK 0	MEAN	289.4	287.7	287.7	286.5	287.5			
	S.D.	10.22	11.48	11.48	10.90	10.04			
	N	10	10	10	10	10			
WEEK 1	MEAN	307.9	303.4	303.4	302.3	289.8*			
	S.D.	14.13	11.38	11.38	15.58	14.72			
	N	10	10	10	10	10			
WEEK 2	MEAN	325.0	315.0	315.0	315.5	306.2			
	S.D.	16.35	14.65	14.65	19.70	17.67			
	N	10	10	10	10	10			
WEEK 3	MEAN	335.5	325.0	325.0	328.8	317.3			
	S.D.	15.86	18.88	18.88	23.42	19.15			
	N	10	10	10	10	10			
WEEK 4	MEAN	353.6	337.7	337.7	341.2	332.7			
	S.D.	22.56	23.93	23.93	26.82	24.45			
	N	10	10	10	10	10			

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

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01075F0M

TABLE : IA- 013

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)
MEAN PARENTAL BODY WEIGHT CHANGE -- GRAMS

WEEK	0 TO 1	MEAN S.D. N	TEST GROUP 0			TEST GROUP 1			TEST GROUP 2			TEST GROUP 3		
			0	50	250	50	250	50	250	50	250	50	250	
WEEK	0 TO 1	18.4 D 6.18 10	15.7 3.59 10	15.8 5.81 10	15.7 3.59 10									
WEEK	1 TO 2	17.1 D 3.59 10	11.7 4.41 10	13.1 6.91 10	11.7 4.41 10									
WEEK	2 TO 3	10.6 D 5.47 10	9.9 7.02 10	13.3 5.23 10	9.9 7.02 10									
WEEK	3 TO 4	18.1 D 9.30 10	12.7 7.28 10	12.5 5.80 10	12.7 7.28 10									
WEEK	0 TO 4	64.2 D 14.58 10	50.0 14.42 10	54.7 18.68 10	50.0 14.42 10									

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

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01075F0F

TABLE : IA- 014

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)

IN RATS -- ORAL ADMINISTRATION (GAVAGE)

MEAN PARENTAL BODY WEIGHTS DURING PREMATING -- GRAMS

WEEK		TEST GROUP 0		TEST GROUP 1		TEST GROUP 2		TEST GROUP 3	
		0 MG/KG BW/D	189.8 D	50 MG/KG BW/D	192.2	250 MG/KG BW/D	191.6	1.000 MG/KG BW/D	192.6
WEEK 0	MEAN		6.39		6.75		5.87		8.05
	S.D.		10		10		10		10
	N								
WEEK 1	MEAN		193.8 D		198.7		196.9		194.0
	S.D.		6.39		7.92		8.26		8.96
	N		10		10		10		10
WEEK 2	MEAN		203.4 D		207.7		207.6		199.0
	S.D.		9.41		7.60		9.06		7.83
	N		10		10		10		10

Statistics: 0=Dunnett-test (two-sided)

* : p<=0.05 ** : p<=0.01

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01075F0F

TABLE : IA- 015

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 MEAN PARENTAL BODY WEIGHT CHANGE DURING PREMATING -- GRAMS

WEEK	0 TO 1	MEAN S.D. N	TEST GROUP 0 0 MG/KG BW/D			TEST GROUP 1 50 MG/KG BW/D			TEST GROUP 2 250 MG/KG BW/D			TEST GROUP 3 1,000 MG/KG BW/D		
			MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N
WEEK	0 TO 1		4.10	3.76	10	6.5	5.00	10	5.3	4.49	10	1.4	2.73	10
WEEK	1 TO 2		9.60	6.74	10	9.0	5.28	10	10.7	6.49	10	4.9	6.98	10
WEEK	0 TO 2		13.70	6.82	10	15.5	5.98	10	16.0	5.77	10	6.4	7.87	10

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

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01075F1

TABLE : IA- 016

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 MEAN MATERNAL BODY WEIGHTS DURING GESTATION -- GRAMS

DAY	MEAN S.D. N	TEST GROUP 0 0 MG/KG BW/D			TEST GROUP 1 50 MG/KG BW/D			TEST GROUP 2 250 MG/KG BW/D			TEST GROUP 3 1,000 MG/KG BW/D		
		MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N
DAY 0		204.3	7.96	9	208.0	7.18	9	207.2	8.72	9	202.9	8.73	6
DAY 7		230.0	8.00	9	233.2	9.46	9	233.0	8.64	9	232.1	7.53	6
DAY 14		255.4	8.97	9	261.1	12.49	9	258.6	8.11	9	238.5*	10.53	6
DAY 20		303.7	15.86	9	313.3	19.29	9	304.4	9.80	9	230.8**	8.34	6

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

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01075F1

TABLE : IA- 017

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION -- GRAMS

DAYS		TEST GROUP 0			TEST GROUP 1			TEST GROUP 2			TEST GROUP 3		
		MEAN	S.D.	N									
0 TO 7		25.7	5.14	9	25.1	6.92	9	25.8	5.18	9	29.2	3.25	6
7 TO 14		25.4	4.17	9	27.9	4.69	9	25.6	3.47	9	6.4**	11.31	6
14 TO 20		48.3	8.47	9	52.2	9.18	9	45.9	9.19	9	-7.7**	7.32	6
0 TO 20		99.4	11.30	9	105.3	15.80	9	97.3	12.78	9	27.9**	7.45	6

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

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01075F1

TABLE : IA- 018

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 MEAN MATERNAL BODY WEIGHTS DURING LACTATION -- GRAMS

DAY	MEAN S.D. N	TEST GROUP 0 0 MG/KG BW/D			TEST GROUP 1 50 MG/KG BW/D			TEST GROUP 2 250 MG/KG BW/D			TEST GROUP 3 1,000 MG/KG BW/D		
		MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N	MEAN	S.D.	N
DAY 0		239.2	14.29	8	234.3	13.67	9	238.6	13.88	9	238.6	13.88	9
DAY 4		241.8	9.09	8	245.2	17.32	9	245.1	10.57	9	245.1	10.57	9
DAY 7		238.2	12.85	8	241.2	9.65	9	238.8	8.97	9	238.8	8.97	9

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

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01075F1

TABLE : IA- 019

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 MEAN MATERNAL BODY WEIGHT CHANGE DURING LACTATION -- GRAMS

DAYS	0 TO 4	0 TO 7	0 TO 7	TEST GROUP 0 0 MG/KG BW/D		TEST GROUP 1 50 MG/KG BW/D		TEST GROUP 2 250 MG/KG BW/D		TEST GROUP 3 1,000 MG/KG BW/D	
				MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
				2.6	13.78	10.9	13.22	6.5	8.23		
				8		9		9			
				-3.6	6.44	-4.0	11.54	-6.2	9.13		
				8		9		9			
				-1.0	16.03	6.9	11.25	0.3	12.37		
				8		9		9			

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

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01075F1

TABLE : IA-

020

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS --- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF COHABITATION DATA

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1.000 MG/KG BW/D
Males placed with females	N 10	10	10	10
mated (A)	N 10 F	10	9	10
male mating index (B)	% 100	100	90	100
did not mate	N 0 F	0	1	0
	% 0.0	0.0	10	0.0
with females pregnant (C)	N 9 F	9	9	6
male fertility index (D)	% 90	90	90	60
without females pregnant	N 1 F	1	1	4
	% 10	10	10	40

Statistics: F=Fisher's exact test (two-sided)
 * : p<=0.05 ** : p<=0.01

(A) DEFINED BY A FEMALE WITH VAGINAL SPERM, OR THAT GAVE BIRTH TO A LITTER, OR WITH PUPS/IMPLANTATIONS IN UTERO

(B) MALE MATING INDEX = $\frac{\text{NUMBER OF MALES WITH CONFIRMED MATINGS}}{\text{NUMBER OF MALES PLACED WITH FEMALES}} \times 100$

(C) DEFINED BY A FEMALE GIVING BIRTH TO A LITTER, OR WITH PUPS/IMPLANTATIONS IN UTERO

(D) MALE FERTILITY INDEX = $\frac{\text{NUMBER OF MALES PROVING THEIR FERTILITY}}{\text{NUMBER OF MALES PLACED WITH FEMALES}} \times 100$

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TABLE : IA-

PROJECT NO. 90R0019/01075; SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)

021

SUMMARY OF FEMALE REPRODUCTION AND DELIVERY DATA

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1.000 MG/KG BW/D
Females on Study	N 10	10	10	10
Females Mated	N 10F1	10	9	10
Female Mating Index	100	100	90	100
Mating days until day 0 pc	MEAN 1.8 D	2.9	2.1	2.9
	S.D. 1.03	0.88	1.05	1.91
	N 10	10	9	10
days 1 to 4	N 10	10	9	9
	100	100	100	90
days 5 to 8	N 0	0	0	1
	0.0	0.0	0.0	10
days 9 to 14	N 0	0	0	0
	0.0	0.0	0.0	0.0
days 15 to 21	N 0	0	0	0
	0.0	0.0	0.0	0.0
Females Pregnant	N 9F1	9	9	6
Female Fertility Index	90	90	100	60
Duration of Gestation (Days)	MEAN 21.8 D	22.0	22.0	22.0
	S.D. 0.46	0.50	0.00	0.00
Implantation sites	TOTAL 86	113	100	19
	MEAN 10.8 D	12.6	11.1	3.2**
	S.D. 1.91	1.51	2.98	0.98
	N 8	9	9	6
Postimplantation Loss	TOTAL 4	6	7	19
	MEAN 0.5 D	0.7	0.8	3.2**
	S.D. 1.07	1.12	1.09	0.98
	N 8	9	9	6
Postimplantation Loss	MEAN 4.7 D	5.5	6.4	100.0**
	S.D. 9.78	9.02	8.58	0.00
	N 8	9	9	6

Statistics: D-Dummett-test (two-sided) F1 = Fisher's exact test (one-sided)

* : p<=0.05 ** : p<=0.01

THE INDICES ARE DEFINED IN THE TEXT

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01075F1

TABLE : IA- 022

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF FEMALE REPRODUCTION AND DELIVERY DATA

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
Females with Liveborn Gestation Index	N 89	N 100	N 100	N 9
with Stillborn Pups	FI 13	FI 11	FI 33	FI 0
with all Stillborn	OFI 0.0	OFI 0.0	OFI 0.0	OFI 0.0
Pups Delivered	MEAN 10.3 D S.D. 2.19 TOTAL 82	MEAN 11.9 D S.D. 1.90 TOTAL 107	MEAN 10.3 D S.D. 2.83 TOTAL 93	MEAN 10.3 D S.D. 2.83 TOTAL 93
Liveborn Live Birth Index	N 81FI 99	N 106 99	N 88 95	N 88 95
Stillborn	N 1FI 1.2	N 1 0.9	N 5 5.4	N 5 5.4

Statistics: D-Dunnett-test (two-sided) FI = Fisher's exact test (one-sided)
* : p<0.05 ** : p<0.01
THE INDICES ARE DEFINED IN THE TEXT

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TABLE : IA- 023

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF LITTER DATA

		TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
(Total Number of) Litters	N	8	9	9	0
Litters with Liveborn Pups	N	8Fi 100	9 100	9 100	
Litters with Stillborn Pups	N	1Fi 13	1 11	3 33	
Litters with all Stillborn Pups	N	0Fi 0.0	0 0.0	0 0.0	
Pups Delivered	TOTAL MEAN S.D.	82 10.3 2.19	107 11.9 1.90	93 10.3 2.83	0
Pups Liveborn	N	81Fi 99	106 99	88 95	
Pups Stillborn	N	1Fi 1.2	1 0.9	5 5.4	
Pups Died	N	1Fi 1.2	2 1.9	9* 9.7	
Pups Sacrificed Moribund	N	0Fi 0.0	0 0.0	0 0.0	
Pups Cannibalized	N	0Fi 0.0	2 1.9	9** 9.7	
Pups Accidental Death	N	0 0.0	0 0.0	0 0.0	
Pups Sacrificed, Maternal Death	N	0 0.0	0 0.0	0 0.0	

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

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01075F1

TABLE : IA-

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)

024

SUMMARY OF LITTER DATA

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1.000 MG/KG BW/D
Pups dead day 0	N 0	N 0	N 1	N 1
	X 0.0	X 0.0	X 1.1	X 1.1
days 1 to 4	N 1	N 4	N 17	N 19
	X 1.2	X 3.8	X 19	X 19
Pups Surviving days 0 to 4	N 80F1	N 102	N 70**	N 80
Viability Index	X 99	X 96	X 80	X 80

Statistics: F1 = Fisher's exact test (one-sided)

* : p<=0.05 ** : p<=0.01

THE INDICES ARE DEFINED IN THE TEXT. Pups Dead = Pups Died + Sacrificed Moribund + Cannibalized

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01075F1

TABLE : IA- 025

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF LITTER DATA

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
Live Pups/Litter				
day 0	MEAN 10.1	MEAN 11.8	MEAN 9.8	
	S.D. 2.10	S.D. 1.92	S.D. 2.99	
	TOTAL 81	TOTAL 106	TOTAL 88	0
day 4	MEAN 10.0	MEAN 11.3	MEAN 7.8	
	S.D. 2.14	S.D. 2.06	S.D. 2.59	
	TOTAL 80	TOTAL 102	TOTAL 70	0
Sex Ratio				
day 0				
- Live Males	3	55.7	56.8	0.0
- Live Females	3	44.3	43.2	0.0
day 4				
- Live Males	3	54.9	58.6	0.0
- Live Females	3	45.1	41.4	0.0

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TABLE : IA- 026

PROJECT NO. 90R0019/03075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF PUP BODY WEIGHTS -- GRAMS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
day 1 males	MEAN S.D. N 6.3 D 0.59 8	6.1 0.40 9	6.1 0.69 9	
day 1 females	MEAN S.D. N 6.1 D 0.56 8	5.8 0.42 9	5.6 0.86 9	
day 1 males+females	MEAN S.D. N 6.2 D 0.57 8	6.0 0.39 9	5.9 0.81 9	
day 4 males	MEAN S.D. N 9.5 D 1.22 8	8.8 1.63 9	9.0 1.20 9	
day 4 females	MEAN S.D. N 9.2 D 1.12 8	8.6 1.52 9	8.2 1.44 8	
day 4 males+females	MEAN S.D. N 9.4 D 1.17 8	8.7 1.58 9	8.9 1.41 9	

RUNTS ON DAY 1
 males 3
 females 0

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

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01075F1

TABLE : IA- 027

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF PUP BODY WEIGHT CHANGES -- GRAMS

DAYS 1 TO 4		TEST GROUP 0		TEST GROUP 1		TEST GROUP 2		TEST GROUP 3	
		0 MG/KG BW/D	50 MG/KG BW/D	50 MG/KG BW/D	250 MG/KG BW/D	1,000 MG/KG BW/D	1,000 MG/KG BW/D	1,000 MG/KG BW/D	1,000 MG/KG BW/D
	males	MEAN S.D. N	3.3 D 0.65 8	2.7 1.26 9	2.9 0.81 9				
	females	MEAN S.D. N	3.2 D 0.60 8	2.8 1.15 9	2.7 0.74 8				
	males+females	MEAN S.D. N	3.2 D 0.62 8	2.7 1.21 9	3.0 0.78 9				

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

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01075F1

TABLE : IA-

PROJECT NO. 90R0019/01075; SCREENING STUDY (OECD NO. 421)
IN RATS -- ORAL ADMINISTRATION (GAVAGE)

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SUMMARY OF PUP NECROPSY OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
Litters Evaluated	N	9	9	0
Pups Evaluated	N	105	84	0
Live	N	104	79	0
Stillborn	N	1	5	0
POST MORTEM AUTOLYSIS				
Pup Incidence	N	0	1	0
	%	0.0	1.2	
Litter Incidence	N	0	1	0
	%	0.0	11	
Affected Pups/Litter	MEAN%	0.0	1.2	
	S.D.	0.00	3.70	
SITUS INVERSUS				
Pup Incidence	N	1	0	0
	%	1.0	0.0	
Litter Incidence	N	1	0	0
	%	11	0.0	
Affected Pups/Litter	MEAN%	0.9	0.0	
	S.D.	2.78	0.00	
ANEURYSM OF AORTA				
Pup Incidence	N	19	47	0
	%	0.0	56	
Litter Incidence	N	6**	9**	0
	%	67	100	
Affected Pups/Litter	MEAN%	17.7**	55.2**	
	S.D.	17.95	21.18	

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

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01075F1

TABLE : IA- 029

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECO NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF PUP NECROPSY OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
Litters Evaluated	N 8	9	9	0
Pups Evaluated	N 82	105	84	0
Live	N 81	104	79	0
Stillborn	N 1	1	5	0
ANEURYSM OF PULMONARY TRUNK				
Pup Incidence	N 0	17	37	
	% 0.0	16	44	
Litter Incidence	N 0Fi	8**	9**	
	% 0.0	89	100	
Affected Pups/Litter	MEAN% 0.0Mi	16.3**	44.5**	
	S.D. 0.00	9.79	20.14	
HIGH AORTIC ARCH				
Pup Incidence	N 0	0	1	
	% 0.0	0.0	1.2	
Litter Incidence	N 0Fi	0	1	
	% 0.0	0.0	11	
Affected Pups/Litter	MEAN% 0.0Mi	0.0	2.8	
	S.D. 0.00	0.00	8.33	
DILATED CAROTID				
Pup Incidence	N 0	19	30	
	% 0.0	18	36	
Litter Incidence	N 0Fi	7**	8**	
	% 0.0	78	89	
Affected Pups/Litter	MEAN% 0.0Mi	18.8**	35.8**	
	S.D. 0.00	14.57	27.94	

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

21-NOV-02

01075F1

TABLE : IA-

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)

030

IN RATS -- ORAL ADMINISTRATION (GAVAGE)
SUMMARY OF PUP NECROPSY OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1.000 MG/KG BW/D
Litters Evaluated	N	9	9	0
Pups Evaluated	N	105	84	0
Live	N	104	79	0
Stillborn	N	1	5	0
DILATED DESCENDING AORTA				
Pup Incidence	N	22	47	
	%	0.0	56	
Litter Incidence	N	6**	9**	
	%	0.0	100	
Affected Pups/Litter	MEAN%	21.1**	55.2**	
	S.D.	0.00	23.35	17.43
ABNORMAL COURSE OF CAROTIDS				
Pup Incidence	N	11	20	
	%	0.0	24	
Litter Incidence	N	4	6**	
	%	0.0	67	
Affected Pups/Litter	MEAN%	10.3	21.9**	
	S.D.	0.00	17.02	24.40
DILATED PULMONARY TRUNK				
Pup Incidence	N	4	0	
	%	0.0	3.8	0.0
Litter Incidence	N	2	0	
	%	0.0	0.0	
Affected Pups/Litter	MEAN%	4.6	0.0	
	S.D.	0.00	9.42	0.00

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

21-MOV-02

01075F1

TABLE : IA- 031

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF PUP NECROPSY OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
Litters Evaluated	N	9	9	0
Pups Evaluated	N	105	84	0
Live	N	104	79	0
Stillborn	N	1	5	0
ANEURYSM OF CAROTID				
Pup Incidence	N	0	5	
	%	0.0	6.0	
Litter Incidence	N	0	4	
	%	0.0	44	
Affected Pups/Litter	MEAN%	0.0Wt	5.0	
	S.D.	0.00	6.39	
ANEURYSM OF DUCTUS ARTERIOSUS				
Pup Incidence	N	1	1	
	%	0.0	1.2	
Litter Incidence	N	1	1	
	%	0.0	11	
Affected Pups/Litter	MEAN%	1.0	1.0	
	S.D.	0.00	3.03	
PERICARDIUM FILLED WITH BLOOD				
Pup Incidence	N	0	1	
	%	0.0	1.2	
Litter Incidence	N	0	1	
	%	0.0	11	
Affected Pups/Litter	MEAN%	0.0Wt	1.1	
	S.D.	0.00	3.33	

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

21-NOV-02

01075F1

TABLE : IA-

032

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF PUP NECROPSY OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
Litters Evaluated	N	9	9	0
Pups Evaluated	N	105	84	0
Live	N	104	79	0
Stillborn	N	1	5	0
DILATED RENAL PELVIS				
Pup Incidence	N	1	1	0
	%	1.0	1.2	0
Litter Incidence	N	1	1	0
	%	11	11	0
Affected Pups/Litter	MEAN%	1.0	0.9	0
	S.D.	3.03	2.78	0
HEMORRHAGIC TESTIS				
Pup Incidence	N	0	1	0
	%	0.0	1.2	0
Litter Incidence	N	0	1	0
	%	0.0	11	0
Affected Pups/Litter	MEAN%	0.0	1.1	0
	S.D.	0.00	3.33	0
AGNATHIA				
Pup Incidence	N	1	0	0
	%	1.0	0.0	0
Litter Incidence	N	1	0	0
	%	11	0.0	0
Affected Pups/Litter	MEAN%	0.9	0.0	0
	S.D.	2.78	0.00	0

Statistics: F1 = Fisher's exact test (one-sided) W1 = Wilcoxon-test (one-sided)
 * : p<=0.05 ** : p<=0.01

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TABLE : IA- 033

PROJECT NO. 90R0019/01075: SCREENING STUDY (OECD NO. 421)
 IN RATS -- ORAL ADMINISTRATION (GAVAGE)
 SUMMARY OF PUP NECROPSY OBSERVATIONS

	TEST GROUP 0 0 MG/KG BW/D	TEST GROUP 1 50 MG/KG BW/D	TEST GROUP 2 250 MG/KG BW/D	TEST GROUP 3 1,000 MG/KG BW/D
Litters Evaluated	N	9	9	0
Pups Evaluated	N	105	84	0
Live	N	104	79	0
Stillborn	N	1	5	0
ASTOMIA				
Pup Incidence	N	1	0	0
	%	1.0	0.0	0.0
Litter Incidence	N	1	0	0
	%	11	0.0	0.0
Affected Pups/Litter	MEAN%	0.9	0.0	0.0
	S.D.	2.78	0.00	0.00
TOTAL PUP NECROPSY OBSERVATIONS				
Pup Incidence	N	50	75	
	%	48	89	
Litter Incidence	N	9**	100	
	%	100	100	
Affected Pups/Litter	MEAN%	48.4**	87.8**	
	S.D.	24.17	12.75	

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

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IB 1

PATHOLOGY REPORT

90R0019/01075

Reproduction/Developmental Toxicity

Feb/19/2003 CEGE

Screening Test (Gavage) in Wistar Rat

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (MALE)

Sacrifice group		F1				
Sex		M				
Dose group		0	1	2	3	
Terminal body weight	g	M	333.21	317.26	320.15	310.63
		SD	20.199	21.768	25.406	24.608
		n	10	10	10	10
Testes	g	M	3.318	3.12	3.076	3.338
		SD	0.307	0.7	0.779	0.161
		n	10	10	10	10
Epididymides	g	M	1.093	1.051	1.028	0.94 **
		SD	0.098	0.153	0.167	0.038
		n	10	10	10	10

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

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IB 2

PATHOLOGY REPORT

90R0019/01075

Reproduction/Developmental Toxicity

Feb/19/2003 CEGE

Screening Test (Gavage) in Wistar Rat

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (FEMALE)

Sacrifice group		F1				
Sex		F				
Dose group		0	1	2	3	
Terminal body weight	g	M	219.489	222.83	221.85	212.37
		SD	12.071	8.164	8.957	8.919
		n	9	10	10	10
Ovaries	mg	M	104.778	100.3	107.0	87.7 *
		SD	15.434	12.12	12.147	8.152
		n	9	10	10	10

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

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IB 3

PATHOLOGY REPORT

90R0019/01075

Reproduction/Developmental Toxicity

Feb/19/2003 CEGE

Screening Test (Gavage) in Wistar Rat

acopat system

RELATIVE WEIGHTS - MEAN VALUES (MALE)

Sacrifice group		F1				
Sex		M				
Dose group		0 1 2 3				
Terminal body weight	%	M	100.0	100.0	100.0	100.0
		n	10	10	10	10
Testes	%	M	0.998	0.991	0.955	1.079
		SD	0.106	0.234	0.226	0.082
	n	10	10	10	10	
Epididymides	%	M	0.329	0.334	0.321	0.304
		SD	0.029	0.058	0.048	0.023
	n	10	10	10	10	

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

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IB 4

PATHOLOGY REPORT

90R0019/01075

Reproduction/Developmental Toxicity

Feb/19/2003 CEGE

Screening Test (Gavage) in Wistar Rat

acopat system

RELATIVE WEIGHTS - MEAN VALUES (FEMALE)

Sacrifice group		F1				
Sex		F				
Dose group		0	1	2	3	
Terminal body weight	%	M	100.0	100.0	100.0	100.0
		n	9	10	10	10
Ovaries	%	M	0.048	0.045	0.048	0.041
		SD	0.007	0.006	0.007	0.004
	n	9	10	10	10	

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

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IB 5

PATHOLOGY REPORT

90R0019/01075

Reproduction/Developmental Toxicity

Feb/19/2003 CEGB

Screening Test (Gavage) in Wistar Rat

acopat system

INCIDENCE OF GROSS LESIONS

Sacrifice group	F1				F			
	M							
Dose group	0	1	2	3	0	1	2	3
Animals in selected Group	10	10	10	10	10	10	10	10
NAD	8	9	9	6	8	9	8	6
General Observations
- Animal not pregnant	1	1	1	4
- Impaired fertility?	1	1	1	4
Testes
- Organ size reduced	.	1	1
Epididymides
- Abscess	1
- Organ size reduced	.	1	1
Uterus
- Inflammation	1	.
- Pregnancy	1	.	.	.

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IB 6

PATHOLOGY REPORT

90R0019/01075

Reproduction/Developmental Toxicity

Feb/19/2003 CEGE

Screening Test (Gavage) in Wistar Rat

acopat system

INCIDENCE AND GRADED SEVERITY OF MICROSCOPIC FINDINGS

Sacrifice group	F1				F			
	M							
Sex	M				F			
Dose group	0	1	2	3	0	1	2	3
<u>Animals in selected Group</u>	10	10	10	10	10	10	10	10
Epididymides	10	1	1	10
- Aspermia	.	1	1
. P.	.	1	1
- Granuloma, spermatog.	1
. P.	1
<u>Ovaries</u>	10	1	1	10
Testes	10	1	1	10
- Tubular atrophy, foc	1
. 1.	1
- Diff.tubular atrophy	.	1	1
. 5.	.	1	1
<u>Uterus</u>	1	.	1	.
- Status of pregnancy	1	.	.	.
. P.	1	.	.	.
- Inflammation	1	.
. 3.	1	.

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PATHOLOGY REPORT
 Reproduction/Developmental Toxicity Screening Test (Gavage) in Wistar Rat

90R0019/01075
 June/24/2002 CEGE

Table IB 7

Differential Ovarian Follicle Count (DOFC)

Group 0	Animal No.	Ovary 1		Ovary 2		Ovary 3		Ovary 4		Ovary 5		Ovary 6		Ovary 7		Ovary 8		Ovary 9		Ovary 10		Ovaries 1 + 2				
		Number of Follicles		Number of Follicles		Number of Follicles		Number of Follicles		Number of Follicles		Number of Follicles		Number of Follicles		Number of Follicles		Number of Follicles		Number of Follicles		Primordial Follicles (P)	Growing Follicles (G)	Primordial + Growing	Antral Follicles (A)	Corpora Lutea (CL)
		P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G					
	101	249	23	272	3	11	229	26	255	1	8	478	49	527	4	19										
	102	114	21	135	3	6	87	27	114	3	6	201	48	249	6	12										
	103	125	34	159	3	10	114	47	161	7	12	239	81	320	10	22										
	104	126	25	151	6	12	77	9	86	1	11	203	34	237	7	23										
	105	58	14	72	2	6	51	10	61	2	8	109	24	133	4	14										
	106	157	19	176	2	8	128	11	139	0	8	285	30	315	2	16										
	107	60	14	74	4	10	76	20	96	8	9	136	34	170	12	19										
	108	176	19	195	1	12	185	32	217	5	12	361	51	412	6	24										
	109	104	24	128	6	10	104	20	124	3	10	208	44	252	9	20										
	110	184	22	206	1	8	153	28	181	2	7	337	50	387	3	15										
		1,353	216	1,568	31	93	1,204	230	1,434	32	91	2,557	445	3,002	63	184										

Table IB 9

Differential Ovarian Follicle Count (DOFC)

Group	Animal No.	Ovary 1				Ovary 2				Ovaries 1 + 2				Corpora Lutea (CL)				
		Number of Follicles				Number of Follicles				Primordial Follicles (P)	Growing Follicles (G)	Primordial + Growing	Antral Follicles (A)					
		P	G	P + G	A	CL	P	G	P + G						A			
2	121																	
	122																	
	123																	
	124																	
	125																	
	126																	
	127		109	27	136	7	9	12	83	18	101	2	14	192	45	237	9	23
	128																	
	129																	
	130																	

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PATHOLOGY REPORT

Reproduction/Developmental Toxicity Screening Test (Gavage) in Wistar Rat

90R0019/01075

June/24/2002 CEGE

Table IB 10

Differential Ovarian Follicle Count (DOFC)

Group	Animal No.	Ovary 1		Ovary 2		Number of Follicles										Ovaries 1 + 2				
		Number of Follicles		Number of Follicles		P			G			P + G			CL	Primordial Follicles (P)	Growing Follicles (G)	Primordial + Growing	Antral Follicles (A)	Corpora Lutea (CL)
		P	G	P	G	A	P	G	A	P	G	A								
3	131	136	26	162	4	10	110	32	142	7	12	246	58	304	11	22				
	132	120	38	158	3	15	82	27	109	2	11	202	65	267	5	26				
	133	101	18	119	3	13	80	24	104	2	15	181	42	223	5	28				
	134	84	26	110	2	14	173	36	209	4	9	257	62	319	6	23				
	135	281	32	323	7	12	254	32	286	7	6	545	64	609	14	18				
	136	142	34	176	3	22	193	37	230	3	14	335	71	406	6	36				
	137	112	35	147	8	13	140	43	183	5	12	252	78	330	13	25				
	138	121	37	158	3	14	107	33	140	4	13	228	70	298	7	27				
	139	136	36	172	3	13	106	35	141	3	18	242	71	313	6	31				
	140	144	29	173	3	14	176	61	237	3	16	320	80	410	6	30				
		1,387	311	1,698	39	140	1,421	360	1,781	40	128	2,808	671**	3,479	79	266**				

** = p ≤ 0.001