Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

tert-butyl 2-ethylperoxyhexanoate

EC Number: 221-110-7
CAS Number: 3006-82-4

CLH-O-0000007217-74-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

1 December 2022

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

tert-butyl 2-ethylhexaneperoxoate (TBPEH)

EC Number: **221-110-7**

CAS Number: **3006-82-4**

Index Number: **/**

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)
14 rue Pierre Marie Curie
F-94701 Maisons-Alfort Cedex
classification.clp@anses.fr

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON *TERT*-BUTYL 2-ETHYLPEROXYHEXANOATE

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON *TERT*-BUTYL 2-ETHYLPEROXYHEXANOATE

1 BACKGROUND INFORMATION

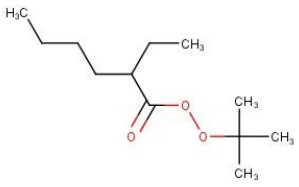
The opportunity to submit a classification proposal for different peroxyesters have been raised by France. Indeed, 5 substances was identified in the peroxyester family: *tert*-butyl-2-ethylperoxylhexanoate (TBPEH), (CAS3006-82-4), *tert*-butyl ethaneperoxoate (TBPA) (CAS 107-71-1), *tert*-butyl peroxypivalate (TBPP) (CAS 927-07-1), *tert*-amyl peroxypivalate (TAPP) (CAS 29240-17-3), *tert*-butyl peroxyneodecanoate (TBPN) (CAS 26748-41-4) and *tert*-butyl 3,5,5-tris(methylperoxy)hexanoate (TBPIN) (CAS 13122-18-4). However, regulatory actions are ongoing at European level, including EOGRTS requested under CCH process for some of these substances and an ECHA's group management work on a wider group "*tert*-alkyl/aryl peroxyesters".

Therefore, the present CLH proposal is only submitted for TBPEH (skin sensitisation and reprotoxicity) awaiting the expected EOGRTS and the GMT conclusion by ECHA before reopening the dossier in order to assess the relevance to propose a CLH report for the peroxyesters category.

2 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*tert*-butyl 2-ethylhexaneperoxoate
Other names (usual name, trade name, abbreviation)	*tert*-butyl 2-ethylperoxyhexanoate hexaneperoxoic acid, 2-ethyl-, 1,1-dimethylethyl ester T-BUTYLPEROXY-2-ETHYLHEXANOATE *tert*-butyl 2-(ethylperoxy)hexanoate *tert*-butyl 2-Ethyl-Perhexanoate *tert*-butyl 2-ethylhexaneperoxoate *tert*-butyl peroxy(2-ethyl)-hexanoate *tert*-butylperoxy-2-ethylhexanoat *Trade name:* Kayaester O, Trigonox 21 LUPEROX® 26 TBPEH Trigonox 21S
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	221-110-7
EC name (if available and appropriate)	*tert*-butyl 2-ethylperoxyhexanoate
CAS number (if available)	3006-82-4
Other identity code (if available)	*InChI=1/C12H24O3/c1-6-8-9-10(7-2)11(13)14-15-12(3,4)5/h10H,6-9H2,1-5H3*
Molecular formula	C12H24O3
Structural formula	
SMILES notation (if available)	CCCCC(CC)C(=O)OOC(C)(C)C
Molecular weight or molecular weight range	216.3172

Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	no information available
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥80%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
tert-butyl 2-ethylperoxyhexanoate EC no 221-110-7 CAS no 3006-82-4	>= 80 - <= 90 % (w/w)	None	Org. Perox. C – H242 Press. Gas (comp) – H280 Skin Irrit. 2 – H315 Eye Irrit. 2 – H319 Skin Sens 1 – H317 Repr. 1B – H360 Repr. 2 – H361 Aquatic Acute 1 – H400 Aquatic Chronic 1 – H410 Aquatic Chronic 2 – H411

Table 3: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
Hydrocarbons, C4, 1,3-butadiene-free, polymd., triisobutylene fraction, hydrogenated EC no 297-629-8 CAS no 93685-81-5		confidential	None	Flam. Liq. 3 – H226 Asp. Tox. 1 – H304 Skin Irrit. 2 – H315 Aquatic Chronic 4 – H413	No

3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

3.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	None										
Dossier submitters proposal	tbd	*tert*-butyl 2-ethylperoxyhexanoate	221-110-7	3006-82-4	Repr. 1B Skin Sens.1B	H360FD H317	GHS08 Dgr	H360FD H317			
Resulting Annex VI entry if agreed by RAC and COM	tbd	*tert*-butyl 2-ethylperoxyhexanoate	221-110-7	3006-82-4	Repr. 1B Skin Sens.1B	H360FD H317	GHS08 Dgr	H360FD H317			

Table 5: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	Harmonized classification proposed: Skin. Sens. 1B – H317	Yes
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	Harmonized classification proposed: Repr. 1B – H360 FD	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

4 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

TBPEH has no current harmonised classification according to CLP Regulation.

RAC general comment

Tert-butyl 2-ethylperoxyhexanoate (hereafter abbreviated as TBPEH) is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 1 000 to < 10 000 tonnes per annum.

This substance is used in polymers and plastic products by consumers, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing (ECHA, 2021).

The industrial uses reported are the following:

- Industrial use of organic peroxides as polymerisation initiators, cross linking agents or curing agents
- Other industrial uses of organic peroxides
- Use of reactive processing aid at industrial site (no inclusion into or onto article)
- Industrial use of chemicals for polymer processing
- Industrial use of coatings and paints
- Industrial use as polymerisation initiator and cross-linking agent
- Use of reactive process regulators in polymerisation processes at industrial site (inclusion or not into /onto article)

On the ECHA website, no public registered data are noted for widespread uses by professionals but various PROCs are indicated in the registered dossier, with PROC 5, 8a, 8b, 9, 10, 11, 13, 15, 19 and 28 (ECHA dissemination, 2021).

Regarding consumer uses, the substance is according to publicly available data on the ECHA website used in adhesives and sealants (PC1), coating and paints, thinners, paint removers (PC9a) and fillers, putties, plasters, modelling clay (PC9b) (ECHA dissemination, 2021).

The lead registrant and members of the Organic Peroxides Consortium stated in their comments during consultation, that they don’t support that there are any consumer or professional uses for TBPEH, and that it is only used under industrial settings, without widespread use.

5 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Regarding proposal related to reproductive toxicity:

There is no requirement for justification that action is needed at Community level.

Regarding proposal related to skin sensitisation: Justification that action is needed at Community level is required:

Differences in self-classification for the following substances:

Among the 797 notifiers, 707 self-classified the substance as Skin. Sens. 1. The others do not classify the substance for skin sensitisation.

6 IDENTIFIED USES

This substance is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 1 000 to < 10 000 tonnes per annum.

This substance is used in polymers and plastic products by consumers, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing (ECHA, 2021).

The industrial uses reported are the following:

- Industrial use of organic peroxides as polymerisation initiators, cross linking agents or curing agents
- Other industrial uses of organic peroxides
- Use of reactive processing aid at industrial site (no inclusion into or onto article)
- Industrial use of chemicals for polymer processing
- Industrial use of coatings and paints
- Industrial use as polymerisation initiator and cross-linking agent
- Use of reactive process regulators in polymerisation processes at industrial site (inclusion or not into /onto article)

No public registered data are noted in ECHA website for widespread uses by professional but various PROC are indicated in the registered dossier, with PROC 5, 8a, 8b, 9, 10, 11, 13, 15, 19 and 28 (ECHA, 2021).

Regarding consumer uses, the substance is used in adhesives and sealants (PC1), coating and paints, thinners, paint removers (PC9a) and fillers, putties, plasters, modelling clay (PC9b) (ECHA, 2021).

7 DATA SOURCES

Data from the Reach registration dossier have been taken into account for elaboration of this CLH report. In addition, a bibliographic search was performed in 2021.

8 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid (100%)	ECHA disseminated website	TBPEH is a colourless organic liquid with perester-odour. Its aggregate state at 20 °C and 1013 hPa is liquid.
Melting/freezing point	-67.3°C	ECHA disseminated website	measured OECD Guideline 102 (Melting point / Melting Range)
Boiling point	-	ECHA disseminated website	technically not feasible The substance decomposes before boiling.
Relative density	0.9 (20°C)	ECHA disseminated website	DIN ISO 3507

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Vapour pressure	2 Pa at 20°C 3 Pa at 25 °C 36 Pa at 50 °C	ECHA disseminated website	Extrapolated value from the Antoine equation
Surface tension	-	ECHA disseminated website	the study does not need to be conducted because based on structure, surface activity is not expected or cannot be predicted
Water solubility	46.3 mg/L (20°C)	ECHA disseminated website	Measured OECD Guideline 105 (Water Solubility)
Partition coefficient n-octanol/water	Log Pow: 4.79 (20°C)	ECHA disseminated website	Determinated by HPLC OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method)
Flash point	78.15°C (101325 Pa)	ECHA disseminated website ISO 3679 (Determination of flash point - Rapid equilibrium closed cup method)	Measured
Flammability	-	ECHA disseminated website	Based on the study results, the molecular structure and experience in handling and use, the substance should not be classified as flammable in contact with water, pyrophoric, self-reactive substance and self-heating substance according to Regulation (EC) No 1272/2008 (CLP). Based on the results of the UN-MTC tests and the decision logic, TBPEH should be classified as Organic Peroxide Type C with H242 (heating may cause a fire) according to Regulation (EC) No 1272/2008 (CLP).
Explosive properties	Non explosive	ECHA disseminated website	TBPEH has no explosive properties because it is not classified as Organic Peroxide Type B (reference CPL regulations 2.15.2.2)
Self-ignition temperature	-	ECHA disseminated website	the study does not need to be conducted for liquid organic peroxides, because the vapours decompose during the conduction of the test
Oxidising properties	-	ECHA disseminated	the study does not need to be

Property	Value	Reference	Comment (e.g. measured or estimated)
		website	conducted for organic peroxides
Granulometry	-	ECHA disseminated website	the study does not need to be conducted because the substance is marketed or used in a non solid or granular form
Stability in organic solvents and identity of relevant degradation products	-	ECHA disseminated website	the study does not need to be conducted because the stability of the substance is not considered to be critical.
Dissociation constant	pKa -4.8	ECHA disseminated website	the study does not need to be conducted because the substance has no ionic structure. By using SPARC Performance Automated Reasoning in Chemistry (v.4.5) the dissociation constant of *tert*.-butylperoxy- 2-ethylhexanoate was calculated revealing a pKa of - 4.85
Viscosity	3.7 mPa.s	ECHA disseminated website	Measured OECD Test Guideline 114 (Viscosity of Liquids)

9 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

10 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

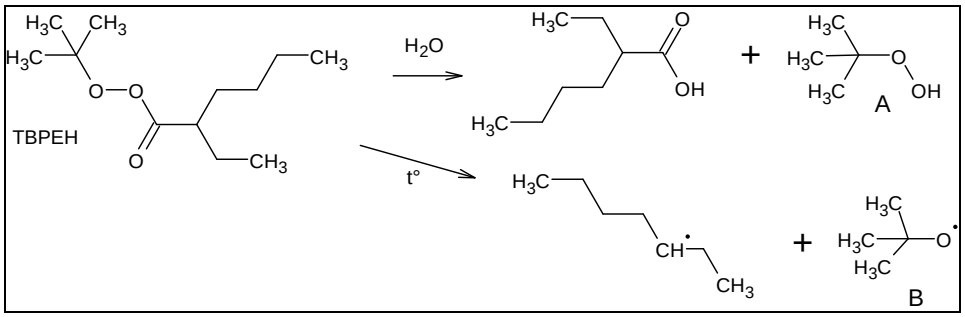
There is no experimental toxicokinetic data available on TBPEH.

TBPEH belongs to the peroxyester family, which is a class of organic peroxides that are relatively unstable under basic or acidic conditions in the presence of water, which catalyses the cleavage of the peroxyester molecule to form an organic acid and conjugate hydroperoxide. Peroxyesters are also expected to be oxidized by intrinsic peroxidases, resulting in the cleavage of the O-O bond (OECD SIDS, 2004).

Therefore, based on the structure of the molecule, the metabolism of TBPEH could possibly include the following pathways:

- enzymatic and non-enzymatic hydrolysis,
- direct reaction with biomolecules due to the reactivity of the peroxyester group of the parent or the hydroperoxide formed after hydrolysis.

The figure below represents the expected degradation products or metabolites of TBPEH:



Estimation of toxicokinetic parameters based on physico-chemical properties:

The molecular weight (< 500) is in favour of absorption. In contrast, water solubility (46.3 mg/L) and log Kow (4.79) are not favourable for significant absorption. Finally, absorption (oral and dermal) is suggested by the presence of systemic effects in toxicological studies performed by oral route but also by the skin sensitising properties of the substance. There is no experimental data by inhalation; the high vapour pressure indicates volatility of the substance and potential respiratory exposure.

When absorbed, TBPEH may be distributed throughout the organism (when considering the low molecular weight, the log Kow and the systemic effects reported). Expected hydrolysis products present a lower molecular weight, a relatively higher water solubility and a lower log Pow value than the parent itself, suggesting the lack of bioaccumulation. Based on these data, excretion is likely to occur mainly via the urine. This is supported by the effects on kidney reported in toxicological studies.

According to the Danish QSAR database, the absorptions from gastrointestinal tract are estimated to be 100% for 1 mg dose and 90% for 1000 mg dose. Dermal absorption is estimated at 0.00146 mg/cm^2/event. The log brain/blood partition coefficient was estimated at 0.5707. TBPEH was not expected to be CYP2C9 and CYP2D6 substrate.

10.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

There is no toxicokinetic data available for TBPEH. Physicochemical properties and data from toxicological studies generally favour systemic absorption. There is no bioaccumulation expected. The metabolism of TBPEH is expected to be mainly via hydrolysis. Excretion of parents and hydrolysis products is expected mainly via the urine.

11 EVALUATION OF HEALTH HAZARDS

11.1 Acute toxicity

Not evaluated in this dossier.

11.2 Skin corrosion/irritation

Irritation properties are summarized in the context of the classification proposal for skin sensitisation. This endpoint has not been assessed in regards to CLP criteria and thus is not open for public consultation.

According to the OECD SIDS related to t-butyl and t-amyl derived alkyl peroxyesters (2004), TBPEH was not irritating to the skin. No additional data is available in the registration dossiers.

11.3 Serious eye damage/eye irritation

Not evaluated in this dossier.

11.4 Respiratory sensitisation

Not evaluated in this dossier.

11.5 Skin sensitisation

Table 7: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Buehler test OECD Guideline 406 Low sensitivity to detect weak sensitizers for Buehler 3 inductions	Hartley-derived albino guinea pigs 10 males, 10 females 10 controls	**TBPEH** Purity: not stated. vehicle: mineral oil Positive control: Hexylcinnamaldehyde	Induction exposure 25 % w/v TBPEH once per week, for 3 consecutive weeks. Challenge exposure: Following a two week rest period, 5 % w/v TBPEH in mineral oil. Rechallenge exposure: Following a one week rest period, 2% w/v TBPEH in mineral oil. Challenge and rechallenge responses in the test animals were compared to those of the challenge control animals. epicutaneous, occlusive	**Skin sensitiser.** Following induction: mild irritation in the test animals. The dermal irritation increased slightly at induction 2 and 3. Following challenge: Dermal scores = 1 in 9/19 (47%) test animals and 3/10 control animals at the 24 hour scoring interval Dermal score = 1 in 3/19 (15%) test animals and 2/10 control animals at the 48 hour scoring interval. Dermal scores = 0 in the remaining test and challenge control animals. Group mean dermal scores similar in the test animals as compared to the challenge control animals. Following rechallenge: Dermal scores = 1 in 5/19 (26%) test animals at the 24 hour scoring interval which do not persist to the 48 hour scoring interval. Dermal scores = 0 in the remaining test and all challenge control animals. Group mean dermal scores slightly higher in the test animals as compared to the challenge control animals. **26% of positive cases following rechallenge** **➔ classification as skin sensitiser 1B**	Unnamed 1996 key study Klimisch score : 2

11.5.1 Short summary and overall relevance of the provided information on skin sensitisation

The dermal skin sensitisation was investigated using TBPEH in a Buehler test using 3 inductions.

The dermal sensitisation potential of TBPEH (purity not stated) was evaluated in Hartley-derived albino guinea pigs. Ten male and ten female guinea pigs were topically treated with 25 % w/v TBPEH in mineral oil, once per week, for 3 consecutive weeks. One test female animal was found dead on study day 27. Gross necropsy observations included dark red mandibular and axillary lymph nodes, an adhesion in the thoracic cavity, mottled lungs, mottled liver, enlarged spleen and congested meningeal vessels in the brain. The

majority of sensitisation study animals gained weight during the test period and generally appeared in good health. Following a two week rest period, a challenge was performed whereby the 19 tested and 10 previously untreated (naive) challenge control guinea pigs were topically treated with 5 % TBPEH in mineral oil. Challenge responses in the test animals were compared to those of the challenge control animals. Following a one-week rest period, a rechallenge was performed whereby the 19 tested and the 10 challenge control guinea pigs were topically treated with 2% w/v TBPEH in mineral oil. Rechallenge responses in the test animals were compared to those of the challenge control animals.

Following induction 1 with 25 % w/v TBPEH in mineral oil, mild irritation was noted in the test animals. The dermal irritation increased slightly at induction 2 and 3. Following challenge with 5 % w/v TBPEH in mineral oil, dermal scores of 1 were noted in 9/19 test animals and 3/10 challenge control animals at the 24 hour scoring interval and in 3/19 test animals and 2/10 challenge control animals at the 48 hour scoring interval. Dermal scores of 0 were noted in the remaining tested and challenge control animals. Group mean dermal scores were noted to be similar in the test animals as compared to the challenge control animals. Following rechallenge with 2 % w/v TBPEH in mineral oil, dermal scores of 1 were noted in 5/19 (26%) test animals at the 24-hour scoring interval; however, the dermal responses did not persist to the 48-hour scoring interval. Dermal scores of 0 were noted in the remaining tested and all challenge control animals. Group mean dermal scores were noted to be slightly higher in the test animals as compared to the challenge control animals.

11.5.2 Comparison with the CLP criteria

According to CLP criteria, classification as Skin Sens. 1B is required in the following cases:

Animal test results for sub-category 1B	
Assay	Criteria
Local lymph node assay	EC3 value > 2 %
Guinea pig maximisation test	≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
Buehler assay	≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

The dermal sensitisation potential of TBPEH was evaluated in a Buehler assay. Ten male and ten female guinea pigs were topically treated with 25 % w/v TBPEH in mineral oil, once per week, for 3 consecutive weeks. The study was concluded of reliability of 2 according to Klimish score. 26% of cases of sensitisation are reported after a topical induction of 25% (> 0.2 % to ≤ 20 %). Therefore a classification Category 1B is warranted for TBPEH.

11.5.3 Conclusion on classification and labelling for skin sensitisation

TBPEH (in mineral oil) was positive in a Buehler test (≥ 15 % to < 60 % animals responding at > 0.2 % to ≤ 20 % topical induction dose).

According to CLP criteria, a classification **Skin Sens. 1B – H317 (May cause an allergic skin reaction)** is warranted for TBPEH.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The dermal sensitisation potential of TBPEH was evaluated in a Buehler assay according to OECD TG 406 (Unnamed, 1996, Klimisch 2). Ten male and ten female Guinea pigs were topically treated with 25 % w/v TBPEH in mineral oil, once per week, for 3 consecutive weeks. Following a two-week rest period, challenge exposure with 5 % w/v TBPEH in mineral oil was performed. As the group mean dermal scores were similar in the test animals compared to the challenge control animals, a re-challenge exposure with 2 % w/v TBPEH in mineral oil was performed following another week of rest period. A valid positive control with hexylcinnamaldehyde was performed concurrently. The study had a reliability of 2 according to Klimisch score. According to the CLH dossier, following rechallenge with 2 % w/v TBPEH in mineral oil, dermal scores of 1 were noted in 5/19 (26 %) test animals at the 24-h scoring interval; however, the dermal responses did not persist to the 48-hour scoring interval. Therefore, the Dossier Submitter (DS) considered that TBPEH (in mineral oil) was positive in the Buehler test (≥ 15 % to < 60 % animals responding at > 0.2 % to ≤ 20 % topical induction dose[1]) and proposed classification as Skin Sensitisation Category 1B for TBPEH.

Comments received during consultation

The lead registrant on behalf of the Organic Peroxides Consortium agreed with the proposed classification for Skin Sensitisation Category 1B.

The proposed classification of Category 1B was supported by one Member State Competent Authority (MSCA). Another MSCA proposed to consider Category 1 without sub-categorisation since for topical induction concentrations of TBPEH ≤ 0.2 % were not tested and therefore a strong potency of TBPEH cannot be excluded.

The latter MSCA pointed to the observation that positive reactions in 3/10 (30 %) control animals after 24 h and in 2/10 (20 %) after 48 h may be due to irritating properties at 5 % challenge concentration whereas no response in control animals was seen at 2 % concentration for re-challenge.

Assessment and comparison with the classification criteria

RAC agrees that the rate of 26 % responsive animals (5/19; dermal score of 1) at a 2 % w/v re-challenge concentration of TBPEH at the 24-h scoring interval warrants a classification for skin sensitising properties. It is noted that the dermal score of the remaining animals was 0 and observed responses in test animals did not persist until the 48-h scoring interval.

Based on the results from the re-challenge with 2 % w/v TBPEH, the criteria for Skin

[1] Note that the DS referred to (> 0.2 % to ≤ 20 %). In this context related to the induction concentration of 25 %, the range cited in brackets is not appropriate.

Sens. 1B are fulfilled with ≥ 15 % (26 % in the study) animals responding at > 20 % (i.e., at 25 % w/v TBPEH) topical induction dose. Based on these results and according to Table 3.8 of the Guidance on the Application of the CLP criteria (CLP guidance, ECHA, 2017), TBPEH is of moderate potency. However, as a topical induction concentration of ≤ 0.2 % TBPEH has not been tested, a higher grade of potency and thereby a classification as Skin Sens. 1A cannot be excluded. Thus, insufficient information is available to appropriately assess the sub-category. RAC concludes that **classification for Skin Sens. 1, H317 (May cause an allergic skin reaction), without a sub-categorisation** is warranted.

11.6 Germ cell mutagenicity

Not evaluated in this dossier.

11.7 Carcinogenicity

Not evaluated in this dossier.

11.8 Reproductive toxicity

11.8.1 Adverse effects on sexual function and fertility

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reproduction/developmental toxicity screening test (OECD TG 421) Wistar rat Males and females, n=10 animals/sex/dose	**TBPEH** purity not specified 0, 100, 300 and 1000 mg TBPEH/kg bw/day, Duration of Exposure : Treatment beginning (females/males): day-1 of pre-pairing -Pre-pairing (females/males): 14 days -Pairing (females/males): until mating (maximum 14 days) -Gestation (females): about 21 days -Parturition (females): expected : on day 21 or 22 post coitum	P0: At 1000 mg/kg bw/day: decreased food consumption in male and female animals during the first week of the pre-pairing period and lactation. Slight decreased bw in males until necropsy; in females: slight increase of bw during gestation but significant decreased bw during lactation. Some dams were noted with ruffled fur and/or bad condition. No test item-related effects were noted during necropsy. Reproduction parameters: All mated females were pregnant. At 1000 mg/kg bw/d: Increase in post-implantation loss and reduction in the number of live pups. F1 offsprings: Reduction in mean body weights of pups and increase in post-natal loss at 1000 mg/kg/day	Anonymous 2008 from ECHA website Klimisch score: 1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	-Lactation (females): until day 4 post partum -Treatment ending: females: on day 3 post partum		
OECD 443, EOGRTS including cohorts 1A and 1B Han:WIST rats P0: n= 24/sex/group, 4 groups Control animals received the vehicle, only. F1 for Cohort 1A n= 20 animals/sex/group F1 for Cohort 1B n= 20 animals/sex/group	**TBPEH** Purity : not specified 0 (vehicle), 100, 300 and 1000 mg/kg bw/day Gavage (oral): once daily Vehicle: sunflower oil Exposure : All animals of the parent (P) generation dosed prior to mating (10 weeks) and throughout mating. In addition, males received the test item or vehicle after mating up to the day before the necropsy (altogether for 153-156 days). Dams additionally exposed through the mating and gestation periods and up to lactation days 21-23 (altogether for 100-129 days).	**<u>Parental generations (P0 and P1):</u>** No mortality related to treatment. No adverse clinical signs of systemic toxicity related to the test item at any dose level except salivation and nuzzling up the bedding material for P0 male and female animals at 300 and 1000 mg/kg bw/day shortly after the administration with variable incidence and duration in a dose related manner. From 300 mg/kg bw/day: elevated weights of kidneys and liver in male and female animals of Cohort 1A and Cohort 1B. <u>at 1000 mg/kg bw/day</u> Reduced body weight in P0 males : -13% of the control on day 152; cohort 1B males: -11% on day 154; cohort 1A males : -12% on day 90 and cohort 1B females : -4% of the control on day 36; cohort 1A females: -6% day on 42. Decreased bw gain in the most cases during the observation period only in P0 and P1 males. No effect on food consumption. Slightly elevated urine volume and lowered pH levels in both sexes in P0 and P1A generations. Slightly lower free triiodothyronine (FT3) and free tyroxine (FT4) levels in P0 and P1 animals as compared to the control. No alterations in the TSH levels, organ weight or histology of thyroid gland. Changes in various organ weights (including elevated liver weight) but not associated with histological alterations. Changes in kidneys: elevated weight (P0 and P1; both sexes) with chronic progressive nephropathy (only P0 males) **<u>Reproduction parameters in P0 and P1 generations:</u>** <u>at 1000 mg/kg bw/day</u> Irregular estrous cycle (P0), number and percentage of female animals in prolonged estrous slightly higher (P1) Reduced reproduction index in P0 and P1 animals. A second mating showed that the male animals of P0 generation – which did not fertilize their partners of main group – mated successfully with non-treated females, suggesting a female effect. Slightly longer mean duration of pregnancy of P0 dams.	Anonymous 2020 Klimisch score: 1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Decreased number of developing follicles and increased number of follicular atresia in P0 females (pregnant or non-pregnant) compared with their control. Not reported in P1. **F1 offspring** at 1000 mg/kg bw/day: Cohort 1B: Percentage of offspring showing signs (no milk in the stomach, cold, found dead, missing, alopecia) higher. Higher extra uterine mortality on PND0 (2% vs 0%) and PND0-21 (9% vs 1%), reduced pup body weight on PND0 (-6.5 %) and bw gain between PN0-21 (- 8.6 %). Similar effect on litter weight (-14% on PND21). Lower percentage of pups with positive response and higher percentage of pups with negative response at surface righting reflex, pinna detachment - but not statistically significant - and eye opening (statistically significant; positive results: 31% versus 58%). Shorter absolute anogenital distance but not when normalized. Cohort 1A: Longer period of vaginal patency (ex. 65% versus 100% at PND34) and appearance of the first cornified vaginal smear (34.3 versus 34.2 day) **F2 offspring** at 1000 mg/kg bw/day: Percentage of found dead F2 offspring higher (+12%). Mortality higher from PND0. Lower BW (-8% on PND4) and BW gain of pups between PND0 and 4 (-13%). Also see for litter based. Shorter absolute anogenital distance but not when normalized.	

Table 9: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
OECD Guideline 408 (90-Day Oral Toxicity Study) Hsd.Brl.Han:	TBPEH Doses tested : 0, 70, 150 and 450	10 animals per sex per dose in the main study. Additionally, 5 animals per sex in	No mortality, no toxic signs in clinical observations and in the course of the functional observation battery. Salivation with variable frequency within a group but in a dose related manner regarding	Anonymous 2013 from ECHA website

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Wistar rats Oral:gavage Exposure time : 90 or 91 days GLP	mg/kg bw/d Vehicle : sunflower oil	the control and high dose group (recovery group).	the incidence and onset. No difference in body weight and body weight gain. Daily mean food consumption similar in all groups. No changes in hematology and biochemistry. No macroscopic or histopathological alterations at necropsy. No change in mean weights of examined organs at any dose level. No effect on the estrous cycle. No effect on the sperm cells (count, motility and morphology).	
OECD guideline 407 (28-Day Oral Toxicity Study) Fischer 344/DuCrl male and female Oral : gavage Exposure time : 28 days GLP	TBPEH Doses tested : 0, 100, 316 and 1000 mg/kg bw/day Vehicle : corn oil	Dose levels selected based on data obtained during the 7-day dose-range-finding toxicity study. Two controls (with and without recovery) and four dose groups (n=5 animals per group and sex).	No mortality. No effect in clinical observations. No change in body weight and weight gain. No difference in food consumption in both sexes. Decrease in number of platelets in the blood of the mid and the high dose females groups and some minor liver changes (higher organ weights) in the high dose groups of both sexes, elevated plasma alkaline phosphatase level in the high dose females group. Indication for renal tubular alterations in the high dose females group.	Anonymous 2009 from ECHA website

11.8.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

TBPEH (purity not stated) was tested in a reproduction/developmental toxicity screening test according to OECD TG 421. TBPEH was administered orally (by gavage) to Wistar rats at repeated doses of 0, 100, 300 and 1000 mg/kg body weight/day, for 14 days during the pre-pairing period, and the pairing, the gestation and the lactation periods until 3 post partum.

All animals survived until scheduled necropsy. Treatment at 1000 mg/kg was associated with decreased food consumption in male and female animals during the first week of the pre-pairing period and during lactation. During pre-pairing period, mean body weight of the males exposed to 1000 mg/kg bw/d was slightly decreased starting on day 3 and continuing until necropsy. In females exposed to 1000 mg/kg bw/day, the mean body weight gain was slightly increased during gestation but statistically decreased during lactation. During the last two days of gestation or the first two days of lactation, seven dams (gestation period) and four dams (lactation period), respectively, were noted periodically to have ruffled fur and / or a generally bad condition. No test item-related effects were noted during necropsy.

For reproduction parameters, all mated females were pregnant leading to a fertility index of 100%. However, treatment at 1000 mg/kg was associated with an increase of post-implantation loss (no numerical value available). Developmental toxicity results obtained are evaluated under section “Adverse effects on development”.

An Extended One-Generation Reproduction Toxicity Study (EOGRTS) (Unnamed, 2020) in the rat according to OECD TG 443 (including production of a second generation) is also available using TBPEH, including the production of a second generation (cohort 1A and 1B; but without cohorts for developmental neurotoxicity and immunotoxicity).

The effect of the test item was examined on the male and female reproductive performance, such as gonadal function, estrous cycle, mating behavior, conception, parturition, gestation, lactation and weaning (P0 and Cohort 1B generation) and on the offspring viability, neonatal health and mortality, growth and development of the offspring (Cohort 1A and Cohort 1B later producing F2 generation) to adulthood following oral (by gavage) administration.

Four groups of Han:WIST rats (n= 24/sex/group) were administered with the test item orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day of TBPEH. Control animals received the vehicle, sunflower oil, only. The suitability of the vehicle at the intended concentrations of the test item was analytically verified up front (concentration and homogeneity). Concentration of the test item in the dosing formulations varied in the acceptable range between 92 % and 107 % of the nominal values and formulations were homogenous thereby confirming the proper dosing.

All animals of the parent (P0) generation were dosed prior to mating (10 weeks) and throughout mating. In addition, males received the test item or vehicle after mating up to the day before the necropsy (altogether for 153-156 days). Dams were additionally exposed through the mating and gestation periods and up to lactation days 21-23 (altogether for 100-129 days).

Clinical observations (clinical signs, body weight, food consumption, estrous cycle) and pathology (clinical and organ pathology) examinations were performed on parental (P) animals for signs of toxicity, with special emphasis on the integrity and performance of the male and female reproductive systems. Estrous cycle was monitored by examining vaginal smears before the mating for two weeks and during the mating period until evidence of mating and on the day of the necropsy. The dams were allowed to litter and rear their offspring up to day 21 post-partum. As the number of pregnancies was low (16/21) in the high dose group, a second mating of the P0 males with untreated females (untreated group; n= 8 naïve females) was performed to clarify if the male fertility of the high group was impaired. Dams and offspring in the untreated group were terminated on post-partum/ PND (post-natal days) 5-8.

All F1 offspring were observed individually for the health, growth, development and function up to and including post-natal day 21 (clinical signs, body weight, surface righting reflex, pinna detachment, eye opening, anogenital distance). Twenty animals/sex/group for Cohort 1A and 20 animals/sex/group for Cohort 1B were randomly selected on post-natal day 21 for follow-up examinations. Dosing of offspring selected for follow-up examinations (Cohort 1A and Cohort 1B) begun on post-natal day 22 and treatment was continued up to the day before the necropsy. The foetal/offspring (from implantation) parameters are further evaluated under section ‘Adverse effects on development’.

F1 offspring (Cohort 1A and Cohort 1B) were observed during adulthood (P1) identically to parental animals – clinical signs, body weight, food consumption, estrous cycle, clinical pathology and organ pathology. Sexual maturity of offspring (Cohort 1A and Cohort 1B) was investigated by observation of balano-preputional separation, vaginal patency and appearance of first cornified vaginal smear. Cohort 1A animals were subjected to necropsy, organ weighing and sperm analysis – one day after the termination of the exposure – on PND 91-97. Cohort 1B animals were mated to produce a second (F2) generation after at least 90-day pre-mating period and were observed identically to parental (P0) animals. F2 offspring were observed and subjected to necropsy up to PND 5-8.

Blood samples were collected for determination of serum levels of thyroid hormones (FT3, FT4 and TSH) from 3-5 F1 pups per litter (where it was feasible) on PND 4, from 1-2 pups/10 litters on PND 22, from 10 dams (P) /group and from 10 parental (P) male animals/group at termination, from 10 male animals/ group and from 10 female animals in Cohort 1A, from all male and female animals in Cohort 1B at termination and from F2 pups on post-natal day 5 or shortly thereafter.

All adult animals (P0, Cohort 1A and Cohort 1B) were subjected to gross pathology with complete tissue preservation one day after the last treatment. Brain, spleen, thymus and mammary tissues were preserved for 10 male and 10 female pups per group – where feasible – in F1 offspring not selected for cohorts on PND22

or shortly thereafter and in F2 offspring on PND5 or shortly thereafter. Special attention was paid to the organs and tissues of the reproductive system for P, F1 or F2 animals.

Selected organ weights were determined in adult animals (P0, P1) (cohort 1B for males: kidneys, brain, testis, epididymides, seminal vesicle, prostate, pituitary gland; for females: brain, kidneys, uterus, ovaries, pituitary gland); (cohort 1A for males : brain liver, heart, thymus, spleen, testes, epididymides, seminal vesicle, prostate, adrenal glands, thyroids, pituitary gland; for females : brain, liver, kidneys, heart, thymus, spleen, uterus, ovaries, adrenal glands, thyroid, pituitary gland) and in offspring (PND22 or shortly thereafter and in F2 offspring on PND5) (brain, spleen, thymus)

Sperm parameters were determined in all control and high dose male animals in P0 generation and in P1 generation (Cohort 1A and Cohort 1B).

Full histopathology examinations were performed on the organs and tissues of adult animals (P0, Cohort 1A and Cohort 1B) in control and high dose groups with special emphasis on sexual organs and tissues. Reproductive organs were also processed and examined histologically in non-mated and non-pregnant female animals and their mating partners (P0 and Cohort 1B) in the low and mid dose groups. In addition, organs showing macroscopic changes were also processed and examined histologically in adult animals in low or mid dose groups (P0, Cohort 1A and Cohort 1B) and in F1 offspring. The kidneys of male animals at 100 and 300 mg/kg bw/day were processed and evaluated histologically based on the organ weight data and histological observations at 1000 mg/kg bw/day.

Based on the low reproduction index and histopathological findings in parental (P0) female animals at 1000 mg/kg bw/day, a quantitative evaluation of primordial, small growing (secondary and tertiary) follicles, as well as corpora lutea was performed in all adult female animals (P0, Cohort 1A and Cohort 1B) in the control, 100, 300 and 1000 mg/kg bw/day.

The results were interpreted comparing treatment groups with respect to controls, which were treated concurrently with vehicle (sunflower oil) only.

There was no test item related mortality in 100, 300 or 1000 mg/kg bw/day groups in adult animals (P0, Cohort 1A and Cohort 1B) during the course of study. Salivation and nuzzling up the bedding material were noted for P0 male and female animals (but not in Cohort 1A or 1B) at 300 and 1000 mg/kg bw/day shortly after the administration with variable incidence and duration in a dose related manner.

The mean body weight gain and mean body weight were slightly reduced in male animals at 1000 mg/kg bw/day (P0 males : -13% of the control, day 152, Cohort 1B males: -11%, day 154; Cohort 1A males : -12%, day 90). The body weight of female animals in Cohort 1A and Cohort 1B at 1000 mg/kg bw/day was slightly lower during the first weeks of the treatment period (cohort 1B females : -4% of the control, day 36; Cohort 1A females: -6%, day 42) and the difference with respect to the control recovered during the following weeks. The food consumption was not adversely affected in male or female animals (P0, Cohort 1A and Cohort 1B) at 100, 300 and 1000 mg/kg bw/day.

There was no treatment-related toxicological relevant effect on hematological and clinical biochemistry parameters in any of the groups, sex and cohorts. At urinalysis, slightly elevated urine volume and lowered pH levels were apparent in both sexes in P0 generation and in both sexes in Cohort 1A at 1000 mg/kg bw/day.

There was a slightly lower FT3 and FT4 levels in males of P0 generation and of Cohort 1A and 1B at 1000 mg/kg bw/day as compared to the control. There were no accompanying alterations in the TSH levels, organ weight or histology of thyroid glands.

Specific macroscopic alterations related to the effect of the test item were not detected in male or female animals at 100, 300 or 1000 mg/kg bw/day at the terminal necropsy (P0, Cohort 1A and Cohort 1B).

Various organ weights were modified. Elevated weights of kidneys were indicative of the test item effect in P0 male animals at 1000 mg/kg bw/day and in male and female animals at 300 and 1000 mg/kg bw/day of P1 generation (cohort 1A and cohort 1B). Elevated liver weights were reported in male and female parental P0 animals at 1000 mg/kg bw/day and in male and female animals of Cohort 1A and Cohort 1B at 300 and 1000 mg/kg bw/day.

Histopathological investigations revealed chronic progressive nephropathy (CPN) in a higher incidence of P0 male animals at 1000 mg/kg bw/day but not in animals in Cohort 1A or Cohort 1B.

Effects on reproduction:

The estrous cycle was irregular in several P0 female animals at 1000 mg/kg bw/day during the two last weeks of the ten weeks pre-mating period. Statistical significance was noted for the lower percentage of female animals with regular cycle (54% versus 83%) and for the lower mean number of days in pro-estrous (1.5 versus 3) at 1000 mg/kg bw/day. The number of female animals in prolonged estrous was also higher than in the control group at 1000 mg/kg bw/day (29% versus 0%). In cohort 1A, the estrous cycle was irregular in several female animals in the control, 100, 300 and 1000 mg/kg bw/day groups during the two weeks observation period. The number and percentage of female animals in prolonged estrous was slightly higher than in the control group at 1000 mg/kg bw/day (15 % versus 0/20).

In the female animals of the control and 1000 mg/kg bw/day groups, the ovaries, uterus, cervix, vagina had a normal structure characteristic of the species, age and phase of the active sexual cycle. However, decreased number of developing follicles and increased number of follicular atresia (mean number follicular atresia: 13.2 versus 5.3 in control at quantitative evaluation of ovaries) were detected by qualitative histological as well as quantitative evaluation in P0 female animals at 1000 mg/kg bw/day compared with their control. The same effects were seen in the females not achieving pregnancy. Similar findings were not detected in female animals in Cohort 1A and Cohort 1B.

The investigated organs of male reproductive system (testes, epididymides, prostate, seminal vesicles, coagulating glands) were histologically normal and characteristic of the sexually mature organism in all P0, Cohort 1A and Cohort 1B male animals at 1000 mg/kg bw/day. Sperm examinations did not reveal any test item related influence on the sperm cell morphology and motility, and total sperm count at 1000 mg/kg bw/day (P0, Cohort 1A and Cohort 1B).

The reproductive performance, represented by the reproduction index (= percentage of pregnant females), was reduced in P0 animals at 1000 mg/kg bw/day (67% versus 91% in controls) and in Cohort 1B animals at 300 mg/kg bw/day (80% versus 95% in controls) and 1000 mg/kg bw/day (56% versus 95% in controls). A second mating showed that the male parental animals (P0) exposed at 1000 mg/kg bw/day group – which did not fertilize their partners of main group – mated successfully with non-treated females (n= 8 naïve females). This information suggests that the decrease in fertility of treated rats originate from alteration of reproductive function of the females.

The slightly longer mean duration of pregnancy of P0 dams at 1000 mg/kg bw/day was statistically significant (22.37 versus 21.97 days).

The mean number of implantation sites were comparable in all groups.

The foetal/offspring (from implantation) parameters are further evaluated below under section ‘Adverse effects on development’.

In summary, under the conditions of the EOGRTS, TBPEH caused:

- effects on reproductive function: irregular estrus cycle (P0 and P1 female), decreased number of developing follicles and increased number of follicular atresia (P0 females), decreased reproduction index (P0, Cohort 1B). These effects were mainly reported at 1000 mg/kg bw/day. At 300 mg/kg bw/day, reproduction index was also decreased in cohort 1B animals;
- general toxicity, including body weight reduction (mainly in males of P0, Cohort 1A, Cohort 1B generations), effects in kidneys (elevated weight, chronic progressive nephropathy (only in P0 males)). These effects were mainly reported at 1000 mg/kg bw/day. At 300 mg/kg bw/day, some changes in organ weights, without histopathological findings, were reported.

Based on data, the NOAEL for parental systemic toxicity and reproductive performance was determined at 100 mg/kg bw/day by the registrants based on slight changes in liver and kidneys weights and disturbed reproductive ability at 300 mg/kg bw/d, respectively. Dossier submitter notes that the NOAEL for parental systemic toxicity set by the registrants is highly conservative in the light of the effects observed at 300 mg/kg bw/day (only changes in organ weights).

Other data: 28-day and 90-day oral (gavage) studies (OECD 407 and OECD 408) have investigated systemic effects of TBPEH in rats. Data below are issued from disseminated ECHA website.

TBPEH was tested in a 28-day repeated dose toxicity study by oral route (gavage) in male and female Fischer, F344/DuCrl rats (n=5) according to OECD guideline 407, including recovery groups for control and high dose animals. The test item was administered at 100, 316 and 1000 mg/kg bw/day. Dose levels administered in the study were selected based on data obtained during a 7-day dose-range-finding toxicity study. The substance caused a decrease in the number of platelets in the blood of the mid and the high dose females groups and some minor liver changes (higher organ weights in the high dose groups of both sexes, elevated plasma alkaline phosphatase level in the high dose females group). There was also a borderline indication for renal tubular alterations in the high dose females group. There was a sex difference in the response to the test substance with different effects in both sexes and a somewhat higher susceptibility of the females. The NOAEL set by the registrants was 316 mg/kg bw/day in males and 100 mg/kg bw/day in females.

TBPEH was tested over a prolonged period of time (90 days) followed by a 28-day recovery period in order to assess reversibility, persistence or delayed occurrence of potential toxicological effects. The substance was administered orally (by gavage) to Hsd.Brl.Han: Wistar rats (n=15 animals/sex in the control and high dose groups, n= 10 animals/sex in the low and middle dose groups) once a day at 0, 70, 150 or 450 mg/kg bw/day. 5 animals per sex in the control and high dose groups were observed without administration for four weeks (recovery observations). There was no substance related mortality, no toxic signs at any dose level in the daily and detailed weekly clinical observations and in the course of the functional observation battery. Salivation was observed in the male and female treated animals with variable frequency within a group but in a dose related manner regarding the incidence and onset. No substance-related effects on body weight, or body weight gain were observed with respect to controls at any dose level during the course of the study. The daily mean food consumption was similar in animals of the control and test item treated groups. There were no abnormalities in the eyes of animals in the high dose group at termination of the treatment. No substance-related changes were observed in investigated hematology, blood coagulation parameters and clinical chemistry examinations. Specific macroscopic and histopathological alterations related to the substance were not detected from necropsy observations. The mean weights (absolute and relative to the body and brain weights) of examined organs were not affected by the test item at any dose level. A substance influence on the estrous cycle was not detected. Sperm analysis did not reveal substance influence on the sperm cells (count, motility and morphology) at 450 mg/kg bw/day dose. The NOAEL set by the registrants was determined at 450 mg/kg bw/day for male and female animals.

11.8.3 Comparison with the CLP criteria

For potential classification on sexual function and fertility, criteria from CLP guidance (ECHA, 2017c) are applied.

- Adverse effects on sexual function and fertility are described as "*Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*." *(ECHA, 2017c).*
- *Known human reproductive toxicant. "The classification of a substance in this Category 1A is largely based on evidence from humans."*

There are no human data. Thus Cat. 1A is not fulfilled for TBPEH.

- *Presumed human reproductive toxicant. "The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic*

information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate" (ECHA, 2017c).

Two experimental studies have specifically investigated effects of TBPEH in rodent on reproductive function.

One reproduction/developmental toxicity screening study performed in rats according to TG 421 did not show clear effects on sexual function and fertility. Regarding the increase of post-implantation loss, this is rather considered as a developmental effect but it cannot be excluded that it is secondary to fertility alteration. In addition, it can be noted that the OECD 421 guideline study is a screening assay. According to the OECD guideline, this protocol is only "*designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition*". Statistical analysis is also rather limited since only 10 animals of both sexes were included.

One Extended One-Generation Reproduction Toxicity Study including cohort 1A and 1B according to TG 443 in Wistar is also available.

Regarding parental toxicity, at 1000 mg/kg bw/day, a slight reduction in mean body weight gain and mean body weight was noted in P0 and P1 generations. This effect is particularly observed in males, with decreases reaching a max. of -13% in P0 on day 152. Some changes were observed in various organ weights (including kidney and liver) at 1000 mg/kg bw/d in both parental generations with no supporting pathological alteration at histology, except for P0 males that presented chronic progressive nephropathy. At this dose, there was also a reduction of thyroid hormones (T3 and T4) in males of both generations and effects in urinalysis (higher volume, lower pH). At 300 mg/kg bw/day, the reported findings mainly consisted on some changes in organ weights.

Regarding fertility, the estrous cycle parameters were changed at the high dose in P0 and 1A cohorts. In P0 females, there was a statistically significant lower percentage of female animals with regular cycle (54% versus 83%) and lower mean number of days in pre-estrous (1.5 days versus 3 days). The number of female animals in prolonged estrous was higher (29% versus 0%). In P1 generation, the number and percentage of female animals at high dose in prolonged estrous was slightly higher (15 % versus 0%). This finding is consistent with observations in the P0 generation and considered substance related.

The reproductive performance was reduced in parental (P0) animals at 1000 mg/kg bw/day and in Cohort 1B animals at 300 and 1000 mg/kg bw/day. In P0 generation, the number of pregnant females (reproduction index = 67%) was statistically lower and the number of non-pregnant females animals was statistically higher (91%) with respect to their control group at 1000 mg/kg bw/day. In P1 generation, a lower reproduction index was also observed at 1000 mg/kg bw/day (56% versus 95%) and 300 mg/kg bw/day (80%). A second mating showed that the male parental animals (P0) exposed at 1000 mg/kg bw/day group – which did not fertilize their partners of main group – mated successfully with non-treated females. This suggests that the decrease in fertility of treated rats originate from alteration of female reproductive functions. A decreased number of developing follicles and increased number of follicular atresia (mean number follicular atresia: 13.2 versus 5.3 in control at quantitative evaluation of ovaries) were observed in pregnant or non-pregnant animals, but only in P0 females at 1000 mg/kg bw/d. Copulatory index was also lower in P1 exposed to 1000 mg/kg bw/day (90% versus 100%).

Finally, a slightly longer mean duration of pregnancy of dams at 1000 mg/kg bw/day was statistically significant (22.37 days versus 21.97 days) in P1 females. The value was at the upper of the historical control range (21.8-22.3 days; 13 studies).

There was no effect on reproductive organs reported in 28-day and/or 90-day studies in rats at tested doses up to 1000 or 450 mg/kg bw/day, respectively. No change in oestral cycles or sperm parameters was noted in the 90-day study with doses up to 450 mg/kg bw/day.

In conclusion:

Clear fertility effects, mainly characterised by a decrease of reproduction index, were consistently found in both generations. In particular, this effect occurred at lower doses in P1 (from 300 mg/kg bw/day) than in P0

(1000 mg/kg bw/day) suggesting that P1 animals are more sensitive to TBPEH toxicity than P0. Other findings that can be related to effects on sexual function and fertility included changes in oestral cycles in both generations, histopathological effects in ovaries in P0 females, lower copulatory index in P1 and slightly longer mean duration of pregnancy were reported at 1000 mg/kg bw/d.

In comparison, general toxicity is slight and particularly restricted to males exposed to TBPEH at 1000 mg/kg bw/day (decreased body weight < -13% mainly in males; histopathological effects in kidney only in P0 males). At 300 mg/kg bw/day, there are only changes in organ weights, without associated histopathological findings.

In summary, fertility effects are consistent between generations and cannot be considered secondary to a general toxicity. So, criteria for classification as Repr. 1B for fertility are fulfilled.

11.8.4 Adverse effects on development

Table 10: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal Developmental Toxicity Study Pregnant female Hsd. Brl. Han: WISTAR rats 24/dose group According to OECD TG 414 Deviations: no GLP: yes	**TBPEH** Purity: confidential 200, 400 and 1000 mg/kg bw/day Exposure: GD 5 to GD 19 Exposure via gavage	Dams: No death, no effect on corrected BW and no necropsy findings in treated groups. Only salivation and decreased food consumption during first 6 days of treatment reported. Offspring: No dose-related significant difference in the intrauterine mortality of the conceptuses, the number of implantations, the number of viable fetuses and their sex distribution. The incidence of visceral abnormalities was statistically significantly ($p<0.05$) higher in the 1000 mg/kg bw/day dose group (3 hydroureter + 1 umbilical hernia). No increase in the incidence of external and visceral variations in treated groups. No increase in skeletal malformations. Slightly but statistically significant ($p<0.01$) increase in the incidence of fetuses with incomplete ossification of the skull-bones and metacarpal/metatarsal at 1000 mg/kg bw/day.	Anonymous 2013 Klimisch score : 1 Key study
Prenatal Developmental Toxicity Study inseminated New Zealand White rabbits (26-27 animals/dose group) According to OECD TG 414 Deviations: no GLP: yes	**TBPEH** Purity: confidential 30, 100 and 300 mg/kg bw/day. Exposure: From GD 6 to GD27	**Dams**: At 30 mg/kg bw/day: 1 abortion At 100 mg/kg bw/day: 2 abortions. Lower bw gain (GD15-18) with reduced food consumption. At 300 mg/kg bw/day: 8 abortions. 4 moribund dams. Bleeding of the vagina in 6 animals. Lower mean body weight until GD24 (-6% on GD24) and bw gain between GD6-12 and GD15-18; reduced food consumption up to GD21. No stat. significant effect when corrected bw and bw gain considered. On GD28: 22, 24, 20 and 17 females with implantation site(s). **Offspring**: 20, 23, 16 and 10 litters evaluated in the control, 30, 100 and 300 mg/kg bw/day group respectively At 100 mg/kg bw/d: No effect on parameters assessed.	Anonymous 2018 Klimisch score :1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		At 300 mg/kg bw/day: Increase of early embryonic death (15% versus 6%) and post-implantation loss (17% versus 8%). Decrease of mean number of viable fetuses (not stat. signif). Significantly lower fetal weight (-15.6%) and crownrump length (-7.3%). No significant difference in incidence of overall fetal malformations among the experimental groups. Increasing skeletal variations: proximal and middle phalanges less than 7/7 ossified (18% versus 7%), misaligned and fused sternebra (2% versus 0%), multiple malformed ribs and vertebrae (3% versus 0%).	
See also section 11.8.1: developmental effects are reported in the OECD TG 421 study and in an EOGRTS performed with TBPEH.			

11.8.5 Short summary and overall relevance of the provided information on adverse effects on development

Two prenatal developmental toxicity studies, in rats and rabbits, are available with TBPEH.

A first prenatal developmental toxicity study (Anonymous, 2013) was performed in rats according OECD TG 414. Groups of 24 sperm-positive female Hsd. Brl. Han: Wistar rats were treated with TBPEH by oral administration daily at three dose levels of 200, 400 and 1000 mg/kg bw/day from day 5 up to and including day 19 post coitum. A control group of 24 sperm positive females was given the vehicle sunflower oil. During the study, mortality was checked for and clinical observations were performed. Body weight and food consumption of the dams were also recorded. The day when sperm was detected in the vaginal smear was regarded as day 0 of gestation. A Caesarean section and gross pathology were performed on gestational day 20. The number of implantations, early and late resorptions, live and dead fetuses in each uterine horn and the number of corpora lutea were recorded. Each fetus was weighed and examined for sex and gross external abnormalities. The placentas were weighed and examined externally. About half of each litter was preserved for visceral examination and the other half of the litters were preserved for skeletal evaluation. At visceral examination the bodies were micro dissected by means of a dissecting microscope. The heads were examined by Wilson's free-hand razor blade method. After cartilage-bone staining the skeletons were examined by means of a dissecting microscope. All abnormalities found during the fetal examinations were recorded. In total, there were 21, 19, 23 and 19 evaluated litters in the control, 200, 400 and 1000 mg/kg groups, respectively.

One pregnant female in the control group was found dead on gestational day 20. No other clinical signs than alopecia in a few females unrelated to the treatment and salivation in the 400 and 1000 mg/kg bw/day groups immediately after treatment were observed. This was attributed to be an effect of the treatment, however as non-adverse. There were no findings observed at necropsy. There was no indication of an effect of the substance on body weight development and food consumption of the dams in the 200 and 400 mg/kg bw/day dose groups. The statistically significantly ($p<0.01$) reduced body weight gain on the first three days of treatment and the statistically significantly ($p<0.01$) reduced food consumption in the first week of treatment in the 1000 mg/kg bw/day dose were not considered adverse, in particular, considering that the corrected body weight and corrected body weight gain were not affected. There was no dose related significant difference in the intrauterine mortality of the conceptuses, the number of implantations, viable fetuses and their sex distribution.

The number of late embryonic death increased slightly in a statistically significant manner ($p<0.05$) in the 400 mg/kg bw/day dose group (sum = 7 versus 1 in the control group; but not significant if the mean percentage value was considered (3%)) and without a statistical significance in the 1000 mg/kg bw/day group (sum = 5; percentage = 2%). Registrants considered this finding as non adverse, since there was in the range of the historical control data of the laboratory (5 studies in Wistar rats using vehicle control groups and treated groups with no adverse effects: "inactive treatment"; 2009-2011): 0.0-1.4% in control group (n = 80 animals) and 0.9-5.0% in "inactive treatment" groups. Dossier submitter considers the use of this latter group as highly questionable. Thus, reference to this group has not been made thereafter.

There was a statistically significant ($p<0.01$) reduction in the body weight of the male and female fetuses in the 1000 mg/kg bw/day group (combined weight = 3.3 g). Registrants considered this finding as non adverse, since this was in the range of the historical control data of the laboratory (3.0-3.4 g in control group).

Absolute placental weight was similar in all experimental groups. There was a statistically significant increase in the relative placental weight in the 1000 mg/kg bw/day dose group (204.3 mg/g). It should be noted that relative placental weights reported in this study (for control and treated groups) were below the historical control level of the laboratory (210.0 – 234.4 mg/g).

Regarding external examination, umbilical hernia occurred in one fetus in the 1000 mg/kg bw/day dose group.

The incidence of visceral abnormalities was statistically significantly ($p<0.05$) higher in the 1000 mg/kg bw/day dose group (4%), mainly due to variations (which are not significantly increased when considered individually: bilateral hydroureter (0, 1, 0, 1 in each group, respectively) and hydroureter with dilated renal pelvis (1, 0, 0, 2, in each group, respectively)).

There was no test item related effect at skeletal examination of the fetuses in the 200 and 400 mg/kg bw/day dose group. The incidence of the fetuses with skeletal variations increased significantly ($p<0.01$) in the 1000 mg/kg bw/day dose group due to the higher incidence of the delayed ossification of skull and metacarpal/metatarsal. Skeletal malformations were found only in the control and 1000 mg/kg bw/day dose group with an incidence of 2 and 1 respectively, thus the test item did not induce skeletal malformations.

The NOAEL were determined by the registrants as follows:

- NOAEL maternal toxicity: 1000 mg/kg bw/day

- NOAEL developmental toxicity: 1000 mg/kg bw/day

A second prenatal developmental toxicity study (Anonymous, 2018) was performed in rabbits according to OECD TG 414. It consisted of oral treatment of inseminated New Zealand White rabbits with TBPEH at dose levels of 30, 100 and 300 mg/kg bw/day respectively from day 6 up to and including day 27 post insemination. Four rabbits went into moribund state at 300 mg/kg bw/day. Eight aborted in the 300 mg/kg bw/day group, two in the 100 mg/kg bw/day group and one in the 30 mg/kg bw/day group. 1, 0, 2, 2 animals in each group died due to technical reason. Overall, there were 22, 24, 20 and 17 females with implantation site on GD28 and 20, 23, 16 and 10 litters evaluated in the control, 30, 100 and 300 mg/kg bw/day group respectively. Thus, it should be noted that the number of litters evaluated at the highest tested dose was rather low compared to control.

At the dose level of 100 mg/kg bw/d, TBPEH caused slightly reduced food consumption and lower body weight gain (only statisticaly significant on GD15-18). Two females aborted at this dose. At 300 mg/kg bw/day, there was a lower mean body weight in dams until GD24 (-6% on GD24; no statistical difference thereafter) and body weight gain between GD6-12 and GD15-18. This was associated with reduced food consumption up to GD21 (ex. -40.4% between GD 18-21; not statistical difference between GD21-GD28). There was also a lower body weight gain at 100 mg/kg bw/d (on GD6-9; GD9-12 and GD15-18; no statistical difference thereafter) with reduced food consumption. However, corrected body weight and body weight gain was not statistically significantly affected. Eight females aborted at this dose.

Early embryonic death (15% versus 6% in control) and post-implantation loss (17% versus 8% in control) (data compared to number of implantations) were increased at 300 mg/kg bw/day. These effects are statistically significant if the number and percent of resorptions are evaluated and not statistically significant if the mean number and SD are calculated, probably due to the high standard deviation) and mean number of

viable fetuses were decreased (sum: 91 viable fetuses in treated group versus 191 in controls; not statistically significant) in the 300 mg/kg bw/day dose group.

There was no significant difference in incidence of overall fetal malformations among the experimental groups. However, significantly lower fetal weight (-15.6%) and crownrump length (-7.3%) were observed in the 300 mg/kg bw/day dose group. At this dose, were also reported increasing skeletal variations (proximal and middle phalanges less than 7/7 ossified (18% versus 7%), misaligned and fused sternebra (2% versus 0%) and multiple malformed ribs and vertebrae (3% versus 0%)).

The NOAELs were determined by the registrants as follows:

- NOAEL maternal toxicity: 30 mg/kg bw/day

- NOAEL developmental toxicity: 100 mg/kg bw/day based on statistical significant changes in body weight and body weight gain, increased early embryonic death, increased post-implantation loss, slight decrease in mean number of viable fetuses, significant lower fetal weight and crown-rump length, increased incidence in number of fetuses with skeletal variations at 300 mg/kg bw/d.

In addition, developmental effects are reported in the OECD TG 421 study and EOGRTS.

In the OECD TG 421 study, treatment at 1000 mg/kg was associated with an increase of post-implantation loss. Post-natal loss was statistically increased (5/10 dams affected), with a reduction of live pups (43 dead and 20 pups missing until day 4 post-partum). The mean body weight of pups was also reduced at this dose, up to day 4 post-partum. The NOAEL set by the registrants was 300 mg/kg bw/day for parental and developmental toxicity. It should be noted that the level of information available for this study is very limited (no numerical value available).

In the EOGRTS, effects on development, characterised by a higher extra-uterine mortality (F1 and F2), a lower survival index (F2), clinical signs (F1 and F2), reduced body weight (F1 and F2) and slower development of reflexes (F1), were reported in offsprings. These effects were mainly reported at 1000 mg/kg bw/day. At 300 mg/kg bw/day, a lower body weight was also reported in pups. Details are summarized below:

The mean number of post-implantation loss, the mean number of total births, mean number of viable pups and live born and the live birth index (live pups/total birth) were comparable in all groups. Delivery data of dams was not adversely affected at 100, 300 or 1000 mg/kg bw/day dose level (P0 and Cohort 1B).

A higher mortality (F1 and F2) was reported on PND0 (2% versus 0% for F1; 12% versus 0% for F2), between PND0-4 (15% versus 3% for F2) and between PND0-21 (9% versus 1% for F1). A reduced body weight on PND0 (-6.5% in F1 and -5% in F2) and body weight gain (-8.6% between PND0-21 for F2; -13% between PND0-4 for F2) were observed in offspring at 1000 mg/kg bw/day. Lower pup body weight was also reported at 300 mg/kg bw/day (-3.2% for F1 and -3.4% for F2 on PND0). Clinical signs, such as no milk in the stomach, cold pups, were reported in the treated groups.

There was some statistical significance in the percentage of pups with positive response or lower percentage of pups with negative response at 100 mg/kg bw/day (pinna detachment, eye opening) or at 300 mg/kg bw/day (eye opening). At 1000 mg/kg bw/day, the percentage of pups with positive response was lower and the percentage of pups with negative response was higher than in the control group at surface righting reflex, pinna detachment and eye opening. Statistical significance was only reached for eye opening on PND14 (44% with positive response compared to 58% in the control group).

Regarding sexual maturity, the balano-preputional separation was completed in all F1 Cohort 1A male animal groups – control, 100, 300 and 1000 mg/kg bw/day – on post-natal day 35, although, the mean body weight was slightly lower with respect to the control group in male animals at 1000 mg/kg bw/day on PND35. In the F1 Cohort 1A female animals, statistical significance was noted for the longer period of vaginal patency at 1000 mg/kg bw/day (ex. 65% versus 100% at PND34) and at the longer period of appearance of the first cornified vaginal smear at 100 and 1000 mg/kg bw/day (34.2 day and 34.3 day versus 32.3 in the control group). The interval between days of vaginal patency and first cornified smear were similar in all groups (control, 100, 300 and 1000 mg/kg bw/day). Statistical significance was detected at the shorter absolute anogenital distance of male and female F1 pups and male F2 pups at 1000 mg/kg bw/day.

However, the normalized anogenital distances were comparable. In F1 pups, nipples/areoles were not visible in any of the examined male offspring in the control or 100, 300 or 1000 mg/kg bw/day groups on post-natal day 13.

Histological investigations did not reveal test item related pathologic changes in the examined organs in F1 offspring.

The NOAEL for offsprings was set by the registrants at 300 mg/kg bw/day based on higher mortality (F1 and F2), lower survival index (F2), clinical signs (F1), reduced body weight (F1 and F2) and slower development of reflexes (F1) for F1, F2 at 1000 mg/kg bw/d. However, the dossier submitter notes that a lower pup body weight was also observed at 300 mg/kg bw/d.

In both studies (OECD 421 and EOGRTS), parental toxicity consisted mainly in slightly decreased body weight, mostly in males (see section 11.8.2).

11.8.6 Comparison with the CLP criteria

For potential classification on development, criteria from CLP guidance (ECHA, 2017c) were applied.

"Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency."

- *Known human reproductive toxicant "The classification of a substance in* ***Category 1A*** *is largely based on evidence from humans" (ECHA, 2017).*

There is no existing epidemiological studies. Therefore, classification as Repr. 1A is not fulfilled.

- *The classification of a substance in this* ***Category 1B*** *is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in* ***Category 2*** *may be more appropriate.*

Data on animals report consistent effects on development.

Two experimental prenatal developmental toxicity studies were performed in rats and in rabbits according to OECD TG 414.

The first prenatal developmental toxicity study (OECD TG 414) in rats tested 0, 200, 400, 1000 mg/kg bw/day of TBPEH. At all doses tested, no effect on corrected body weight and body weight gain was observed in rats. Thus, no maternotoxicity was evidenced up to 1000 mg/kg bw/day. Regarding developmental toxicity, at 1000 mg/kg bw/day, there was a slight but statistically significant ($p<0.01$) reduction in mean body weight of male and female fetuses (within historical control data according to registrants). Incidence of skeletal variations (incomplete ossification of the skull-bones and metacarpal/metatarsal) was also increased at this dose.

The second prenatal developmental toxicity study (OECD TG 414) in rabbits tested 0, 30, 100 and 300 mg/kg bw/d of TBPEH. Abortion and moribund animals were found at the highest tested dose. In dams, body weight (until GD24), body weight gain (GD6-12 and 15-18) and food consumption (until GD21) were lower than control group at 300 mg/kg bw/day. However, there was no statistically significant effect when

corrected body weight and body weight gain were considered. Regarding developmental toxicity, at 300 mg/kg bw/d, increase of early embryonic death and post-implantation loss (statistically significant if number and percent of resorptions evaluated) was noted. A slight decrease (without a statistical significance) in mean number of viable fetuses was observed. Fetal weight and crown-rump length were significantly lower. Incidence in number of fetuses with skeletal variations (proximal and middle phalanges less than 7/7 ossified, misaligned and fused sternebra, multiple malformed ribs and vertebrae) was observed.

Developmental toxicity of TBPEH was also assessed in a reproduction/developmental toxicity screening test (OECD TG 421) and in an extended one generation study, including the production of a second generation (cohort 1A and 1B) (OECD TG 443).

In the OECD TG 421 study, TBPEH was administered orally (by gavage) to Wistar rats at repeated doses of 0, 100, 300 and 1000 mg/kg body weight/day, for 14 days during the pre-pairing period, and the pairing, the gestation and the lactation periods until 3 post partum. At 1000 mg/kg bw/day, reduction of body weight and/or body weight gain was reported in males during the entire study and in females during lactation. This was associated with a decreased food consumption. Treatment at 1000 mg/kg was associated with an increase of post-implantation. Post-natal loss was also statistically increased, with a reduction of live pups (until day 4 post-partum). The mean body weight of pups also was reduced at this dose, up to day 4 post-partum.

In the OECD TG 443 study, rats were administered with TBPEH orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day. TBPEH did not cause overt general toxicity in the mother up to the highest tested dose but interfered with development of the offsprings. Indeed, at 1000 mg/kg bw/day, higher post-natal mortality (observed from PND0) and reduction of body weight in both F1 and F2 offsprings were reported. There were also effects on post-natal development regarding absolute anogenital distance and eye opening, at this dose. At the mid dose of 300 mg/kg bw/day, lower body weight of F1 and F2 pups was also noted. In comparison, parental general toxicity is slight and particularly restricted to males exposed to TBPEH at 1000 mg/kg bw/day (decreased body weight < -13% mainly in males; histopathological effects in kidney only in P0 males). At 300 mg/kg bw/day, there are only changes in organ weights (in particular increase of kidney and liver weights) in both sexes, without associated histopathological findings.

The developmental adverse effect findings are consistent among studies and generations and occurred in the absence of overmaternotoxicity. They are visible in two species and cannot be considered as secondary non-specific consequence of other toxic effects. Therefore, the criteria for classification as Repr. 1B for development are fulfilled for TBPEH.

11.8.7 Adverse effects on or via lactation

11.8.8 Short summary and overall relevance of the provided information on effects on or via lactation

There are no experimental data specifically related to adverse effects on or via lactation. However, some information can be derived from the OECD TG 421 study and the EOGRTS (see sections above). Post-natal losses and decreased body weight of pups were found in both studies.

In the OECD TG 421 study in rats, treatment at 1000 mg/kg was associated with an increase of post-natal loss and a reduction of live pups (until day 4 post-partum). The mean body weight of pups was also reduced at this dose, up to day 4 post-partum.

In the extended one generation according to OECD TG 443 in rats, at 1000 mg/kg bw/day, a higher percentage of offspring showed signs (no milk in the stomach, cold, found dead, missing, alopecia). There was also a higher extra-uterine mortality on PND 0 (2% versus 0% in F1 pups; 12% versus 0% in F2 pups), between PND0-4 (15% versus 3% in F2 pups) and between PND 0-21 (9% versus 1% in F1 pups). Reduction of body weight on PND 0 (-6.5 % for F1 pups; -5% for F2 pups) and of body weight gain between PND0-4 for F2 pups (-12.8%) and between PND0 - PND21 for F1 pups (- 8.6 %) were observed. At 300

mg/kg bw/day, F1 pups showed lower body weight (-6.5%) at PND0, due to male body weight and F2 pups showed a lower body weight (-3% on PND4).

11.8.9 Comparison with the CLP criteria

The two criteria suggested by ECHA (2017c) were checked for classification for adverse effects on or via lactation:

1. *"Substances which are absorbed by women and have been shown to interfere with lactation. This relates to effects in the mother that impact adversely on the breast milk, either in terms of the quantity produced or the quality of the milk produced (i.e. the composition). Any effect on the quantity or quality of the breast milk is likely to be due to systemic effects in the mother. However, overt maternal toxicity may not be seen (e.g. the substance may just affect the transfer of a nutrient into the milk with no consequence for the mother)."*

There is no study investigating the quantity and quality of the milk produces, or any suggestion in studies available that TBPEH can have an impact on breast milk production.

2. *"Substances which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child. This relates to the ability of the substance (including metabolites), to enter the breast milk in amounts sufficient to cause a concern. When the effect on the offspring is caused by the substance (or metabolite) after transport through the milk then the maternal toxicity has no relevance for classification."*

- *"Ideally, studies will be available which inform directly on whether the substance causes adverse effects in the offspring due to an adverse effect on lactation. One generation or multi-generation reproductive toxicity studies, which involve direct exposure or exposure via the milk of the offspring postnatally, usually provide information on this." (ECHA, 2017c).*

There is no study investigating the analysis and presence of metabolites of TBPEH in milk.

From the EOGRTS, an inadequate nursing behaviour of dams can be suggested considering the lower pup body weight during lactation and the higher extra-uterine mortality with the fact that some pups did not have milk in the stomach. In contrast, the fact that the observed adverse effects occurred from PND0 supports that they are consecutive to exposure during gestation. Overall, it cannot be clearly distinguished if these post-natal effects were caused by gestational exposure and/or lactation exposure.

A classification on or via lactation is not warranted for TBPEH.

11.8.10 Conclusion on classification and labelling for reproductive toxicity

Classification for reproductive toxicity addresses adverse effects on sexual function and fertility, developmental effects and adverse effects on or via lactation.

<u>Adverse effects on sexual function and fertility</u>

Impairment of fertility is reported in the EOGRTS 443 with decreased number of developing follicles and increased number of follicular atresia observed in pregnant or non-pregnant animals, irregular estrous cycle and lower reproduction index. These effects occurred in the absence of overtoxicity in parental animal. So a classification as Repr. 1B – H360F is warranted.

<u>Adverse effects on development</u>

In the OECD TG 421 study, rats were administered with TBPEH orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day. Treatment at 1000 mg/kg was associated with an increase of post-implantation. Post-natal loss was also statistically increased, with a reduction of live pups (until day 4 post-partum). The mean body weight of pups also was reduced at this dose, up to day 4 post-partum.

In the OECD TG 443 study, rats were administered with TBPEH orally (by gavage) once a day at 0 (vehicle), 100, 300 and 1000 mg/kg bw/day. TBPEH did not cause overt toxicity in the mother up to the

highest tested dose but interferes with development of the offsprings. This include, at 1000 mg/kg bw/day, higher post-natal mortality (observed from PND0) and reduction of body weight in both F1 and F2 offsprings. There were also effects on post-natal development regarding absolute anogenital distance and eye opening, at this dose. At the mid dose of 300 mg/kg bw/day, lower body weight of F1 and F2 pups was also noted.

Therefore, these studies can be used by themself for classification purpose.

Two experimental Prenatal Developmental Toxicity Studied were performed in rat and in rabbit according guideline 414. The first OECD TG 414 study was performed in rat exposed to 0, 200, 400, 1000 mg/kg bw/day of TBPEH. Even of at all doses tested, no effect on corrected body weight and body weight gain was observed in rats. A slight but statistically significant reduction in mean body weight of male and female fetuses, and a higher incidence of skeletal variations was noted. The second OECD TG 414 study was performed in rabbits exposed to 0, 30, 100, 300 mg/kg bw/day of TBPEH. At high dose, abortions occurred. Moribund animals and clinical signs were also reported. Mean body weight of the animals was lower until GD24 and body weight gain decreased at GD6-12 and GD15-18. But there was no statistically significant effect when corrected body weight and body weight gain were considered. Food consumption was reduced up to GD21. Increase of early embryonic death and post-implantation loss, and a slight decrease in mean number of viable fetuses were observed. Fetal weight and crown-rump length were significantly lower. Incidence in external variations increased significantly.

The available data in the four *in vivo* studies showed clear evidence of an effect of TBPEH on pup development. TBPEH should therefore be classified as Repr. 1B – H360D.

Adverse effects on or via lactation

There was no specific study assessing effects via or on lactation. From the EOGRTS, an inadequate nursing behaviour of dams can be suggested considering the lower pup body weight during lactation and the higher extra-uterine mortality with the fact that some pups did not have milk in the stomach. In contrast, the fact that the observed adverse effects occurred from PND0 support that they are consecutive to exposure during gestation. Overall, post-natal effects could not clearly be distinguished from effects caused by gestational exposure.

A classification on or via lactation is not warranted for TBPEH.

Table 57: Summary table of comparison between main adverse effects reported on general toxicity, fertility and development

Protocol	Parental toxicity	Fertility effect	Developmental toxicity
OECD 421 study in rats Dosage: 0, 100, 300, 1000 mg/kg bw/day Anonymous, 2008	**At 1000 mg/kg bw/day** Clinical signs in females at the end of gestation and beginning of lactation. Decreased BW in males during the whole study and in females during lactation. Decreased food consumption.	/	**At 1000 mg/kg bw/day** Increase of post-implantation loss, postnatal loss. Mean BW up to day 4 post-partum reduced.
OECD 443 in rats Dosage: 0, 100, 300, 1000 mg/kg bw/day Anonymous, 2020	**At 1000 mg/kg bw/day** **P0 generation** No mortality. Clinical signs as salivation and nuzzling up the bedding material. Lower BW in males (Day 152; -13% of the control) with decreased BW gain in the most cases during the observation period. No significant effect on BW and BW gain in females. Changes in various organ weights. Chronic progressive nephropathy in males, with increase kidney weight and changes of some urinalysis parameters (higher volume of urine and lower pH). Lower mean FT3 and FT4 in males. **P1 (second parental generation)** F1 cohort 1B: No test-item related mortality. BW reduced in males (-11%) and females from PND22-36 (max. -8% on PDN29) and higher on lactation day 4 (L4) (2%) (no effect on BW gain). Changes in various organ weights. No pathological alteration at histology. Reduction in T3 and T4 in males (and T4 in females). F1 cohort 1A: No test-item related mortality.	**At 1000 mg/kg bw/day** **P0 generation** Decreased number of developing follicles and increased number of follicular atresia (13.2 versus 5.3) Lower number animals with regular cycle (54% versus 83%), lower number of days in proestrous (1.5 versus 3). Reproduction index: 67% versus 91% Slightly longer mean duration of pregnancy of dams (22.37 versus 21.97 days). **P1 generation** Number and percentage of female animals in prolonged estrous slightly higher (15 %). Lower reproduction index (56% versus 95%). Copulatory index lower as 2 males failed to mate (95% versus 100%). **At 300 mg/kg bw/day:** Reproduction index reduced	**At 1000 mg/kg bw/day** **Cohort F1B generation pups** Higher percentage of offspring with clinical signs. Higher mortality on PND 0 (2% versus 0%) and between PND 0-21 (9% versus 1%). Reduced BW on PND 0 (-6.5 %). BW gain between PND0 - PND21 depressed (- 8.6 %). Similar effect on litter weight (-14% on PND21). Lower percentage of pups with positive response and higher percentage of pups with negative response at surface righting reflex, pinna detachment - but not statistically significant - and eye opening (statistically significant; positive results: 31% versus 58%). Shorter absolute anogenital distance but not when normalized. **Cohort 1A:** Longer period of vaginal patency and appearance of the first cornified vaginal smear (34.3 versus 34.2 day) **F2 generation** Clinical signs. Percentage of found dead F2 offspring higher (+12%) from PND0. Lower BW (-8% on PND4) and BW gain of pups between PND0 and 4 (-13%). Shorter absolute anogenital distance but not when normalized. **At 300 mg/kg bw/day:** Lower BW in F1 pups (-6.5% at PND0; due to male) and in F2 pups

	BW reduced in males (-12% on day 90) (with some decreases in BW gain) and females from PND22-42 (max. -10% on PDN25). No pathological alteration at histology. Higher volume of urine in males, lower pH of urine in females. Elevated kidney weight. Reduction of T3 and T4 in males. **At 300 mg/kg bw/day:** Some changes in organ weights in P0 and P1. Lower pH of urine in cohort 1A females. Reduction of T3 and T4 in cohort 1B males.	(80% versus 95%)	(-3% on PND4).
OECD 414 study in rats Dosage: 0, 200, 400, 1000 mg/kg bw/day Anonymous, 2013	**At all doses:** No effect on corrected BW and BW gain	**Not applicable**	**At 1000 mg/kg bw/day** Incidence of skeletal abnormalities increased with a statistical significance due to the increase in the variations (incomplete ossification of the skull-bones and metacarpal/metatarsal).
OECD 414 study in rabbits Dosage: 0, 30, 100, 300 mg/kg bw/day Anonymous, 2018	**At 300 mg/kg bw/day:** Abortions increased significantly (8 versus 0 in control). Moribund animals (16% versus 0%). Clinical signs reported. Mean body weight of the animals lower until GD24 (-6% on GD24). Decreased BW gain GD6-12 and GD15-18. No significant effect when corrected BW and BW gain considered. Reduced food consumption up to GD21.	**Not applicable**	**At 300 mg/kg bw/day:** Increase of early embryonic death and post-implantation loss (statistically significant if number and percent of resorptions evaluated): 41 versus 20 and 36 versus 13, respectively. 4 dams with total post-implantation loss Lower fetal weight (-15.6%) and crown-rump length (-7.3%) with one litter completely affected (i.e. 10/10 fetuses). Incidence of external variations increased due to growth retardation (15% versus 3%). Increase incidence of fetuses with skeletal abnormalities (42 versus 27%): proximal and middle phalanges less than 7/7 ossified, misaligned and fused sternebra, multiple malformed ribs and vertebrae

In summary, a classification as Repr. 1B – H360 FD is warranted for TBPEH.

RAC evaluation of reproductive toxicity

ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

Summary of the Dossier Submitter's proposal

For assessing the adverse effects on sexual function and fertility of TBPEH, the DS cited 4 studies:

- A reproduction/developmental toxicity screening test according to OECD TG 421 (Anonymous, 2008; Klimisch 1) with TBPEH (purity not specified) which did not show clear effects on sexual function and fertility up to 1 000 mg/kg bw/d. Increases in post-implantation loss were reported, but the DS considered this rather as a developmental effect, although the DS could not exclude that it is secondary to adverse fertility effects. The DS indicated that no full study report was available.
- An Extended One-Generation Reproduction Toxicity Study (EOGRTS) according to OECD TG 443 (Anonymous, 2020; Klimisch 1) with TBPEH (purity not specified) in the rat according to OECD TG 443 (including production of a second generation), in which impairment of fertility characterised by a decrease of reproduction performance and reproductive index in parental (P0) animals and Cohort 1B animals was observed. A second mating showed that the male parental animals (P0), which did not fertilise the treated females of the same dose group, mated successfully with non-treated females, suggesting that the decrease in fertility of treated animals originates from an alteration of reproductive function of the females. Other findings that can be related to effects on sexual function and fertility included irregular oestrous cycles at the high dose in P0 animals, histopathological effects in ovaries in pregnant and non-pregnant P0 females (decreased number of developing follicles and an increased (mean) number of follicular atresia), a lower copulatory index in F1 males and females, and a slightly longer mean duration of pregnancy. No abnormalities were observed in male reproductive organs or on sperm counts, morphology, or motility.

  General toxicity was reported to be slight and particularly restricted to males exposed to TBPEH at 1 000 mg/kg bw/d. It consisted of lower (absolute) mean body weight (up to -13 %) mainly in males; increased relative organ weights of liver and kidneys in both parental generations, chronic progressive nephropathy observed only in P0 males (necropsy on day 153-156), higher urine volume and lower urine pH and reductions of thyroid hormones T3 and T4. At 300 mg/kg bw/d, there were changes in relative organ weights (histopathology not evaluated).
- A 28-d oral (gavage) study with TBPEH (purity not specified) in rats according to OECD TG 407 (Anonymous, 2009), in which no effects on reproductive organs were reported at tested doses up to 1 000 mg/kg bw/d.
- A 90-d oral (gavage) study with TBPEH (purity not stated) in rats according to OECD TG 408 (Anonymous, 2013), in which no effects on reproductive organs,

oestrous cycle or sperm parameters were reported at tested doses up to 450 mg/kg bw/d.

The DS concluded that the fertility effects observed in the EOGRTS are consistent between the generations and cannot be considered secondary to general toxicity. Hence, the DS considered that classification of TBPEH as Repr. 1B; H360F is warranted.

Comments received during public consultation

Two MSCAs supported the classification of TBPEH as Repr. 1B, H360F. The lead registrant on behalf of the Organic Peroxides Consortium also agreed with the proposed classification as Repr. 1B, H360F.

Assessment and comparison with the classification criteria

RAC agrees that the results of the available EOGRTS (according to OECD TG 443; Anonymous, 2020) with TBPEH (unknown purity) in rats (Cohort 1B animals were mated to produce a second (F2) generation) showed clear adverse effects on sexual function and fertility induced by TBPEH treatment, without evidence of severe general systemic toxicity.

The adverse effects included:

Oestrus cycle:

- P0 females: a statistically significantly lower percentage of females with regular oestrus cycle (54 % versus 83 %; effect was dose-dependent), a lower mean number of days in pre-oestrous (1.5 days versus 3 days; effect was dose-dependent and gained significancy at 1 000 mg/kg bw/d) and a higher number of females in prolonged oestrous (29 % versus 0 %; effect was dose-dependent) at 1 000 mg/kg bw/d.
- F1 females: slightly higher number and percentage in prolonged oestrous at the high dose (15 % versus 0 %); irregular oestrus cycle observed in all groups, including controls.

Reproductive performance:

- P0 animals: decreased reproductive index (67 % pregnant females versus 91 % in controls) at 1 000 mg/kg bw/d.
- F1 animals: lower copulatory index at 1 000 mg/kg bw/d (males: 90 % versus 100 % in controls; females: 95 % versus 100 % in controls).
- F1 females: a dose-dependently lower reproductive index at 300 mg/kg bw/d and 1 000 mg/kg bw/d (80 % and 56 % pregnant females, respectively, versus 95 % in controls); statistically significant at 1 000 mg/kg bw/d.
- A second mating showed that the male parental animals (P0) exposed at 1 000 mg/kg bw/d that did not fertilise the treated females of the same dose group, mated successfully with non-treated females (n = 8), suggesting that the decrease in fertility of treated rats originate from alterations of female reproductive functions.

Female histopathology of reproductive organs:

- P0 females: decreased number of developing follicles.
- P0 females: increased mean number of follicular atresia in pregnant and non-pregnant animals at 1 000 mg/kg bw/d (13.2 versus 5.3 in controls at quantitative evaluation of ovaries).
- P0 females: follicular cyst in ovaries at 1 000 mg/kg bw/d, one or both sides (3/24 animals).
- F1 females (Cohort 1A): significantly increased number of primordial and primary follicles in all treatment groups.

Pregnancy:

- P0 females: slightly, but statistically significantly, longer mean duration of pregnancy (22.37 versus 21.97 days in controls) at 1 000 mg/kg bw/d (value reported is at upper limit of the historical control data (HCD) range (21.8-22.3 d; 13 studies, no further details on HCD)).

General toxicity:

- P0 and F1 animals: slight reduction in mean body weight gain and mean body weight (bw) at 1 000 mg/kg bw/d, particularly in males (P0 males: throughout the study duration starting at pre-mating day 28, max. of -13 % bw at day 152 (bw gain days 0-152: -28.6 % compared to controls); F1 males: throughout the study duration, max. -11.3 % at day 154 (bw gain not reported); P0 females: no effects on bw, except for lactation day 21 (+5.5 %), slightly increased bw gain at days 0-69 (+2.4 %, n.s.) of the premating period; no effects on bw gain during gestation; statistically significant increase in bw gain during lactation (lactation days 0-21: +82.9 %); F1 females: statistically significant decrease in bw only up to postnatal day (PND) 36, max. at day 22: -8.9 % bw, no significant effects on F1 female bw and bw gain afterwards, except for a higher mean bw gain at GD0-7 and lactation days 0-4).
- P0 and F1 animals: some changes in various organ weights including kidney (relative weight; P0: +51 % in males and +10 % in females; F1: +44 % in males and +15 % in females) and liver (relative weight; P0: up to +18 % in males and +25 % in females; F1: no data reported) at 1 000 mg/kg bw/d with no supporting pathological alteration at histopathology evaluation, except for P0 males that presented chronic progressive nephropathy. At 300 mg/kg bw/d, there were only changes in organ weights (kidney: relative weight; P0: +16 % in males; F1: +13 % in males and +10 % in females and liver: relative weight; P0: +6 % in males; F1: no data reported) without associated histopathological findings. No further details reported.
- P0 males: reduction in thyroid hormones (% free T3 and % free T4, but no effect on TSH; no weight or histopathology effects in thyroid glands)
- F1 animals: reduction in % free T3 in males at ≥ 300 mg/kg bw/d and % free T4 in males and females at 1 000 mg/kg bw/d; the latter was significantly increased in males at 100 and 300 mg/kg bw/d; TSH below detection limit at all doses; no histopathology effects in thyroid glands; dose-dependent increase in relative thyroid to brain weight.
- P0 and F1A males and females: effects on urinalysis (higher volume, lower pH) at 1 000 mg/kg bw/d.

Other relevant studies performed with TBPEH, including a reproduction/developmental toxicity screening test (OECD TG 421), a 28-d and a 90-d oral toxicity study (OECD TG 407 and 408, respectively), did not result in adverse effects on male or female fertility and/or female oestrus cycling. However, exposure during pre-mating is much shorter in a screening study (14 days) and lower doses were used in the 90-day oral toxicity study (≤ 450 mg/kg bw/d) compared to the EOGRTS (exposure for ten weeks pre-mating, ≤ 1 000 mg/kg bw/d).

Overall, RAC concludes that treatment with TBPEH induced adverse effects on sexual function and fertility that cannot be considered secondary to other non-specific toxic effects. TBPEH treatment led to reduced number of pregnant females, changes in oestrous cycle and histopathological effects in the ovaries, as well as a slightly prolonged pregnancy period in rats without concurrent severe systemic toxicity. In conclusion, RAC agrees with the DS that **classification of TBPEH as Repr. 1B; H360F, is warranted.**

ADVERSE EFFECTS ON DEVELOPMENT

Summary of the Dossier Submitter's proposal

The DS considered that the criteria for classification in Category 1B for adverse effects on development (Repr. 1B; H360D) are fulfilled for TBPEH, as the developmental effects observed in two pre-natal developmental toxicity (PNDT) studies (according to OECD TG 414) in rats and rabbits, respectively, in a reproductive screening test (OECD TG 421) and an EOGRTS (OECD TG 443) in rats are consistent between the studies, species and generations. In addition, effects occurred in the absence of overt maternal toxicity and were hence not considered as secondary, non-specific consequence of other toxic effects.

In the PNDT study in Wistar rats (Anonymous, 2013; Klimisch 1), animals were exposed via oral gavage to doses of 0, 200, 400, 1 000 mg/kg bw/d (purity confidential), on gestation days (GD) 5-19.

Effects observed in this study included a slight but statistically significant decrease in mean foetal body weight at 1 000 mg/kg bw/d (6 %; said to be within HCD of the laboratory, no details provided), as well as an increase in the incidence of combined skeletal variations (32 % at 1 000 mg/kg bw/d versus 6 % in controls, significant for the sum of foetuses and non-significant for litter means: 6 per litter versus 3.7 per litter in controls): incomplete ossification of the skull bones (13 % versus 0 % in controls; marked = three bones or more in 7 % of pups versus 0 % in control pups) and incomplete ossification of metacarpal/metatarsal, less than three ossified (6 % versus 0 % in controls).

In addition, non-significant increase in the incidence of bilateral hydroureter (1, 0, 1 at 200, 400, 1 000 mg/kg bw/d, respectively, versus 0 in controls) or with unilateral hydroureter with dilated renal pelvis (0, 0, 2 at 200, 400, 1 000 mg/kg bw/d, respectively, versus 1 in the controls) were observed; the bilateral and unilateral hydroureters combined were 1, 0, and 3 at 200, 400, 1 000 mg/kg bw/d, respectively, versus 1 in controls. Based on the hydroureter findings, the percentage of visceral variations (4 % at 1 000 mg/kg bw/d versus 1 % in control and low dose groups) were

significantly increased. No maternal toxicity was observed in this study. No effect on corrected body weight and body weight gain was observed at any of the doses. During the first days of treatment there was a transient reduction in food consumption (GD5-8: -22 %, GD8-11: -14 %) and body weight gain (GD5-8) at the dose level of 1 000 mg/g bw/d followed by increased body weight gain on GD8-11 (no effect on absolute body weight).

In the PNDT study in New Zealand White rabbits (Anonymous, 2018; Klimisch 1), animals were exposed to 0, 30, 100 and 300 mg/kg bw/d (purity confidential) on GD6-27.

In this study, 8 dams (32 %) out of 25 pregnant aborted and 4 additional moribund dams (16 %) were found at 300 mg/kg bw/d. Bleeding from the vagina (associated to abortion or post-implantation loss) and other clinical signs of maternal toxicity (gastro-intestinal tract findings, weakness, reduced activity) were observed at this dose. In dams of this treatment group, lower body weight (significant on GD9-24), transient body weight loss (significant at GD6-12 and GD15-18), lower body weight gain (GD6-12 and 15-18) and lower food consumption (significant from GD6 to 21) compared to controls were seen, but terminal body weight and body weight gain (throughout the study) were according to the CLH report not affected when corrected for gravid uterine weight. At 100 mg/kg bw/d, reduced food consumption, lower body weight gain (significant on GD15-18) and 2 animals (10 %) that aborted were observed. Abortion in one dam (4 %) was seen at 30 mg/kg bw/d versus none in the control group. Single incidences of blood in the bedding were observed for dams at 30 and 100 mg/kg bw/d, both aborted.

An increase of early embryonic death (36 (15 %) at the dose level of 300 mg/kg bw/d versus 13 (6 %) in controls) and post-implantation loss (41 (17 %) at 300 mg/kg bw/d versus 20 (8 %) in controls (statistically significant for both number and percentage of resorptions) at this dose was noted, and a decrease in absolute number of viable foetuses (sum: 91 viable foetuses (38 % of sum of implantations) at 300 mg/kg bw/d versus 191 viable foetuses in control group (81 % of sum of implantations); reported as not statistically significant) was observed as well.

There were 4 dams with total post-implantation loss in the 300 mg/kg bw/d group and the number of viable foetuses per litter was non-significantly lower than in controls (6.5 compared to 8.3 in controls). Foetal weight (-15.6 %) and crown-rump length (-6.7 cm (-7.3 %) in mean crown-rump-length (male + female) compared to controls) were significantly lower at 300 mg/kg bw/d. The number of foetuses with retarded body weight and crown rump length was significant higher (14 % each versus 2 % and 3 %, respectively, for both effects). Higher incidence in numbers of foetuses with skeletal variations due to increased incidences of delayed ossified proximal and middle phalanges (18 % of foetuses affected versus 7 % in controls, not significant on a litter basis), misaligned and fused sternebrae (2 % of litters affected versus 0 % in controls, no effect on the numbers of foetuses with this effect) and multiple malformed ribs and vertebrae (3 % of foetuses versus 0 % in controls, significant also on a litter base in 2 litters (20 %) versus none in controls) were observed at 300 mg/kg bw/d. No significant or dose-related effects were observed at 100 mg/kg bw/d. No effect on the ureters (compared with some hydroureters in the rat PNDT study) were seen.

In the OECD TG 421 study in Wistar rats (Anonymous, 2008; Klimisch 1), 0, 100, 300 and 1 000 mg/kg bw/d of TBPEH (purity not specified) was administered orally (by

gavage) for 14 days during the pre-pairing, pairing, gestation, and lactation period until PND3. At 1 000 mg/kg bw/d, a slight reduction in mean body weight (no details whether significancy was reached) from day 3 until necropsy and significantly decreased food consumption during the first pre-mating week only was reported in parental males. Decreased food consumption and lower body weight gain was seen in the lactation phase (sacrifice on day 4 post-partum). Clinical signs (fur and/or bad general health condition) was noted during the last two gestation days (seven dams) and the first two days of the lactation period (four dams). No treatment-related effects were seen on the fertility index, number of corpora lutea, implantation rate or gestation length. Treatment at 1 000 mg/kg bw/d was associated with an increase in post-implantation loss (4.9 % versus 1.5 % in the control group, statistically significant), post-natal loss (5/10 dams affected, statistically significant) and a reduction in live pups (43 dead and 20 pups missing) until day 4 post-partum. The mean body weight of pups was reduced at the high dose, up to PND4. Study authors reported that post-implantation losses and effects on pup viability and weight were considered as test-item related. No further quantitative details are available.

In the OECD TG 443 study in Wistar rats (Anonymous, 2020; Klimisch 1), TBPEH at doses of 0, 100, 300 and 1 000 mg/kg bw/d (purity not specified) did not cause overt general toxicity in the P0 or F1 (Cohort 1B) dams up to the highest tested dose but interfered with development of the offspring. At 1 000 mg/kg bw/d, higher post-natal mortality rates than in controls were reported in F1 (2 % on PND0; 9 % on PND0-21) and F2 offspring (12 % on PND0, 15 % on PND0-4). The mean body weight (PND0-21 in F1 pups, PND0-4 in F2 pups) and litter weight gain (PND4-21 in F1 pups, PND0-4 in F2 pups) was significantly lower at the high dose level. There were also effects on absolute anogenital distance (AGD) (significant reduction in absolute but not normalised AGD) and eye opening on PND14 (significant lower percentage of animals) at this dose. At the mid dose of 300 mg/kg bw/d, lower mean body weight in F2 pups (no effect in F1 pups) was also noted.

The mean body weight remained significantly lower during the offspring's development on PND22-90 in Cohort 1A males and on PND22-42 in Cohort 1A females. Food consumption during this period was unaffected in females or transiently lower in males.

Parental general toxicity, on the other hand, was slight and particularly restricted to P0 and F1 Cohort 1A males exposed to TBPEH at 1 000 mg/kg bw/d (lower body weight, up to -13 % versus controls, mainly in males; histopathological effects in kidney only in P0 males). At 300 mg/kg bw/d, changes in organ weights (in particular relative increase of kidney and liver weights) in both sexes were noted, without associated histopathological findings. Historical control data are reported to be available and used for the evaluation. However, no details on the source of HCD and on means/medium values and their ranges were presented.

Comments received during consultation

One MSCA supported classification of TBPEH as Repr. 1B; H360D. Another MSCA noted that the study descriptions lack a number of quantitative data that are considered necessary for an assessment of the reproductive toxicity on development of TBPEH. A more detailed reporting in the dossier would have been appreciated by this MSCA and a

recommendation for a more thorough evaluation (e.g., of maternal toxicity, post-natal loss, etc.) was suggested in order to show why classification for developmental toxicity is warranted. The DS acknowledged that the level of details is limited for the OECD TG 421 study since there was no access to the full study report but that detailed results of the EOGRTS are available in Annex I of the dossier and that RAC will have access to this information. The DS further highlighted the effects reported in the EOGRT study: "Results of EOGRTS show clear effects on development at the high dose: post-natal mortality (max 15 % versus 3 % in control), lower bw (max -13 %) and delayed development (surface righting reflex, pinna detachment, eye opening and AGD). These effects are consistent between F1 and F2 offspring and more pronounced in the F2 generation. Effects on parents are detailed in section 11.8.2 and all numerical data are available in Annex I". Overall, the DS concluded that there are clear and severe developmental effects reported in three different studies that cannot be linked to maternal toxicity, justifying a classification as Repr. 1B; H360D.

One comment from a Company/Manufacturer was received, in which the classification of TBPEH as Repr. 1B; H360D was questioned, and an additional weight of evidence consideration based on all of the available studies was provided (see the RCOM document for responses by RAC). In their analysis, the commenter argued that the effects upon which the proposed classification is based are secondary to a fertility alteration and/or occurred at doses in presence of maternal toxicity. In their view, no classification for development is warranted.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the results of the PNDT study and the EOGRTS in rats showed clear adverse effects on the development that were induced by TBPEH treatment without evidence of severe general systemic toxicity.

With regard to developmental toxicity and malformations the observed adverse effects were:

PNDT study in rat:

- Developmental effects: A slight but statistically significant decrease in mean foetal body weight and a higher incidence of skeletal variations (delayed ossification of skull, metacarpal and metatarsal bones) and visceral alterations (hydroureter) at 1 000 mg/kg bw/d were seen but reported as being within HCD of the laboratory.
- Maternal toxicity: A transient reduction in food consumption (GD5-11) and body weight gain (GD5-8) was observed at 1 000 mg/kg bw/d followed by increased body weight gain on GD8-11. Apart from that, there was no indication of maternal toxicity.
- In conclusion, it cannot be excluded that the observed skeletal variations are secondary to lower pup weight. The observed effect on the pup weight is slight (6 % lower than control values). The pup weights were reported to be within the HCD range, which were not given in the study report. This effect could be considered as treatment related as no maternal toxicity was observed. While the effect on pup weight is consistently seen in other rat studies (OECD TG 421 and the EOGRTS), no increase in post-implantation losses was observed in this study at the dose level of 1 000 mg/kg bw/d. The incidence of the hydroureter is

significantly increased, but the overall increase in the incidence of this variation is low (4 % in total compared to 1 % in controls). Data on HCD are not available.

PNDT study in rabbit:

- Developmental effects: Early embryonic death[2] (15 % versus 6 % in control) and post-implantation loss (17 % versus 8 % in control) (relative to sum of implantations) were increased at the highest dose of 300 mg/kg bw/d. It is likely that these effects resulted in lower absolute number of viable foetuses compared to the control group.

  Foetal weight and crown-rump length were significantly lower at 300 mg/kg bw/d; the relative number of affected foetuses was higher.

  At 300 mg/kg bw/d a higher incidence in numbers of foetuses with skeletal variations were seen. No significant or dose-dependent effects were seen at 100 mg/kg bw/d.
- Maternal toxicity: Moribundity (16 %), weakness, reduced activity and gastro-intestinal tract findings were signs of severe maternal toxicity in rabbits at 300 mg/kg bw/d. Also the high rates of abortion (32 %) and vaginal bleeding were considered to be signs of maternal toxicity in this study (see discussion below). The 10 % incidence of abortion at 100 mg/kg bw/d was also considered indicative of maternal toxicity in this study (with significantly lower food consumption on GD 9-12). A significant and markedly lower food consumption (with very low mean food consumption reduced to a level equivalent of about 30 % of the food consumption of controls) on GD6-21, linked to lower body weight gain on GD6-18 and lower body weight on GD9-24 (causing a bw loss on GD9-21) was observed at the high dose. The corrected body weight at study termination, although reported as comparable to the controls, was only assessed for those 10 dams that did not show abortions, total post-implantation losses or early embryonic deaths.
  Considering the mean corrected body weight and bw gain on GD28 (reflecting the entire duration of pregnancy) for these 10 dams, the mean and median values were non-significantly lower than for controls. However, a reduced food consumption, although less severe than for those dams with abortion, moribundity, etc., was also noted in these 10 dams during the early phase of pregnancy. The median values for the bw gain on GD6-9 and GD9-12 were reduced (significant for GD6-9), concordantly significantly lower food consumption was seen on GD6-9 and GD9-12. Lower foetal weight and crown-rump length, reported for pups of these 10 dams, are likely to be secondary to lower food consumption and bw gain in the early pregnancy.
- In conclusion, due to the high incidence of dams at 300 mg/kg bw/d that aborted or were in a moribund health condition it cannot be excluded that the post-implantation losses and effects on pup development are secondary effects of the maternal toxicity. No treatment-related increase of effects on pup development were seen at 100 mg/kg bw/d or lower. No treatment-related malformations were identified.

[2] *Nishimura, 2001: In NZW rabbits implantation occurs on GD 6-9 depending on the region of the uterus blastocysts are attached to; early embryonic deaths may in rabbits (unlike rats) be seen as developmental effects; organogenesis progresses in parallel.*

OECD TG 421 screening study in rat:

- Developmental effects: Treatment at 1 000 mg/kg bw/d was associated with an increase of post-implantation loss (4.9 % versus 1.5 % in the control group, no details were given in the Annex I to the CLH report), post-natal loss (5/10 dams affected) and a reduction in live pups (43 dead and 20 pups missing until PND4). The mean body weight of the pups was reduced at the high dose, up to PND4.

- Maternal toxicity: At 1 000 mg/kg bw/d there were no signs of general toxicity in the dams during the gestation period. Slightly reduced food consumption was transiently observed during the first week of premating period. Mean body weight of the dams at 1 000 mg/kg bw/d was slightly increased during gestation, while their mean body weight gain was statistically significantly decreased during lactation (sacrificed on PND4) (no details presented). During the last two days of gestation or the first two days of lactation, seven dams (gestation) and four dams (lactation), respectively, were noted periodically to have ruffled fur and/or a generally bad condition. These effects are considered as likely to be attributable to the high number of dead pups (and missing pups that could either be post-implantation losses or stillborn pups cannibalised by their dams).

- In conclusion, the observed pup effects could not be attributed to maternal toxicity and are considered as substance-related developmental effects.

Extended one-generation study in rat:

- Developmental effects: Higher post-natal mortality than in controls were reported in F1 (2 % on PND0 versus 0 % in controls; 9 % on PND0-21 versus 1 % in controls) and in F2 pups (12 % PND0 versus 1 % in controls; 15 % on PND0-4 versus 3 % in controls).

  A reduced body weight on PND0 (-6.5 % in F1 and -5 % in F2 pups) and body weight gain (-8.6 % on PND0-21 in F1 pups; -12 % on PND0-4 in F1 pups; -13 % on PND0-4 in F2 pups) were observed in offspring at 1 000 mg/kg bw/d. Lower pup body weight was also reported at 300 mg/kg bw/d (-3.2 % for F1 and -3.4 % for F2 pups on PND0). Clinical signs, such as no milk in the stomach and cold pups, were reported in the treated groups.

  The mean body weight remained significantly lower during the offspring development on PND22-90 in Cohort 1A males (-12 % on PND90) and on PND22-42 (-6 % on PND42) in Cohort 1A females.

  No effect on post-implantation loss in P0 dams up to 1 000 mg/kg bw/d.

  No detailed information on HCD available.
- Maternal toxicity: No signs of overt general toxicity were observed in the dams up to the highest tested dose of 1 000 mg/kg bw/d.
- In conclusion, the observed pup effects could not be attributed to maternal toxicity and are considered as substance-related developmental effects. The observed effects were considered as treatment-related as no data on laboratory HCD ranges/mean values are available.

RAC concludes on the basis of the PNDT study and the EOGRTS in the rat, supported by

evidence from the OECD TG 421 screening study, that TBPEH induced developmental toxicity which was not secondary to maternal toxicity. At oral doses of 1 000 mg/kg bw/d, TBPEH consistently caused lower pup body weight at PND0 (PNDT, EOGRTS) or PND0-4 (OECD TG 421 study) and/or body weight gain (PND0-21 in F1 and PND0-4 in F2 pups, EOGRTS) were seen in the three rat studies. Reduction in mean body weight continued on PND22-90 in Cohort 1A males and on PND22–42 in Cohort 1A females. Lower body weight gain was also noted in F1 and F2 pups at 300 mg/kg bw/d (EOGRTS).

Increased pup mortality rates were seen at 1 000 mg/kg bw/d TBPEH in the OECD TG 421 study (PND0, PND0-4) and the EOGRTS (PND0 in F1 and F2 pups; PND0-21 in F1 pups and PND0-4 in F2 pups).

Increases in post-implantation losses were observed in the OECD TG 421 study. However, only limited quantitative data are available and information on HCD is lacking in the study report summary. An increased rate of 4.9 % post-implantation losses at 1 000 mg/kg bw/d TBPEH versus 1.5 % in controls was reported in the RCOM document. This effect was also seen in the PNDT on rabbits, but not in the PNDT or EOGRTS in rats. In the OECD TG 421 study, the reporting on a reduction in live pups (43 dead and 20 pups missing until PND4) raises uncertainties about the cause of dead or missing pups. Due to the lack of numerical data, the uncertainties on the dead/missing pups and the lack of HCD, the study report is less informative.

With regard to the PNDT rabbit study, RAC considers that it cannot be excluded that the effects at 300 mg/kg bw/d were due to the severe maternal toxicity observed in pregnant rabbits at this dose level. Beyond the abortions, moribundity, vaginal bleeding, weakness, reduced activity, and gastro-intestinal tract findings, the affected dams at this dose level consumed significantly less food from GD6 until GD21 (around 30 % of the control values). Literature data show that a markedly lower food consumption alone may cause abortions and post-implantation loss in dams and may have effects on pup weight and viability depending on the level of caloric restriction and the onset/duration of the anorexic period in pregnancy (Matsuoka et al., 2006; Matsuzawa et al., 1981). Reduced crown-rump length and foetal weight in the offspring of those 10 dams that did not show abortions, post-implantation losses and moribundity were considered as likely related to less severe, but still significantly lower food consumption throughout the early phase of pregnancy (GD6-12). Based on the uncertainty whether a treatment-relationship for the observed effects in rabbits up to 300 mg/kg bw/d are secondary to the maternal toxicity, this study is given less weight.

Historical control data were reported to be available (indicated in Annex I to the CLH report) for all studies except the OECD TG 421 study. However, no detailed information on the ranges/mean/medium values, the number of studies and animals and the use of relevant strains and time windows were given.

No treatment-related increase in foetal malformations was observed.

Taking all available data into account, developmental toxicity was consistently seen in pups in the available rat PNDT and EOGRTS at the high dose of 1 000 mg/kg bw/d, including significantly reduced pup weight. In F1 and F2 pups of the EOGRTS, post-natal mortality rates were significantly higher at PND21 in the F1 and at PND0 and PND4 in the F2 generation. In addition, the body weight gain was consistently lower at PND21 (F1 pups) and PND4 (F1 and F2 pups). Moreover, the pup body weight remained significantly lower up to PND42 (Cohort 1A, female pups) and PND90 (Cohort 1A, male pups). Increased rates of post-implantation losses and supporting evidence on lower

body weight and post-natal loss comes from the less informative study report on the OECD TG 421 study in rats.

A non-significantly reduced pup body weight was also seen at 300 mg/kg bw/d in F1 and F2 pups of the EOGRTS. Due to the dose selection, no information is available for doses between 300 and 1 000 mg/kg bw/d in the EOGRTS and between 400 and 1 000 mg/kg bw/d in the PNDT rat study.

Overall, RAC agrees with the DS that **classification for TBPEH as Repr. 1B; H360D is warranted**.

ADVERSE EFFECTS ON OR VIA LACTATION

Summary of the Dossier Submitter's proposal

There are no experimental data specifically related to adverse effects on or via lactation. However, some information can be derived from the OECD TG 421 study and the EOGRTS (see sections above). Post-natal losses and decreased body weight of pups were found in both the OECD TG 421 study and the EOGRTS.

In the EOGRTS, an inadequate nursing behaviour of dams may be suggested considering the lower pup body weight during lactation and the higher extra-uterine mortality including the fact that some pups did not have milk in the stomach. However, as these observed adverse effects occurred already at PND0, they may be due to exposure during gestation.

Overall, the DS stated that it cannot be clearly distinguished if the observed adverse effects were caused by gestational exposure and/or by lactation exposure. Hence, classification of TBPEH for adverse effects on or via lactation is considered not warranted.

Comments received during consultation

One MSCA agreed that data are insufficient for concluding on classification for adverse effects on or via lactation for TBPEH.

Assessment and comparison with the classification criteria

There are no experimental data specifically related to adverse effects on or via lactation. Information from the OECD TG 421 study and the EOGRTS (see sections above) may indicate an inadequate nursing behaviour of TBPEH treated dams, as post-natal losses, and decreased body weight of pups, as well as pups with no milk in the stomach were found in one or both studies.

In the OECD TG 421 study in rats, treatment at 1 000 mg/kg bw/d was associated with an increase of post-natal loss and a reduction of live pups (until day 4 post-partum). The mean body weight of pups was also reduced at this dose, up to day 4 post-partum.

In the EOGRTS in rats, a higher percentage of offspring showed signs such as no milk in the stomach, cold, found dead, missing and alopecia at 1 000 mg/kg bw/d that could

have contributed to the higher extra-uterine mortalities on PND0 and between PND0 and PND4 (F2 pups) and between PND0 and PND21 (F1 pups). Mortality was accompanied by statistically significant lower body weight at PND0 and PND4 (F1 and F2 pups) and in F1 pups through PND21. Similarly, statistically significant reductions in body weight gain (PND0-21 in F1 pups; PND0-4 in F2 pups) were observed at that dose.

At 300 mg/kg bw/d, F1 and F2 pups had a lower body weight on PND0 and F2 pups showed a lower body weight (-3 %) on PND4 as well. At this dose, F1 pups also had a statistically significantly lower body weight gain between PND7 and PND14 due to a reduced body weight gain in female F1 pups.

Taking the available data into account, RAC agrees with the DS that it cannot be clearly distinguished if the observed adverse effects were caused by gestational exposure and/or by lactation exposure. Hence, **classification of TBPEH for adverse effects on or via lactation is not warranted due to inconclusive data**.

11.9 Specific target organ toxicity-single exposure

Not assessed in this dossier.

11.10 Specific target organ toxicity-repeated exposure

Not assessed in this dossier.

11.11 Aspiration hazard

Not assessed in this dossier.

12 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

13 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

14 ADDITIONAL LABELLING

Not assessed in this dossier.

15 REFERENCES

OECD. SIDS INITIAL ASSESSMENT PROFILE (SIAP), CoCAM 6, September 30 - October 3, 2014, by NL ICCA.

See confidential Annex II.

Additional references
Matsuoka, T, Mizoguchi Y, Serizawa K, Ishikura T, Mizuguchi H, Asano Y (2006) Effect of stage and degree of restricted feeding on pregnancy outcome in rabbits, Journal of Tox Sciences. 2006; 31 (2) 169-175. https://doi.org/10.2131/jts.31.169 Matsuzawa T, Nakata M, Goto I, Tsushima M (1981) Dietary deprivation induces fetal loss and abortion in rabbits. Toxicology. 1981; 22(3):255-9. Doi: 10.1016/0300-483x(81)90088-3 Nishimura, M. (2001), Timing of implantation in New Zealand White rabbits. Congenital Anomalies, 2001; 41: 198-203. https://doi.org/10.1111/j.1741-4520.2001.tb00833.x

16 ANNEXES

Annex I for study summaries.

Confidential Annex II.

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),

Annex VI, Part 2

International Chemical Identification:

tert-butyl 2-ethylhexaneperoxoate (TBPEH)

EC Number: 221-110-7

CAS Number: 3006-82-4

Index Number: none

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)

14 rue Pierre Marie Curie

F-94701 Maisons-Alfort Cedex

classification.clp@anses.fr

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1.1 Skin sensitisation

1.1.1 Animal data

1.1.1.1 [Anonymous, 1996]

Study reference:

Anonymous, 1996 from ECHA website

Detailed study summary and results:

Test type

Buehler test OECD 406 guideline (skin sensitisation), GLP compliant.

Test substance

- TBPEH (*tert*.-Butylperoxy- 2-ethylhexanoate)
- EC number 221-110-7
- CAS number 3006-84-2
- Purity not stated
- Batch number not specified

Test animals

- Hartey derived albino guinea pig sex
- 10 animals per sex per dose
- Age at the study initiation : Young adult. Weight not provided.

Administration/exposure

- *Control group and treatment :* TBPEH in mineral oil
- *Route of induction and challenge induction*
  - epicutaneous occlusive
  - with occluded patch
  - *type of patch used substance :* not specified
- *Details of study design*

RANGE-FINDING STUDY

On the day prior to dose administration, four topical range-finding guinea pigs were weighed and the hair removed from the right and left side of the animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures. On the following day, four concentrations of the test article were prepared and each concentration was applied to the clipped area of each topical range-finding animal. Following patch application, the trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the patches and the animal was returned to its cage. Approximately six hours after patch application, the elastic wrap, tape and patches were removed. The test sites were then wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages.

MAIN STUDY

On the day prior to the first induction dose administration, the hair was removed from the left side of the 20 test animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures.

Induction

On the following day (day 0), the appropriate concentration of the test article was prepared and applied to the clipped area of the animals. Following patch application, the trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the patch and the animal was returned to its cage. Approximately 6 hours after patch application, the elastic wrap, tape and patches were removed. The test sites were then wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages.

Exposure: Ten male and ten female guinea pigs were topically treated with 25 % w/v TBPEH in mineral oil, once per week, for 3 consecutive weeks.

Dermal Observations: The test sites were graded for dermal irritation at approximately 24 and 48 hours following patch application using the Dermal Grading System.

The induction procedure was repeated on study day 7 and on study day 14 so that a total of three consecutive induction exposures were made to the 20 test animals.

Challenge

On the day prior to challenge dose administration, the hair was removed from the right side of the 19 test and 10 control animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures. On the following day (day 28), the appropriate concentration of the test article was prepared and applied to a naive site within the clipped area of the animals. Following patch application, the trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the patch and the animal was returned to its cage. Approximately 6 hours after patch application, the elastic wrap, tape and patches were removed. The test sites were then wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages.

Exposure: Following a two week rest period, a challenge was performed whereby the 19 test and 10 previously untreated (naive) challenge control guinea pigs were topically treated with 5 % w/v TBPEH in mineral oil.

Dermal Observations: The test sites were graded for dermal irritation at approximately 24 and 48 hours following patch application using the Dermal Grading System.

Rechallenge

A rechallenge was conducted in order to clarify the results of the challenge phase. On the day prior to rechallenge dose administration, the hair was removed from the left side of the 19 test and 10 control animals

with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures. On the following day (day 35), the appropriate concentration of the test article was prepared and applied to a naive site within the clipped area of the animals.

Following patch application, the trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the patch and the animal was returned to its cage. Approximately 6 hours after patch application, the elastic wrap, tape and patches were removed. The test sites were then wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages.

Exposure: Following a one week rest period, a rechallenge was performed whereby the 19 test and the 10 challenge control guinea pigs were topically treated with 2% w/v TBPEH in mineral oil.

Dermal Observations: The test sites were graded for dermal irritation at approximately 24 and 48 hours following patch application using the Dermal Grading System.

Challenge and rechallenge responses in the test animals were compared to those of the challenge control animals.

Positive control substance(s): yes : Hexylcinnamaldehyde

Results and discussion

Following induction : mild irritation in the test animals. The dermal irritation increased slightly at induction 2 and 3.

One test animal (1431/F) was found dead on study day 27. Gross necropsy observations included dark red mandibular and axillary lymph nodes, an adhesion in the thoracic cavity, mottled lungs, mottled liver, enlarged spleen and congested meningeal vessels in the brain. The majority of sensitization study animals gained weight during the test period and generally appeared in good health.

Following challenge : dermal scores of 1 in 9/19 test animals and 3/10 control animals at the 24 hour scoring interval and in 3/19 test animals and 2/10 control animals at the 48 hour scoring interval. Dermal scores of 0 in the remaining test and challenge control animals. Group mean dermal scores similar in the test animals as compared to the challenge control animals.

Following rechallenge: dermal scores of 1 in 5/19 test animals at the 24 hour scoring interval; which do not persist to the 48 hour scoring interval. Dermal scores of 0 in the remaining test and all challenge control animals. Group mean dermal scores slightly higher in the test animals as compared to the challenge control animals.

Historical Control demonstrates that the test design could detect potential mild to moderate contact sensitizers.

Positive control results:

Using Hexylcinnamaldehyde as a mild to moderate positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has compiled historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). 95% of animals induced with Hexylcinnamaldehyde elicited a contact sensitization response following challenge with Hexylcinnamaldehyde, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

<u>For test group :</u>

1st reading at 24 hours after challenge:

Dose level: 5 % TBPEH

No. with + reactions: 9

Total no. in group:19

Clinical observations: Discrete or patchy erythema

2nd reading at 48 hours after challenge:

Dose level: 5 % TBPEH

No. with + reactions: 3

Total no. in group:19

Clinical observations: Discrete or patchy erythema

For control challenge

1st reading at 24 hours after challenge:

mineral oil (vehicle)

No. with + reactions: 3

Total no. in group:10

Clinical observations: Discrete or patchy erythema

2nd reading at 48 hours after challenge:

mineral oil (vehicle)

No. with + reactions: 2

Total no. in group:10

Clinical observations: Discrete or patchy erythema

For test group

1st reading at 24 hours after rechallenge:

Dose level: 2 % t-Butyl Peroctoate

No. with + reactions: 5

Total no. in group:19

Clinical observations: Discrete or patchy erythema

2nd reading at 48 hours after rechallenge:

Dose level: 2 % t-Butyl Peroctoate

No. with + reactions: 0

Total no. in group:19

Clinical observations: none

For control rechallenge

1st reading at 24 hours after rechallenge:

mineral oil (vehicle)

No. with + reactions: 0

Total no. in group:10

Clinical observations: none

2nd reading at 48 hours after rechallenge:

mineral oil (vehicle)

No. with + reactions: 0

Total no. in group:10

Clinical observations: none

1.2 Reproductive toxicity

1.2.1 Animal data

Adverse effects on sexual function and fertility

1.2.1.1 [Anonymous, 2008]

Study reference:

Anonymous 2008 from ECHA website

Detailed study summary and results:

Test type

Reproduction/developmental toxicity screening test (OECD TG 421)

GLP compliance

Test substance

- t-Butylperoxy-2-ethylhexanoate (TBPEH)
- EC No. 221-110-7
- CAS No. 3006-82-4
- purity not specified
- Batch number : 48683458

Test animals

- Wistar rat
- n=10 animals/sex/dose
- no positive control
- age and weight at study initiation :

  Age at delivery: 10 weeks

  Weight (start of treatment): Males: 289 - 344 grams; Females: 186 - 208 grams

Administration/exposure

- Gavage – oral
- Duration of Exposure :

The test item was administered orally, by gavage, once daily. All animals received a dose volume of 4 mL/kg body weight with a daily adjustment of the individual volume to the actual body weight. Control animals were dosed with the vehicle alone. After a pre-pairing period of 14 days, males and females were paired overnight, in the ratio of 1 male : 1 female. The female was placed with the same male until mating occurred or two weeks had elapsed. The day on which spermatozoa were found in the vaginal smear or a vaginal plug was observed was designated day 0 post coitum. After mating was ascertained, the animals were separated and housed individually. The females were allowed to litter and rear their progeny to day 4 of lactation.

- Frequency of treatment**:** once daily
- Doses tested : 0, 100, 300 and 1000 mg TBPEH /kg bw/day,
- rationale for dose level selection : Dose levels were selected in conjunction with the Sponsor, based on the results of a preliminary dose range finding study, where 1000 mg/kg/day was used as highest dose level.
- rationale for animal assignment: The rat is a suitable rodent species for development toxicity studies required by regulatory authorities. The oral route is one possible route for human exposure.
- historical control data if available : no
- vehicle vehicle: sunflower oil, justification of choice of vehicle (if other than water) : sunflower oil was used as the vehicle for the test item in the dose groups. The test item is miscible in aliphatic solvents, immiscible in water at 20°C.
- test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation

Analytical verification of doses or concentrations: yes

Details on analytical verification of doses or concentrations:

The test item concentrations were determined by HPLC coupled to an UV/VIS detector and quantified with the area under the peak

Description of test design:

- *details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy)*

After a pre-pairing period of 14 days, males and females were paired overnight, in the ratio of 1 male : 1 female. The female was placed with the same male until mating occurred or two weeks had elapsed. The day on which spermatozoa were found in the vaginal smear or a vaginal plug was observed was designated day 0

post coitum. After mating was ascertained, the animals were separated and housed individually. The females were allowed to litter and rear their progeny to day 4 of lactation

- **Details on study schedule:**

- Acclimatization (females/males): 7 days (minimum)
- Treatment beginning (females/males): day-1 of pre-pairing
- Pre-pairing (females/males): 14 days
- Pairing (females/males): until mating (maximum 14 days)
- Gestation (females): about 21 days
- Parturition (females): expected : on day 21 or 22 post coitum
- Lactation (females): until day 4 post partum
- Treatment ending: -females: on day 3 post partum; -males: one day prior to the actual day of necropsy (after at least 28 days of treatment)
-Termination: -females: on day 4 post partum; -males: after the first dams had reached day 4 post partum

Examinations

Parental animals: Observations and examinations:

- All animals were checked at least twice daily for any mortalities. All rats found dead were subjected to a detailed macroscopic examination to establish, if possible, the cause of death.
- All animals were observed at least twice daily for signs of reaction to treatment and/or symptoms of ill health. Additionally, the females were observed for signs of difficult or prolonged parturition.
- The animals were weighed daily during the entire study.

The observations and examinations performed in parental males are detailed in following:
- Clinical observations
- Food consumption
- Body weights
- Body weight gain
- Necropsy findings
- Organ weights
- Histopathological examinations

The observations and examinations performed in parental females are detailed in following:
- Clinical observations
- Food consumption
- Body weights
- Body weight gain
- Fertility index
- Number of females paired
- Number of females mated
- Number of non pregnant females
- Duration of gestation
- Gestation index
- Birth index
- Organ weights
- Necropsy findings
- Histopathological examinations

Oestrous cyclicity (parental animals): not examined

Litter observations:

The litters were examined for litter size, live birth, stillbirth and any gross anomalies. The sex ratio of the pups was recorded at birth and at day 4.The dams and pups were observed daily for survival and behavioural abnormalities in nesting and nursing. The efficiency of the suckling was observed by presence of milk in the pups stomach.

In detail:
- Mean pup weight on postnatal days 0 and 4
- Number of pups born alive and number of alive pups on day 4
- Viability index
- Sex ratio

Postmortem examinations (parental animals):

Males were sacrificed after they had been treated until the postmortem examination first dams had reached day 4 post partum. Females were sacrificed on day 4 post partum. The animals were examined macroscopically for any structural abnormalities or pathological changes, with special attention paid to the organs of the reproductive system. The number of implantation sites and corpora lutea was recorded for all dams with litters.

Histology: Sperm parameters (parental animals)

Parameters examined in male parental generations: testes and epididymides (with special emphasis on stages of spermatogenesis and histopathology of interstitial testicular cell structure).
Microscopic observations regarding sperm parameters included:
In testes observation of the following indications: tubular atrophy, Sertoli C.Vacuolat., pyknosis single cell, cellular debris, Stage I, Stage VIII, Stage XI und Stage XIV.

Postmortem examinations (offspring):

Dead pups and pups killed at day 4 of lactation were examined macroscopically.

Statistics:

The following statistical methods were used to analyze body weights, food consumption, and reproduction data:
• Means and standard deviations of various data were calculated.

• Univariate one-way analysis of variance was used to assess the significance of intergroup differences.
• If the variables could be assumed to follow a normal distribution, the Dunnett's t-test, based on a pooled variance estimate, was used for inter-group comparisons (i.e. single treatment groups against the control group).
• The Steel test (many-one rank test) was applied when the data could not be assumed to follow a normal distribution.
• Fisher’s Exact test for 2x2 tables was applied if the variables could be dichotomized without loss of information.

Reproductive indices:

The following reproductive indices were calculated: percentage mating, female fertility index, conception rate and gestation index.

Offspring viability indices:

The following pup mortality and sex ration indices were calculated: sex ratio, birth index and viability index.

Results

Results: P0 (first parental generation)

General toxicity (P0)

Details on results (P0)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)

All male and female animals survived until scheduled necropsy. In group 4 (1000 mg/kg bw/d), all male and female animals were noted that they "moved the head through the bedding material after administration of test item" starting on day 5 of the pre-pairing period until the end of study. Additionally, one dam salivated starting in the pre-pairing period until the end of the gestation period. One dam had an inflamed right eye with lacrimation starting during the gestation period until the end of the lactation period. During the last two days of gestation or the first two days of lactation, seven dams (gestation period) and four dams (lactation period), respectively, were noted periodically to have ruffled fur and / or a generally bad condition.

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)

During pre-pairing period, mean body weight of the males in group 4 (1000 mg/kg bw/d), was slightly decreased starting on day 3 and continuing until necropsy.

During gestation period, mean body weight of the dams in group 4 (1000 mg/kg bw/d), was slightly increased. During lactation period, the mean body weight gain of the dams was statistically significantly decreased, this might reflect the generally bad conditions that were already noted (signs and symptoms).

In group 4 males (1000 mg/kg bw/d), the mean food consumption was statistically significantly decreased during the first week of the pre-pairing period. During after pairing period mean food consumption was similar to that of the control group.

In group 4 females (1000 mg/kg bw/d), the mean food consumption was statistically significantly decreased during the first week of the pre-pairing period and during lactation period.

REPRODUCTIVE FUNCTION (PARENTAL ANIMALS)

All mated females were pregnant. In groups 2, 3, and 4, mating performance was not influenced by treatment with the test item. The fertility index was 100.0% in all groups. In groups 2, 3, and 4, the number of corpora lutea, the implantation rate, and the gestation length were not influenced by treatment with the test item. The post-implantation loss was statistically significantly increased in group 4 (1000 mg/kg bw/d), and was considered test item-related. The postnatal loss was statistically significantly increased in group 4 (1000 mg/kg bw/d), and was considered test item related, as 5/10 dams were affected.

ORGAN WEIGHTS (PARENTAL ANIMALS)

Organ weights recorded in males at necropsy showed no test item-related differences between groups. A few statistically significant deviations in mean testes weight were considered incidental, reflecting the usual biological variability of individual values.

HISTOPATHOLOGY (PARENTAL ANIMALS)

No test item-related microscopic findings were noted.

Results: F1 generation

Details on results (F1)

CLINICAL SIGNS (OFFSPRING)

In group 4 (1000 mg/kg bw/d), forty-three pups were found dead and another 20 pups were missing until day 4 post partum. This was considered most likely test item related.

BODY WEIGHT (OFFSPRING)

In group 4 pups (1000 mg/kg bw/d), the mean body weight up to day 4 post partum was reduced.

NECROPSY
At scheduled necropsy, no test item-related findings were noted.

1.2.1.2 [Anonymous, 2020]

Study reference:

Anonymous 2020

Detailed study summary and results:

Test type

EOGRTS (Extended One-Generation Reproductive Toxicity Study) (2019) rat, OECD 443

GLP compliant

SPECIFICATION OF STUDY DESIGN FOR EXTENDED ONE-GENERATION REPRODUCTION TOXICITY STUDY WITH JUSTIFICATIONS:

- Premating exposure duration for parental (P0) animals : 10 weeks as requested by the authorities

- Basis for dose level selection: based on NOAELs obtained in repeated dose toxicity studies

- Inclusion/exclusion of extension of Cohort 1B: in the course of the study an impairment of the female reproductive performance was observed which triggered the extension of Cohort 1B to produce the F2

- Termination time for F2: as given in the guideline (PND 4)

- Inclusion/exclusion of developmental neurotoxicity Cohorts 2A and 2B : not triggered by available data with the test item

- Inclusion/exclusion of developmental immunotoxicity Cohort 3: not triggered by available data with the test item

- Route of administration: gavage

Test substance

- t-Butylperoxy-2-ethylhexanoate (TBPEH)
- EC No. 221-110-7
- CAS No. 3006-82-4
- Purity : not specified

Test animals

- Han:WIST rats
- P0 : n= 24/sex/group, 4 groups

- *Age and weight at study initiation :*

Age at study initiation: P0 males and females: not older than 9 weeks

Weight at study initiation: (P) Males: 256-309 g; Females: 151-184 g

- Control animals received the vehicle, only.
- F1 for Cohort 1A n= 20 animals/sex/group
- F1 for Cohort 1B n= 20 animals/sex/group
- randomly selected on post-natal day 21 for follow-up examinations.
- Dosing of F1 offspring selected for follow-up examinations (Cohort 1A and Cohort 1B) begun on post-natal day 22 and treatment was continued up to the day before the necropsy.
- F1 offspring (Cohort 1A and Cohort 1B) were observed identically to parental animals – clinical signs, body weight, food consumption, estrous cycle, clinical pathology and organ pathology. Sexual maturity of offspring (Cohort 1A and Cohort 1B) was investigated by observation of balano-preputional separation, vaginal patency and appearance of first cornified vaginal smear.
- Cohort 1A animals were subjected to necropsy, organ weighing and sperm analysis – one day after the termination of the exposure – on PND 91-97.
- Cohort 1B animals were mated to produce a second (F2) generation after at least 90-day pre-mating period and were observed identically to parental (P) animals.
- F2 offspring were observed and subjected to necropsy up to PND 5-8.

Blood samples were collected for determination of serum levels of thyroid hormones (FT3, FT4 and TSH) from 3-5 F1 pups per litter (where it was feasible) on PND 4, from 1-2 pups/10 litters on PND 22, from 10 dams (P)/group and from 10 parental (P) male animals/group at termination, from 10 male animals/group and from 10 female animals in Cohort 1A, from all male and female animals in Cohort 1B at termination and from F2 pups on post-natal day 5 or shortly thereafter.

All adult animals (P, F1 Cohort 1A and Cohort 1B) were subjected to gross pathology with complete tissue preservation one day after the last treatment. Brain, spleen, thymus and mammary tissues were preserved for 10 male and 10 female pups per group – where feasible – in F1 offspring not selected for Cohorts on PND22 or shortly thereafter and in F2 offspring on PND5 or shortly thereafter.

Special attention was paid to the organs and tissues of the reproductive system for P, F1 or F2 animals.

Selected organ weights were determined in adult animals (P, F1) and in offspring (PND22 or shortly thereafter and in F2 offspring on PND5).

Sperm parameters were determined in all control and high dose male animals in P generation and in F1 generation (Cohort 1A and Cohort 1B).

Full histopathology examinations were performed on the organs and tissues of adult animals (P, F1 Cohort 1A and Cohort 1B) in control and high dose groups with special emphasis on sexual organs and tissues

Reproductive organs were also processed and examined histologically in non-mated and non-pregnant female animals and their mating partners (P and Cohort 1B) in the low and mid dose groups.

In addition, organs showing macroscopic changes were also processed and examined histologically in adult animals in low or mid dose groups (P, F1 Cohort 1A and Cohort 1B) and in F1 offspring. The kidneys of male animals at 100 and 300 mg/kg bw/day were processed and evaluated histologically based on the organ weight data and histological observations at 1000 mg/kg bw/day.

Based on the low reproduction index and histopathological findings in parental (P) female animals at 1000 mg/kg bw/day, a quantitative evaluation of primordial, small growing (secondary and tertiary) follicles, as

well as corpora lutea was performed in all adult female animals (P, F1 Cohort 1A and Cohort 1B) in the control, 100, 300 and 1000 mg/kg bw/day

Administration/exposure

- Gavage – oral once daily
- Duration of Exposure :

All animals of the parent (P) generation dosed prior to mating (10 weeks) and throughout mating.

In addition, males received the test item or vehicle after mating up to the day before the necropsy (altogether for 153-156 days).

Dams were additionally exposed through the mating and gestation periods and up to lactation days 21-23 (altogether for 100-129 days).

P0 males: 153 - 156 days
P0 females: 114 - 129 days; not mated and non-pregnant females and dams without living pups administered for 100 or 103 days.

F1
Cohort 1A: approx. 90 days (13 weeks)
Cohort 1B: approx. 120 days (17 weeks)

- Frequency of treatment : daily, 7 days/week
- Doses tested : 0 (vehicle), 100, 300 and 1000 mg TBPEH/kg bw/day doses corresponding to concentrations of 0, 20, 60 and 200 mg /mL TBPEH
- rationale for dose level selection : based on NOAELs obtained in repeated dose toxicity studies
- Rationale for animal assignment: random
- *control group and treatment*
- Control group : yes, concurrent vehicle
- *historical control data if available :* yes
- *positive control :* not applicable
- *vehicle :* sunflower oil, suitability of the vehicle at the intended concentrations of the test item was analytically verified up front (concentration and homogeneity), application volume = 5 mL/kg bw.
- *justification of choice of vehicle (if other than water):* The test item is not stable in water. Therefore, sunflower oil was used for preparing formulations appropriate for oral administration. Sunflower oil is a suitable vehicle to facilitate formulation analysis for the test item. Concentration in vehicle: 20, 60, 200 mg/mL. Treatment volume: A constant treatment volume of 5 mL dose preparation/kg body weight was administered in all groups.
- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation*

Analytical verification of doses or concentrations: yes

Details on analytical verification of doses or concentrations:

A sufficient stability and homogeneity in the chosen vehicle was verified over the range of relevant concentrations at the appropriate frequency of preparation. Recovery from sunflower oil was 104 % and 98 % of nominal concentrations at ca. 2 mg/mL and ca. 500 mg/mL, respectively. The test item was stable at the intended concentrations for 24 hours at room temperature and for three days in a refrigerator (5 ± 3°C).

Description of test design:

- *details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy)*

- Impregnation procedure: cohoused
- If cohoused:
- M/F ratio per cage: 1/3
- Length of cohabitation: in the mornings for two to four hours
- Further matings after two unsuccessful attempts: no
- Verification of same strain and source of both sexes: yes
- Proof of pregnancy: vaginal plug and/or sperm in vaginal smear referred to as day 0 of pregnancy

Details on study schedule:

- Selection of parents from F1 generation when pups were 21 days of age.

- Age at mating of the mated animals in the study: P0 animals first mating: 19 weeks; P0 males second mating (high dose and control): 20 weeks; P1/F1 animals: 13 weeks

Clinical observations (clinical signs, body weight, food consumption, estrous cycle) and pathology (clinical and organ pathology) examinations were performed on parental (P) animals for signs of toxicity, with special emphasis on the integrity and performance of the male and female reproductive systems. Estrous cycle was monitored by examining vaginal smears before the mating for two weeks and during the mating period until evidence of mating and on the day of the necropsy.

The dams were allowed to litter and rear their offspring up to day 21 post-partum.

As the number of pregnancies was low in the high dose group, a second mating of the P males with untreated females (untreated group; n= 8 naïve females) was performed to clarify if the male fertility of the high group was impaired. Dams and offspring in the untreated group were terminated on post-partum/ PND (post-natal days) 5-8.

All F1 offspring were observed individually for the health, growth, development and function up to and including post-natal day 21 (clinical signs, body weight, surface righting reflex, pinna detachment, eye opening, anogenital distance).

Examinations

Parental animals: Observations and examinations:

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: daily

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: same as weighing

BODY WEIGHT: Yes
- Time schedule for examinations:
Parental males were weighed on the first day of dosing (day 0) and weekly thereafter.
Parental females were weighed on the first day of dosing (Day 0) then weekly, on gestation days 0, 7, 14 and 21 and on post-partum days 0 (within 24 hours after parturition), 4, 7, 14 and 21. Body weight of the female animals was additionally weighed on gestation day 10 in order to give accurate treatment volumes, but these data were not evaluated statistically. Body weight data were reported individually for adult animals.

F1 animals selected for follow-up examinations were weighed on post-natal day 22, then twice a week during the two weeks following weaning, and once weekly thereafter.
For selected F1offspring, the body weight was recorded on the day when they attain puberty (completion of balano-preputial separation or vaginal patency).
Fasted body weight was measured on the day of necropsy for all animals (P and F1).

FOOD CONSUMPTION AND COMPOUND INTAKE: Yes
Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

WATER CONSUMPTION AND COMPOUND INTAKE: Yes
Time schedule for examinations: daily by visual inspection

Oestrous cyclicity (parental animals):

Estrous cycle was monitored by examining vaginal smears from each parental female animal daily for two weeks before the mating started. Vaginal smear was also prepared and estrous cycle was monitored daily during the mating period until evidence of copulation. Vaginal smear was also prepared on the day of the necropsy of parental animals. Vaginal smears were examined for all F1 Cohort 1A females selected for follow-up examinations after the onset of vaginal patency until the first cornified smear is recorded thus determining the time interval between these events. Estrous cycle of F1 adult female animals was examined for a period of two weeks commencing on PND77 and PND84 in Cohort 1A and Cohort 1B, respectively, including necropsy days. Vaginal smears were stained with 1 % aqueous methylene blue solution. After drying, the smears were examined with a light microscope.

Sperm parameters (parental animals):

Sperm parameters were measured in all control and high dose male animals in P generation and in F1 generation in Cohort 1A.

The one-side testes and epididymides were used for examinations. The weights of one-side testes and epididymides were determined and recorded.

Sperm from the ductus deferens was collected for evaluation of sperm motility and morphology at necropsy. Both numbers of motile and immotile sperms were recorded. Two samples were prepared from each animal. For the determination of sperm motility, the mean percentage of motile sperm was determined. A morphological evaluation of ductus deferens sperms sample was performed from the same animals. Sperm was examined as fixed, wet preparations and classified as either normal or abnormal (isolated heads, misshapen heads and/or tails). The epididymis was used for numeration of cauda epididymis sperm reserves. The total number of sperm in homogenization was numerated. The testis and epididymidis were frozen and numeration was performed later.

Litter observations:

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

PARAMETERS EXAMINED
The following parameters were examined in F1 / F2 offspring: number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, weight gain, physical or behavioural abnormalities, anogenital distance (AGD), presence of nipples/areolae in male pups.

GROSS EXAMINATION OF DEAD PUPS: yes, for external and internal abnormalities; possible cause of death was determined for pups born or found dead

ASSESSMENT OF DEVELOPMENTAL NEUROTOXICITY: No

ASSESSMENT OF DEVELOPMENTAL IMMUNOTOXICITY: No

Postmortem examinations (parental animals):

SACRIFICE
- Male animals: All surviving animals as soon as possible
- Maternal animals: All surviving animals after the litter was weaned (P0); at PND4 (F1, cohort 1B)

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

HISTOPATHOLOGY / ORGAN WEIGHTS
The following tissues were prepared for microscopic examination and weighed in high dose and control animals:

Parental animals and adult F1 animals of Cohort 1A:
- uterus (with oviducts and cervix)
- ovaries
- testes
- epididymides
- prostate (dorsolateral and ventral parts combined)
- seminal vesicles with coagulating glands as one unit (with their fluids)
- brain
- liver
- kidneys
- heart
- spleen
- thymus
- pituitary
- thyroid glands (post-fixation)
- adrenal glands

Animals of Cohort 1B:
- uterus (with oviducts and cervix)
- ovaries
- testes
- epididymides
- prostate (dorsolateral and ventral parts combined)
- seminal vesicles with coagulating glands as one units (with their fluids)
- brain
- pituitary

Postmortem examinations (offspring):

SACRIFICE
- The F1 offspring not selected as parental animals were sacrificed at 21 days of age, and all F2 offspring on PND 4 or shortly thereafter.
- These animals were subjected to postmortem examinations macroscopic and microscopic examination as follows:

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

HISTOPATHOLOGY / ORGAN WEIGTHS
The tissues indicated above were prepared for microscopic examination and weighed, respectively.

A determination of spleenic subpopulation analysis was considered not required.

Statistics:

The statistical evaluation of appropriate data was performed with the statistical program package SPSS PC+4.0.

The homogeneity of variance between groups was checked by Bartlett's homogeneity of variance test.

Where no significant heterogeneity was detected, a one-way analysis of variance (ANOVA) was carried out. If the obtained result was significant, Duncan Multiple Range test was used to assess the significance of inter-group differences. Getting significant results at Bartlett's test the Kruskal-Wallis analysis of variance was used and the inter-group comparisons were performed using Mann-Whitney U-test. Chi2 test was performed if feasible. Frequency of toxic response, pathological and histopathological findings by sex and dose were calculated.

Reproductive indices:

Copulatory Index (Measure of animals ability to mate):
Males: Number of males with confirmed mating / Total number of males cohabited x 100
Females: Number of sperm positive females / Total number of females cohabited x 100

Fertility Index (measure of male's ability to produce sperm that can fertilize eggs and measure of female's ability to become pregnant):
Males: Number of males impregnating a females / Total number of males with confirmed mating x 100
Females: Number of pregnant females / Number of sperm positive females x 100

Gestation Index (Measure of pregnancy that provides at least one live pup):
Number of females with live born pups / Number of pregnant females x 100

Offspring viability indices:

Post-implantation mortality: Number of implantations – Number of liveborns / Number of implantation x 100

Post-natal mortality: Number of liveborns – Number of live pups on PND 13 / Number of liveborns x 100

Survival Index: Number of live pups on PND 13 / Number of liveborns x 100

Sex ratio: Number of pups examined – Number of pups males (females) / Number of pups examined x 100

Results

Results: P0 (first parental generation)

General toxicity (P0)

Mortality:

There was no test item related mortality in parental animals in 100, 300 or 1000 mg/kg bw/day groups (male or female) during the course of study.

Clinical observations:

Salivation and nuzzling up the bedding material were detected in male and female animals at 300 and 1000 mg/kg bw/day with variable incidence and duration. These observations were related to the treatment/test item and were considered to be toxicologically not relevant because of the transient occurrence and short duration after the administration.

The parental male animals were normal in control group during the entire observation period. Reddish colored hair on the forelimbs was noted for one parental male animal (1/24) at 100 mg/kg bw/day as individual findings between Days 63 and 75. Salivation (10/24 at 300 mg/kg bw/day, 24/24 at 1000 mg/kg bw/day) and nuzzling up the bedding material (7/24 at 300 mg/kg bw/day, 24/24 at 1000 mg/kg bw/day) were observed in parental male animals with variable incidence and in a dose related manner shortly after the administration for some days/weeks.

Parental female animals in the control (24/24) and 100 mg/kg bw/day (24/24) groups were normal during the pre-mating, mating, post-mating and gestation periods. Alopecia on the abdomen was detected in one control dam (1/21) between lactation days 16 and 20. Salivation and nuzzling up the bedding material were noted for parental female animal at 300 mg/kg bw/day (6/24) and at 1000 mg/kg bw/day (24/24) during the pre-mating period, as well as during the post-mating period (8/8) and gestation period (3/16 and 12/16, respectively) only at 1000 mg/kg bw/day. In one dam at 1000 mg/kg bw/day, sanguineous vaginal orifice was observed on lactation day 1 probably as a late consequence of delivery. Alopecia was also observed in some female animals at 1000 mg/kg bw/day as follows:

- in two dams (2/16) under the right ear then both ears from Day 35 up to lactation day 17 or on the forelimbs and base of the tail (1/16) between lactation days 5 and 21;

- in non-pregnant female animals (2/8) between the ears between Day 63 and 94 and on the chest on Days 98 and 99;

Alopecia on the skin is a species-specific finding, which is also observed in untreated experimental rats of this strain with similar age. These were individual findings with low incidence in animals of control or lower dose groups and were thus not considered related to the treatment.

Detailed weekly observations

The behavior and physical condition of animals was not adversely affected by the test item at any dose level (100, 300 or 1000 mg/kg bw/day) based on the weekly detailed clinical observations during the entire treatment period.

The reddish hairs on the forelimbs, as observed at the daily observations, were also detected in parental male animal at 100 mg/kg bw/day at the weekly observations on days 63 and 69.

Alopecia – as described above – were also observed at the detailed weekly clinical observations as follows:

- in two dams (2/16) at 1000 mg/kg bw/day: under the right ear then both ears by weekly interwall from Day 35 up to lactation day 14 or on the forelimbs and base of the tail (1/16) on lactation days 7, 14, 21 and 22;

- in non-pregnant female animals (2/8): between the ears by weekly interval between Day 63 and 92 and on the chest on Day 100

Table 1: summary of daily clinical observations in parent (P) males (pre-mating and post-mating periods)

Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Normal	24/24	23/24	12/24	0/24
Hairs: Reddish colored	0/24	1/24	0/24	0/24
Salivation	0/24	0/24	10/24	24/24
Nuzzling up the bedding material	0/24	0/24	7/24	24/24

Remark: Frequency of observations: number of animals with observation/number of animals examined

Table 2: summary of daily clinical observations in parent (P) females (pre-mating, mating, gestation and lactation periods

Pre-mating and mating periods

Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Normal	24/24	24/24	14/24	0/24
Salivation	0/24	0/24	6/24	24/24
Nuzzling up the bedding material	0/24	0/24	6/24	24/24
Alopecia	0/24	0/24	0/24	3/24

Post-mating period

Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Normal	3/3	1/1	1/1	0/8
Salivation	0/3	0/1	0/1	8/8
Nuzzling up the bedding material	0/3	0/1	0/1	8/8
Alopecia	0/3	0/1	0/1	2/8

Gestation period

Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Normal	21/21	23/23	23/23	4/16
Salivation	0/21	0/23	0/23	3/16
Nuzzling up the bedding material	0/21	0/23	0/23	12/16
Alopecia	0/21	0/23	0/23	1/16

Lactation period

Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Normal	20/21	23/23	23/23	14/16
Alopecia	1/21	0/23	0/23	2/16
Sanguineous vaginal orifice	0/21	0/23	0/23	1/16

Remark: Frequency of observations: number of animals with observation/number of animals examined

Table 3: Summary of weekly clinical observations in parent (P) males

Time of observations	Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Day 0	Normal	24/24	24/24	24/24	24/24
Day 7	Normal	24/24	24/24	24/24	24/24
Day 14	Normal	24/24	24/24	24/24	24/24
Day 21	Normal	24/24	24/24	24/24	24/24
Day 28	Normal	24/24	24/24	24/24	24/24
Day 35	Normal	24/24	24/24	24/24	24/24
Day 42	Normal	24/24	24/24	24/24	24/24
Day 49	Normal	24/24	24/24	24/24	24/24
Day 56	Normal	24/24	24/24	24/24	24/24
Day 63	Normal Hairs: Reddish colored	24/24 0/24	23/24 1/24	24/24 0/24	24/24 0/24
Day 69	Normal Hairs: Reddish colored	24/24 0/24	23/24 1/24	24/24 0/24	24/24 0/24
Day 76	Normal	24/24	24/24	24/24	24/24
Day 83	Normal	24/24	24/24	24/24	24/24
Day 90	Normal	24/24	24/24	24/24	24/24
Day 97	Normal	24/24	24/24	24/24	24/24
Day 104	Normal	24/24	24/24	24/24	24/24
Day 111	Normal	24/24	24/24	24/24	24/24
Day 118	Normal	24/24	24/24	24/24	24/24
Day 125	Normal	24/24	24/24	24/24	24/24
Day 132	Normal	24/24	24/24	24/24	24/24
Day 139	Normal	24/24	24/24	24/24	24/24
Day 146	Normal	24/24	24/24	24/24	24/24
Day 152	Normal	24/24	24/24	24/24	24/24
Day 153	Normal	6/6	/	/	12/12
Day 154	Normal	6/6	/	/	12/12
Day 155	Normal	6/6	12/12	12/12	/
Day 156	Normal	6/6	12/12	12/12	/

Remarks: Frequency of observations: number of animals with observation/number of animals examined

Table 4: Summary of weekly clinical observations in parent (P) females

Pre-mating, mating and post-mating periods

Time of observations	Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Day 0	Normal	24/24	24/24	24/24	24/24
Day 7	Normal	24/24	24/24	24/24	24/24
Day 14	Normal	24/24	24/24	24/24	24/24
Day 21	Normal	24/24	24/24	24/24	24/24
Day 28	Normal	24/24	24/24	24/24	24/24
Day 35	Normal Alopecia	24/24 0/24	24/24 0/24	24/24 0/24	23/24 1/24
Day 42	Normal Alopecia	24/24 0/24	24/24 0/24	24/24 0/24	23/24 1/24
Day 49	Normal Alopecia	24/24 0/24	24/24 0/24	24/24 0/24	23/24 1/24
Day 56	Normal Alopecia	24/24 0/24	24/24 0/24	24/24 0/24	23/24 1/24
Day 63	Normal Alopecia	24/24 0/24	24/24 0/24	24/24 0/24	22/24 2/24
Day 69	Normal Alopecia	24/24 0/24	24/24 0/24	24/24 0/24	22/24 2/24
Day 70-76	Normal Alopecia	7/7 0/7	5/5 0/5	6/6 0/6	10/11 1/11
Day 77-83	Normal Alopecia	3/3 0/3	1/1 0/1	1/1 0/1	7/8 1/8
Day 84-90	Normal Alopecia	3/3 0/3	1/1 0/1	1/1 0/1	7/8 1/8
Day 91-96	Normal Alopecia	2/2 0/2	1/1 0/1	1/1 0/1	7/8 1/8
Day 97	Normal	1/1	/	/	6/6
Day 99	Normal Alopecia	/ /	1/1 0/1	/ /	/ /
Day 100	Normal Alopecia	3/3 0/3	1/1 0/1	1/1 0/1	7/8 1/8

Remarks: Frequency of observations: number of animals with observation/number of animals examined
/ = No data

Gestation period

Time of observations	Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
G0	Normal	21/21	23/23	23/23	15/16
	Skin: Alopecia	0/21	0/23	0/23	1/16
G7	Normal	21/21	23/23	23/23	15/16
	Skin: Alopecia	0/21	0/23	0/23	1/16
G14	Normal	21/21	23/23	23/23	15/16
	Skin: Alopecia	0/21	0/23	0/23	1/16
G21	Normal	21/21	23/23	23/23	15/16
	Skin: Alopecia	0/21	0/23	0/23	1/16

Lactation period

Time of observations	Observations	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
L0	Normal	21/21	23/23	23/23	15/16
	Skin: Alopecia	0/21	0/23	0/23	1/16
L7	Normal	21/21	23/23	23/23	14/16
	Skin: Alopecia	0/21	0/23	0/23	2/16
L14	Normal	21/21	22/22	22/22	14/16
	Skin: Alopecia	0/21	0/22	0/22	2/16
L21	Normal	21/21	22/22	22/22	15/16
	Skin: Alopecia	0/21	0/22	0/22	1/16
L22 or L23 or L24	Normal	21/21	22/22	22/22	15/16
	Skin: Alopecia	0/21	0/22	0/22	1/16

Remarks: Frequency of observations: number of animals with observation/number of animals examined
G = Gestation day
L = Lactation day

Body weight and weight changes:

The body weight development was continuously reduced in parental male animals administered with 1000 mg/kg bw/day.

The mean body weight was comparable to the control in male animals at 100 and 300 mg/kg bw/day during the entire observation period (pre-mating, mating and post-mating periods). Some sporadic statistically significant difference with respect to the control was detected in the mean body weight gain of male animals at 100 and 300 mg/kg bw/day (lower or higher). However, these minor differences in the mean body weight gain had no influence on the mean body weight of male animals.

Statistical significances were detected at the permanently lower mean body weight in male animals at 1000 mg/kg bw/day from Day 28 up to termination of the study (Day 152; -13% of the control). The mean body weight gain of male animals at 1000 mg/kg bw/day was lower than in the control group by weekly interval in the most cases during the observation period and if summarized for the whole study (between Days 0 and 152). The difference to the control reached statistical significance in several cases in male animals at 1000 mg/kg bw/day.

The mean body weight and body weight gain was comparable in the control and test item treated female animals at 100, 300 and 1000 mg/kg bw/day during the pre-mating, gestation and lactation periods. Statistical significance was only noted for the slightly higher mean body weight of dams at 1000 mg/kg bw/day on lactation day 21. Similarly, some sporadic statistical significance was observed at the lower or higher mean body weight gain of female animals during the pre-mating period at 100, 300 or 1000 mg/kg bw/day and between lactation days 14 and 21 at 1000 mg/kg bw/day and if summarized for lactation period (between lactation days 0 and 21). These changes in body weight gain had no toxicologically relevance as there was no significant influence on the mean body weight.

Therefore, these minor statistically significant differences with respect to the control in male animals at 100 and 300 mg/kg bw/day and in female animals at 100, 300 and 1000 mg/kg bw/day had no toxicological significances during this study.

Table 5: Summary of body weight of parent (P) male (pre-mating period, mating and post-mating period)

Pre-mating period

Group		Body weight (g) Pre-mating days 0	7	14	21	28	35	42	49	56	63	69
Control	Mean	284.3	313.5	340.6	360.5	379.8	395.4	409.3	422.3	433.4	443.3	451.8
	SD	12.97	18.13	22.37	25.19	29.83	32.41	35.14	36.38	39.16	40.60	42.48
	n	24	24	24	24	24	24	24	24	24	24	24
100	Mean	284.4	314.4	345.0	363.8	385.2	400.6	413.8	426.7	439.2	449.1	458.5
mg/kg bw/day	SD	13.47	18.91	23.93	28.11	31.95	34.94	37.02	39.78	40.82	43.38	45.35
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	0	0	1	1	1	1	1	1	1	1	1
300	Mean	282.9	315.5	346.1	367.7	388.2	403.0	414.4	427.1	438.1	444.2	452.3
mg/kg bw/day	SD	13.07	16.77	20.70	25.48	29.89	33.11	34.70	36.98	39.05	40.28	41.59
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	0	1	2	2	2	2	1	1	1	0	0
1000	Mean	283.0	306.0	327.8	345.5	360.3	370.8	379.3	390.1	395.6	398.8	403.4
mg/kg bw/day	SD	14.00	18.23	23.40	26.97	28.98	30.94	30.47	31.28	33.27	31.96	32.42
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	0	-2	-4	-4	-5	-6	-7	-8	-9	-10	-11
						*	*	**	**	**	**	**
		NS	NS	NS	NS	DN	DN	DN	DN	DN	DN	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Mating and post-mating periods

Group		Body weight (g) Mating days 76	83	90	Post-mating days 97	104	111	118	125	132	139	146	152
Control	Mean	458.5	463.3	467.9	474.7	479.4	486.5	490.8	499.4	507.4	508.5	512.9	516.2
	SD	43.92	44.95	46.00	46.93	47.50	48.28	49.80	50.14	52.32	53.60	53.91	55.22
	n	24	24	24	24	24	24	24	24	24	24	24	24
100	Mean	463.4	469.8	475.3	482.7	490.0	496.0	501.6	512.1	517.7	519.7	525.2	531.8
mg/kg bw/day	SD	46.59	46.59	48.83	50.89	52.75	55.84	57.47	60.23	61.57	63.04	64.43	66.54
	n	24	24	24	24	24	24	24	24	24	24	24	24
	± %	1	1	2	2	2	2	2	3	2	2	2	3
300	Mean	458.7	464.5	469.9	474.2	479.3	486.1	491.3	502.5	503.2	506.8	513.6	518.4
mg/kg bw/day	SD	42.73	43.14	44.38	44.53	45.94	46.20	47.33	49.93	50.66	52.05	52.92	54.36
	n	24	24	24	24	24	24	24	24	24	24	24	24
	± %	0	0	0	0	0	0	0	1	-1	0	0	0
1000	Mean	409.0	412.8	412.0	421.7	426.8	431.3	435.3	441.3	437.6	440.0	445.2	448.6
mg/kg bw/day	SD	31.84	32.69	33.82	35.33	35.87	37.23	37.91	37.52	37.50	38.26	39.58	39.72
	n	24	24	24	24	24	24	24	24	24	24	24	24
	± %	-11	-11	-12	-11	-11	-11	-11	-12	-14	-13	-13	-13
		**	**	**	**	**	**	**	**	**	**	**	**
		DN	DN	DN	DN	DN	DN	DN	DN	DN	DN	DN	DN

Table 6: Summary of body weight of parent (P) females (pre-mating period)

Group		Body weight (g) Pre-mating days										
		0	7	14	21	28	35	42	49	56	63	69
Control	Mean	167.1	181.1	190.2	198.5	208.6	213.6	217.1	220.3	225.1	228.3	233.0
	SD	7.13	9.28	8.68	8.43	9.99	11.02	10.27	10.15	10.75	10.08	11.30
	n	24	24	24	24	24	24	24	24	24	24	24
100	Mean	167.3	180.2	195.0	201.1	211.4	214.7	221.2	224.8	229.5	233.1	239.1
mg/kg bw/day	SD	8.06	10.37	11.63	12.11	10.83	11.50	12.44	14.22	11.81	14.36	17.97
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	0	0	3	1	1	1	2	2	2	2	3
300	Mean	168.5	182.3	195.4	201.8	211.9	215.8	220.4	224.0	229.5	234.0	239.8
mg/kg bw/day	SD	7.43	8.62	8.54	10.27	11.29	10.32	10.78	12.43	12.69	12.92	13.67
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	1	1	3	2	2	1	1	2	2	2	3
1000	Mean	168.3	178.8	193.2	198.9	209.3	215.3	222.2	223.9	228.3	233.3	235.8
mg/kg bw/day	SD	9.87	10.06	13.67	14.55	15.82	15.88	16.28	17.44	16.58	16.72	18.07
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	1	-1	2	0	0	1	2	2	1	2	1
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 7: Summary of body weight of parent (P) females (gestation and lactation periods)

Group		Body weight (g) on gestation days				Body weight (g) on lactation days				
		0	7	14	21	0	4	7	14	21
Control	Mean	234.3	252.5	276.1	349.3	263.9	267.5	276.0	291.6	284.8
	SD	9.40	10.53	11.97	22.22	13.44	13.70	14.38	12.12	13.68
	n	21	21	21	21	21	21	21	21	21
100	Mean	240.8	259.4	281.9	353.5	267.6	271.3	279.7	299.5	291.9
mg/kg bw/day	SD	15.86	16.03	17.21	25.61	17.29	19.10	17.55	21.10	15.47
	n	23	23	23	23	23	23	23	22	22
	± %	3	3	2	1	1	1	1	3	2
300	Mean	238.7	257.4	281.5	353.7	264.6	272.0	280.4	300.3	289.0
mg/kg bw/day	SD	13.18	13.05	12.04	22.30	12.50	12.56	13.85	17.09	11.44
	n	23	23	23	23	23	23	23	22	22
	± %	2	2	2	1	0	2	2	3	1
1000	Mean	240.5	261.2	284.3	352.2	262.0	271.8	281.4	300.4	300.4
mg/kg bw/day	SD	15.35	15.71	17.34	28.79	19.25	17.25	19.38	18.16	17.61
	n	16	16	16	16	16	16	16	16	16
	± %	3	3	3	1	-1	2	2	3	5
										**
		NS	NS	NS	NS	NS	NS	NS	NS	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 8: Summary of body weight gain of parent (P) males (pre-mating period, mating and post-mating period)

Pre-mating period

Group		Body weight gain (g) Pre-mating days 0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-69
Control	Mean	29.3	27.0	19.9	19.3	15.6	13.9	13.0	11.1	9.9	8.5
	SD	6.81	5.77	5.05	5.92	4.38	3.98	3.04	4.17	3.19	4.16
	n	24	24	24	24	24	24	24	24	24	24
100	Mean	30.0	30.6	18.8	21.4	15.4	13.2	12.9	12.5	9.9	9.4
mg/kg bw/day	SD	7.12	6.12	7.38	7.35	4.47	4.35	3.98	4.16	4.58	4.29
	n	24	24	24	24	24	24	24	24	24	24
300	Mean	32.5	30.6	21.6	20.5	14.8	11.4	12.8	11.0	6.1	8.1
mg/kg bw/day	SD	6.67	5.20	5.98	5.70	4.29	5.14	4.45	4.38	3.27	3.01
	n	24	24 *	24	24	24	24	24	24	24 **	24
1000	Mean	23.0	21.8	17.8	14.8	10.5	8.5	10.8	5.5	3.2	4.7
mg/kg bw/day	SD	8.07	9.97	5.97	5.64	4.03	4.09	4.10	4.57	6.34	3.56
	n	24 **	24 *	24	24 *	24 **	24 **	24	24 **	24 **	24 **
		DN	U	NS	DN	DN	DN	NS	DN	U	DN

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Mating and post-mating periods

Group		Body weight gain (g) Mating days 69-76	76-83	83-90	Post-mating days 90-97	97-104	104-111	111-118	118-125	125-132	132-139	139-146	146-152	SUM 0-152
Control	Mean	6.8	4.8	4.6	6.8	4.7	7.1	4.3	8.6	8.0	1.1	4.4	3.3	231.9
	SD	5.37	4.66	4.84	4.47	3.24	4.88	3.72	3.82	3.40	3.34	3.24	7.51	46.50
	n	24	24	24	24	24	24	24	24	24	24	24	24	24
100	Mean	4.9	6.4	5.5	7.4	7.3	6.0	5.5	10.5	5.5	2.0	5.5	6.6	247.4
mg/kg bw/day	SD	6.16	4.85	4.66	3.81	4.78	4.66	3.91	4.80	6.53	5.88	4.65	4.28	58.13
	n	24	24	24	24	24	24	24	24	24 *	24	24	24	24
300	Mean	6.3	5.8	5.4	4.3	5.1	6.8	5.3	11.1	0.8	3.5	6.9	4.8	235.5
mg/kg bw/day	SD	4.51	3.82	3.92	4.32	4.27	4.09	3.87	3.69	4.44	3.51	3.62	4.04	44.67
	n	24	24	24	24 *	24	24	24	24 *	24 **	24	24	24	24
1000	Mean	5.6	3.8	-0.8	9.6	5.2	4.5	4.0	6.0	-3.7	2.4	5.2	3.4	165.6
mg/kg bw/day	SD	6.01	4.11	6.40	4.43	3.90	4.16	4.62	3.95	5.21	5.29	4.26	3.81	30.11
	n	24	24	24 **	24 *	24	24	24	24 *	24 **	24	24	24	24 **
		NS	NS	DN	DN	NS	NS	NS	DN	U	NS	NS	NS	U

Table 9: Summary of body weight gain of parent (P) females (pre-mating periods)

Group		Body weight gain (g) Pre-mating days										SUM
		0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-69	0-69
Control	Mean	14.0	9.1	8.3	10.1	5.0	3.5	3.1	4.9	3.2	4.7	65.9
	SD	3.81	5.15	5.00	5.85	4.13	3.89	3.95	4.23	4.19	6.06	8.67
	n	24	24	24	24	24	24	24	24	24	24	24
100	Mean	12.9	14.8	6.1	10.3	3.3	6.5	3.6	4.8	3.6	6.0	71.8
mg/kg bw/day	SD	5.38	6.14	4.99	3.25	5.19	5.63	5.23	4.83	5.70	6.03	15.94
	n	24	24 **	24	24	24	24	24	24	24	24	24
300	Mean	13.8	13.1	6.4	10.1	3.9	4.5	3.6	5.5	4.5	5.8	71.3
mg/kg bw/day	SD	3.78	4.55	4.60	4.97	4.99	5.00	4.43	3.64	6.67	5.67	11.45
	n	24	24 *	24	24	24	24	24	24	24	24	24
1000	Mean	10.5	14.4	5.7	10.5	6.0	6.9	1.7	4.4	5.0	2.5	67.5
mg/kg bw/day	SD	5.89	6.33	4.58	6.60	5.08	5.34	5.19	5.18	4.37	6.74	12.21
	n	24 *	24 **	24	24	24	24 *	24	24	24	24	24
		DN	DN	NS	NS	NS	DN	NS	NS	NS	NS	NS

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 10: Summary of body weight gain of parent (P) females (gestation and lactation periods)

Group		Body weight gain (g) between gestation days				Body weight gain (g) between lactation days				
		0-7	7-14	14-21	0-21	0-4	4-7	7-14	14-21	0-21
Control	Mean	18.2	23.6	73.2	115.0	3.7	8.4	15.7	-6.8	21.0
	SD	4.93	4.59	12.75	16.05	10.49	6.69	10.05	11.08	14.95
	n	21	21	21	21	21	21	21	21	21
100	Mean	18.7	22.4	71.6	112.7	3.7	8.3	18.6	-7.6	25.3
mg/kg bw/day	SD	4.26	4.70	13.21	17.19	11.84	7.58	8.31	8.57	12.19
	n	23	23	23	23	23	23	22	22	22
300	Mean	18.7	24.1	72.2	115.0	7.4	8.5	19.6	-11.3	24.9
mg/kg bw/day	SD	8.36	7.91	16.61	21.60	9.50	5.78	8.80	10.44	9.70
	n	23	23	23	23	23	23	22	22	22
1000	Mean	20.7	23.1	67.9	111.7	9.8	9.7	19.0	-0.1	38.4
mg/kg bw/day	SD	7.67	4.30	15.24	19.15	10.96	4.81	11.40	8.71	13.30
	n	16	16	16	16	16	16	16	16 *	16 **
		NS	NS	NS	NS	NS	NS	NS	DN	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Food consumption and compound intake (if feeding study):

The food consumption was not adversely affected in parental male or female animals at 100, 300 and 1000 mg/kg bw/day. Considering the body weight changes and food consumption of male animals at 1000 mg/kg bw/day, a slightly reduced feed efficiency is presumed during the post-mating period. The mean daily food

consumption of parental male animals was slightly lower than in the control group at 100 mg/kg bw/day between Days 104 and 118 and at 300 mg/kg bw/day between Days 11 and 118.

In the male animals at 1000 mg/kg bw/day, the mean daily food consumption was slightly higher than in the control group from week 10 (between Day 63-69) until the end of the study reaching statistical significances in most cases by weekly interval.

In the parental female animals, slightly higher mean daily food consumption was statistically significant at 100 mg/kg bw/day between days 7-14, 35-42, 63-69 and at 300 mg/kg bw/day between Day 7-14 during the pre-mating period. In the parental female animals at 1000 mg/kg bw/day, statistical significance with respect to the control was observed at the lower mean daily food consumption between Days 0 and 7 and at the slightly higher mean daily food consumption between Days 7-14 and 63-69. The mean daily food consumption was similar in the control and test item administered female animals during the gestation and lactation period except for dams at 1000 mg/kg bw/day during one week of lactation period. The lower mean daily food intake of dams at 1000 mg/kg bw/day reached statistical significance between lactation days 7 and 14.

These slight differences with respect to the control were of low degree and not consistent during the treatment period. Therefore, these were not considered to be toxicologically relevant.

Table 11: Summary of food consumption in parent (P) males

Group		Daily mean food consumption (g / animal /day)																			
		Pre-mating day										Post-mating days									
		0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-69	76-83	83-90	90-97	97-104	104-111	111-118	118-125	125-132	132-139	139-146	146-152
Control	Mean	23.6	23.0	22.1	21.2	20.3	20.3	19.0	17.9	17.6	17.9	15.8	16.9	17.3	18.2	18.8	20.7	17.9	17.5	17.3	16.7	15.9
	SD	2.09	2.13	1.72	1.74	1.60	1.36	1.24	1.48	1.37	1.45	0.94	1.94	1.34	1.25	1.17	1.39	1.02	1.26	1.26	1.29	1.77
	n	12	12	12	12	12	12	12	12	12	12	8	12	12	12	12	12	12	12	12	12	12
100	Mean	23.4	23.5	22.3	21.6	20.1	20.3	19.1	18.5	18.2	17.5	16.3	16.8	17.6	17.6	17.5	18.3	17.4	17.6	17.3	16.6	16.8
mg/kg bw/day	SD	2.08	2.19	2.33	2.24	1.81	1.52	1.55	1.44	1.39	1.31	1.63	1.46	1.28	1.63	1.40	1.32	1.22	1.10	1.21	1.45	1.27
	n	12	12	12	12	12	12	12	12	12	12	9	12	12	12	12	12	12	12	12	12	12
	± %	-1	2	1	2	-1	0	1	3	4	-2	3	-1	2	-3	-7 *	-11 **	-3	0	0	-1	5
300	Mean	23.5	23.4	22.5	21.7	20.8	20.4	18.9	18.4	17.6	17.4	15.9	16.7	18.0	17.7	18.1	18.8	17.6	17.8	17.8	16.6	16.9
mg/kg bw/day	SD	1.08	1.31	1.31	1.32	1.39	1.31	1.23	1.06	1.36	1.29	0.86	0.81	0.95	0.86	0.78	1.06	1.27	1.25	1.07	1.33	1.25
	n	12	12	12	12	12	12	12	12	12	12	8	12	12	12	12	12	12	12	12	12	12
	± %	0	2	2	2	3	0	-1	3	0	-3	1	-1	4	-3	-3	-9 **	-2	1	3	-1	6
1000	Mean	22.0	22.7	21.6	21.0	20.6	20.1	18.7	18.9	18.1	19.0	17.6	18.7	20.1	19.8	19.9	20.8	18.5	18.7	18.6	17.6	18.2
mg/kg bw/day	SD	2.10	1.80	1.52	1.26	1.65	1.80	1.67	1.23	1.46	1.38	1.36	2.02	2.00	1.66	1.78	2.08	1.75	1.99	1.73	1.62	1.88
	n	12	12	12	12	12	12	12	12	12	12	9	11	12	12	12	12	12	12	12	12	12
	± %	-7	-1	-2	-1	1	-1	-1	5	3	7 *	11 *	11	16 **	9 **	6 *	1	4	6	7 *	6	14 **
		NS	NS	NS	NS	NS	NS	NS	NS	NS	DN	DN	NS	DN	DN	DN	DN	NS	NS	DN	NS	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 12: Summary of food consumption in parent (P) females

Group		Daily mean food consumption (g / animal /day) Pre-mating days									
		0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-69
Control	Mean	13.9	14.0	14.8	14.5	13.9	13.7	12.7	12.2	12.4	12.6
	SD	0.82	0.62	0.88	0.96	0.90	0.78	0.83	0.98	1.04	0.95
	n	12	12	12	12	12	12	12	12	12	12
100 mg/kg bw/day	Mean	14.1	15.0	14.8	15.0	14.5	15.1	13.4	13.3	13.5	13.9
	SD	0.62	0.74	0.67	0.58	1.26	1.64	0.63	1.12	1.30	1.55
	n	12	12	12	12	12	12	12	12	12	12
	± %	1	6 **	1	3	5	10 *	5	9	9	11 *
300 mg/kg bw/day	Mean	14.4	15.6	15.3	15.4	14.5	14.2	13.3	12.9	12.6	13.3
	SD	1.23	2.80	3.07	3.24	3.21	2.18	2.19	1.86	1.22	1.31
	n	12	12	12	12	12	12	12	12	12	12
	± %	3	11 *	4	7	5	3	5	6	2	6
1000 mg/kg bw/day	Mean	12.5	15.3	14.6	14.7	14.6	14.3	13.5	13.2	13.3	13.9
	SD	1.04	0.93	1.26	1.18	1.11	1.03	1.10	1.06	1.51	1.45
	n	12	12	12	12	12	12	12	12	12	12
	± %	-10 **	9 **	-1	2	5	4	6	8	7	11 *
		DN	U	NS	NS	NS	U	NS	NS	NS	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Gestation period

Group		Daily mean food consumption (g / animal /day) Gestation day		
		0-7	7-14	14-21
Control	Mean	14.9	17.3	20.2
	SD	1.63	1.31	2.02
	n	21	21	21
100 mg/kg bw/day	Mean	15.2	17.4	20.0
	SD	1.81	1.42	1.88
	n	23	23	23
	± %	2	1	-1
300 mg/kg bw/day	Mean	15.1	16.9	20.0
	SD	2.37	1.58	2.00
	n	23	23	23
	± %	1	-2	-1
1000 mg/kg bw/day	Mean	14.6	17.2	19.8
	SD	1.63	1.05	2.27
	n	16	16	16
	± %	-2	0	-2
		NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Lactation period

Group		Daily mean food consumption (g / animal / day) between lactation day 0-4	4-7	7-14	14-21
Control	Mean	24.7	39.1	55.4	69.0
	SD	5.48	4.82	6.04	4.64
	n	21	21	21	21
100 mg/kg bw/day	Mean	25.0	37.3	53.7	68.5
	SD	4.87	8.23	5.54	6.74
	n	23	23	22	22
	± %	1	-5	-3	-1
300 mg/kg bw/day	Mean	25.7	38.5	54.0	68.1
	SD	4.95	4.72	4.06	4.30
	n	23	23	22	22
	± %	4	-2	-3	-1
1000 mg/kg bw/day	Mean	23.3	35.0	50.3	65.0
	SD	4.5	5.1	5.0	6.8
	n	16	16	16	16
	± %	-6	-10	-9	-6
				**	
		NS	NS	DN	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Haematological findings:

There were no test item related adverse changes in the examined hematological parameters in parental male or female animals at 100, 300 or 1000 mg/kg bw/day.

In the male animals, statistical significance was detected at the slightly shorter mean prothrombin time (PT) at 1000 mg/kg bw/day when compared to the control. All other examined parameters were comparable to the control in male animals at 100, 300 and 1000 mg/kg bw/day.

Statistical significance was detected at the slightly higher mean percentage of reticulocytes (RET) in female animals at 100 and 1000 mg/kg bw/day and at the slightly higher mean hematocrit value (HCT) at 300 mg/kg bw/day when compared to the control. All other examined hematological and blood coagulation parameters were comparable in female animals in the control and 100, 300 and 1000 mg/kg bw/day groups.

The individual values PT, RET and HCT were well within the historical control range in male or female animals, where relevant. There were no related changes in other hematological parameters. Therefore, the differences in these parameters were considered to have little or no toxicological relevance.

Table 13: Summary of hematology and blood coagulation of parent (P) males

Group		WBC [x10⁹/L]	NEU [%]	LYM [%]	MONO [%]	EOS [%]	BASO [%]	RBC [x10¹²/L]	HGB [g/L]	HCT [L/L]	MCV [fL]	MCH [pg]	MCHC [g/L]	PLT [x10⁹/L]	RET [%]	PT [sec]	APTT [sec]
Control	Mean	5.83	26.42	68.17	2.87	2.13	0.08	8.97	159.40	0.47	52.78	17.74	336.70	714.10	1.77	10.71	13.49
	SD	0.94	5.90	6.22	0.74	0.47	0.04	0.32	5.02	0.01	1.24	0.39	4.55	112.21	0.29	0.11	1.32
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
100	Mean	5.65	27.18	67.42	2.60	2.35	0.12	8.94	158.40	0.47	52.48	17.74	338.00	752.00	2.06	10.80	13.33
mg/kg bw/day	SD	1.48	5.85	6.91	0.70	0.95	0.08	0.48	6.62	0.02	1.52	0.47	9.23	119.85	0.48	0.47	1.98
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-3	3	-1	-9	10	50	0	-1	-1	-1	0	0	5	16	1	-1
300	Mean	5.34	24.73	69.86	2.86	2.13	0.05	8.68	158.90	0.47	53.78	18.35	341.10	751.10	1.91	10.70	13.72
mg/kg bw/day	SD	1.24	5.31	6.44	0.76	0.76	0.05	0.37	4.48	0.01	2.16	1.02	7.67	89.78	0.28	0.16	1.68
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-8	-6	2	0	0	-38	-3	0	-1	2	3	1	5	8	0	2
1000	Mean	5.01	27.17	67.09	3.40	1.96	0.05	8.86	156.50	0.47	53.39	17.69	331.30	724.10	2.13	10.50	13.02
mg/kg bw/day	SD	0.62	4.97	5.40	0.70	0.68	0.07	0.44	4.95	0.01	2.32	0.78	4.62	70.04	0.45	0.18	1.32
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-14	3	-2	18	-8	-38	-1	-2	0	1	0	-2	1	20	-2 **	-3
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	U	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 14: Summary of hematology and blood coagulation in parent (P) females

Group		WBC [x10⁹/L]	NEU [%]	LYM [%]	MONO [%]	EOS [%]	BASO [%]	RBC [x10¹²/L]	HGB [g/L]	HCT [L/L]	MCV [fL]	MCH [pg]	MCHC [g/L]	PLT [x10⁹/L]	RET [%]	PT [sec]	APTT [sec]
Control	Mean	6.52	45.66	49.18	3.60	1.07	0.09	8.41	161.70	0.49	57.95	19.24	332.30	958.20	1.40	10.06	12.58
	SD	1.46	14.37	15.26	1.21	0.34	0.06	0.58	9.53	0.03	2.20	0.91	6.15	100.75	0.41	0.24	1.08
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
100	Mean	6.84	45.60	48.13	3.92	1.80	0.11	8.44	158.20	0.48	57.17	18.80	328.60	926.20	1.90	9.97	12.83
mg/kg bw/day	SD	1.38	6.29	6.98	1.05	2.04	0.10	0.36	2.53	0.01	1.34	0.70	5.82	133.06	0.35	0.26	1.44
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	5	0	-2	9	68	22	0	-2	0	-1	-2	-1	-3	36 **	-1	2
300	Mean	6.46	41.73	53.33	3.37	0.95	0.14	8.83	167.70	0.51	57.68	19.02	329.70	950.90	1.63	9.92	12.27
mg/kg bw/day	SD	2.01	15.19	15.93	0.85	0.65	0.13	0.46	6.52	0.02	2.51	1.02	6.57	167.74	0.39	0.14	1.14
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-1	-9	8	-6	-11	56	5	4	5 *	0	-1	-1	-1	17	-1	-2
1000	Mean	5.94	45.29	48.83	4.16	1.19	0.11	8.53	163.10	0.50	58.34	19.11	327.40	883.90	1.97	9.90	12.67
mg/kg bw/day	SD	1.94	13.19	14.15	1.25	1.12	0.09	0.34	8.72	0.02	0.88	0.44	4.22	146.20	0.37	0.18	1.10
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	±%	-9	-1	-1	16	11	22	1	1	2	1	-1	-1	-8	41 **	-2	1
		NS	NS	NS	NS	NS	NS	NS	NS	DN	NS	NS	NS	NS	DN	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Clinical biochemistry findings:

The examined clinical chemistry parameters were not adversely affected in parental male or female animals at 100, 300 or 1000 mg/kg bw/day.

Clinical chemistry investigations revealed a slightly lower mean activity of aspartate aminotransferase (AST) at 300 mg/kg bw/day and lower mean concentration of total protein (TPROT) at 1000 mg/kg bw/day in the male animals.

In the female animals, a statistically significant difference with respect to the control was detected at the lower mean concentration of creatinine (CREA) at 100 mg/kg bw/day. All examined parameters were comparable with the control in the female animals at 300 mg/kg bw/day. In the female animals at 1000

mg/kg bw/day, lower mean concentration of creatinine and higher mean concentrations of urea and cholesterol (CHOL) were observed when compared to the control. The changes in AST, TPROT, CREA, UREA and CHOL were judged to be related with corresponding organ weight increases (liver and kidney).

Table 15: Summary of clinical chemistry of parent (P) males

Group		ALT [U/l]	AST [U/l]	TBIL [µmol/l]	CREA [µmol/l]	UREA [mmol/l]	GLUC [mmol/l]	CHOL [mmol/l]	Na^+ [mmol/l]	K^+ [mmol/l]	ALB [g/l]	TPROT [g/l]
Control	Mean	30.00	92.20	1.33	34.50	5.22	6.61	1.50	144.05	4.52	44.86	64.12
	SD	3.89	13.21	0.29	8.24	1.07	0.61	0.26	1.74	0.38	2.00	3.12
	n	10	10	10	10	10	10	10	10	10	10	10
100	Mean	30.40	95.90	1.15	29.00	4.83	6.61	1.65	144.76	4.72	44.14	61.68
mg/kg bw/day	SD	4.22	12.51	0.45	3.94	0.71	0.51	0.28	1.51	0.24	1.80	2.99
	n	10	10	10	10	10	10	10	10	10	10	10
	±%	1	4	-14	-16	-7	0	10	0	4	-2	-4
300	Mean	27.40	80.60	1.31	31.70	4.66	6.25	1.77	144.47	4.56	43.32	62.54
mg/kg bw/day	SD	4.22	9.91	0.23	3.97	0.63	0.83	0.39	1.88	0.32	1.84	2.77
	n	10	10	10	10	10	10	10	10	10	10	10
	±%	-9	-13 *	-2	-8	-11	-5	18	0	1	-3	-2
1000	Mean	34.10	99.00	1.16	29.80	5.47	6.18	1.51	144.28	4.71	44.45	60.66
mg/kg bw/day	SD	6.94	9.03	0.31	2.78	0.73	0.52	0.26	0.84	0.25	1.35	2.25
	n	10	10	10	10	10	10	10	10	10	10	10
	±%	14	7	-13	-14	5	-7	1	0	4	-1	-5 *
		NS	DN	NS	NS	NS	NS	NS	NS	NS	NS	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 16: Summary of clinical chemistry of parent (P) females

Group		ALT [U/l]	AST [U/l]	TBIL [µmol/l]	CREA [µmol/l]	UREA [mmol/l]	GLUC [mmol/l]	CHOL [mmol/l]	Na^+ [mmol/l]	K^+ [mmol/l]	ALB [g/l]	TPROT [g/l]
Control	Mean	49.90	141.80	1.05	32.60	8.94	6.25	1.97	142.10	4.57	41.39	54.91
	SD	11.69	28.54	0.29	2.50	1.01	0.75	0.40	1.59	0.22	1.65	3.41
	n	10	10	10	10	10	10	10	10	10	10	10
100	Mean	57.70	145.80	0.82	29.20	9.60	5.94	1.87	141.15	4.42	40.94	54.64
mg/kg bw/day	SD	12.72	19.22	0.24	5.16	1.10	0.58	0.38	1.45	0.29	1.78	1.74
	n	10	10	10	10	10	10	10	10	10	10	10
	±%	16	3	-22	-10 *	7	-5	-5	-1	-3	-1	0
300	Mean	51.00	144.70	1.27	30.70	9.82	6.22	2.11	141.42	4.28	42.56	56.64
mg/kg bw/day	SD	14.16	27.43	0.59	2.11	1.74	1.04	0.36	1.31	0.38	2.20	3.12
	n	10	10	10	10	10	10	10	10	10	10	10
	±%	2	2	21	-6	10	0	7	0	-6	3	3
1000	Mean	53.30	126.50	1.03	25.90	10.69	6.11	2.61	140.89	4.54	41.68	56.17
mg/kg bw/day	SD	11.76	16.67	0.39	3.31	1.92	0.64	0.65	2.05	0.69	2.85	4.11
	n	10	10	10	10	10	10	10	10	10	10	10
	±%	7	-11	-2	-21 **	20 *	-2	33 **	-1	-1	1	2
		NS	NS	NS	U	DN	NS	DN	NS	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Urinalysis findings:

There were no test item related adverse changes in the examined urine parameters in parental male or female animals at 100, 300 or 1000 mg/kg bw/day.

Statistically significantly higher mean volume (male, +65 % and female, +32 %) and lower pH in males and females (without reaching statistical significance) of the urine with respect to their control were observed at 1000 mg/kg bw/day. In the parental male animals, the lower mean pH of the urine at 100 and 300 mg/kg bw/day were statistically significant.

At 1000 mg/kg bw/day, the volume of urine was higher and the pH of the urine was lower than in the control group. Positive sediment was detected in all male animals at 1000 mg/kg bw/day due to the presence of larger amount of crystals (uric acid and amorphous crystals). The examined urine parameters were comparable in the parental female animals in the control, 100 and 300 mg/kg bw/day groups. Statistical significance was detected for the higher mean volume of the urine of female animals at 1000 mg/kg bw/day when compared to the control.

Table 17: Summary of urinalysis of parent (P) males

Group		Volume (mL)	Color	Clarity	pH	Glucose	Nitrite	Protein	Ketone	Urobili-nogen	Bilirubin	Blood (Ery/µL)	Spec. Gravity	Leu (Leu/µL)	Sediment
Control	Mean	4.0	Norm	Clear	6.6	Neg	Neg	Pos	Neg	Norm	Neg	Neg	1024.5	Neg	Neg
	SD	1.9		or	1.3				or			or	10.4		or
	n	10		Cloudy	10				Pos			Pos	10		Pos
100	Mean	3.4	Norm	Clear	5.5	Neg	Neg	Pos	Neg	Norm	Neg	Neg	1027.5	Neg	Neg
mg/kg bw/day	SD	1.4		or	0.8				or				4.9		or
	n	10		Cloudy	10				Pos				10		Pos
					**										
300	Mean	4.0	Norm	Clear	5.0	Neg	Neg	Pos	Pos	Norm	Neg	Neg	1030.0	Neg	Neg
mg/kg bw/day	SD	0.8			0.0								0.0		
	n	10			10								10		
					**										
1000	Mean	6.6	Norm	Clear	5.0	Neg	Neg	Pos	Pos	Norm	Neg	Neg	1030.0	Neg	Pos
mg/kg bw/day	SD	1.2		or	0.0								0.0		
	n	10		Cloudy	10								10		
		**			**										
		DN			DN								NS		

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 18: Summary of urinalysis of parent (P) females

Group		Volume (mL)	Color	Clarity	pH	Glucose	Nitrite	Protein	Ketone	Urobili-nogen	Bilirubin	Blood (Ery/µL)	Spec. Gravity	Leu (Leu/µL)	Sediment
Control	Mean	13.1	Norm	Clear	6.0	Neg	Neg	Neg	Neg	Norm	Neg	Neg	1019.5	Neg	Neg
	SD	3.0		or	0.7			or					6.4		
	n	10		Cloudy	10			Pos					10		
100	Mean	15.3	Norm	Clear	6.1	Neg	Neg	Neg	Neg	Norm	Neg	Neg	1016.0	Neg	Neg
mg/kg bw/day	SD	4.2		or	0.9			or					5.2		or
	n	10		Cloudy	10			Pos					10		Pos
300	Mean	13.6	Norm	Clear	5.6	Neg	Neg	Neg	Neg	Norm	Neg	Neg	1021.0	Neg	Neg
mg/kg bw/day	SD	3.7		or	0.8			or					5.2		or
	n	10		Cloudy	10			Pos					10		Pos
1000	Mean	17.2	Norm	Clear	5.4	Neg	Neg	Neg	Neg	Norm	Neg	Neg	1023.0	Neg	Neg
mg/kg bw/day	SD	4.2		or	0.7			or					5.4		
	n	10		Cloudy	10			Pos					10		
		*													
		DN			NS								NS		

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Behaviour (functional findings): not examined

Immunological findings: not examined

Organ weight findings including organ / body weight ratios:

Table 19: Summary of organ weight of parent (P) males

Group		Body weight	Organ weight (g) Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididy-mides	Seminal vesicles†	Prostate	Adrenal glands	Thyroids	Pituitary
Control	Mean	502.6	2.30	11.27	2.46	1.15	0.33	0.71	3.80	1.64	1.95	0.61	0.064	0.021	0.009
	SD	54.63	0.08	1.43	0.23	0.08	0.07	0.09	0.28	0.18	0.44	0.14	0.009	0.004	0.002
	n	24	24	24	24	24	24	24	24	24	23	24	24	24	24
100 mg/kg bw/day	Mean	520.3	2.31	11.72	2.70	1.16	0.30	0.71	3.71	1.74	2.17	0.66	0.063	0.022	0.010
	SD	65.37	0.11	1.51	0.34	0.11	0.06	0.09	0.24	0.14	0.43	0.15	0.013	0.004	0.002
	n	24	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	4	0	4	10 **	1	-9	0	-2	6 *	11	9	-1	4	4
300 mg/kg bw/day	Mean	506.5	2.28	12.02	2.81	1.15	0.29	0.72	3.88	1.73	1.89	0.57	0.069	0.022	0.009
	SD	53.91	0.08	1.44	0.29	0.09	0.07	0.08	0.27	0.15	0.42	0.12	0.009	0.005	0.002
	n	24	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	1	-1	7	14 **	0	-10	1	2	5	-3	-6	9	2	-3
1000 mg/kg bw/day	Mean	431.3	2.19	12.64	3.22	1.09	0.27	0.71	3.82	1.57	2.12	0.48	0.065	0.021	0.009
	SD	38.05	0.10	1.45	0.49	0.10	0.06	0.09	0.28	0.13	0.53	0.09	0.013	0.005	0.002
	n	24	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	-14 **	-5 **	12 **	31 **	-6 *	-16 *	-1	0	-5	9	-20 *	3	-3	0
		DN	DN	DN	U	DN	DN	NS	NS	DN	NS	DN	NS	NS	NS

REMARKS : † = Seminal vesicles with coagulating gland
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight relative to body weight (%) Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididy-mides	Seminal vesicles†	Prostate	Adrenal glands	Thyroids	Pituitary
Control	Mean	0.463	2.241	0.491	0.230	0.065	0.142	0.764	0.329	0.394	0.122	0.0128	0.0042	0.0019
	SD	0.050	0.118	0.041	0.017	0.012	0.018	0.084	0.040	0.097	0.029	0.0022	0.0008	0.0004
	n	24	24	24	24	24	24	24	24	23	24	24	24	24
100 mg/kg bw/day	Mean	0.450	2.259	0.522	0.224	0.058	0.138	0.722	0.339	0.425	0.128	0.0122	0.0042	0.0019
	SD	0.059	0.187	0.057	0.021	0.013	0.021	0.083	0.042	0.102	0.025	0.0022	0.0006	0.0004
	n	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	-3	1	6	-3	-11	-3	-5	3	8	5	-5	0	0
300 mg/kg bw/day	Mean	0.455	2.377	0.558	0.227	0.058	0.142	0.774	0.344	0.376	0.114	0.0137	0.0043	0.0018
	SD	0.045	0.183	0.062	0.013	0.015	0.014	0.093	0.040	0.077	0.029	0.0019	0.0009	0.0004
	n	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	-2	6 **	14 **	-1	-10	0	1	4	-5	-6	7	1	-4
1000 mg/kg bw/day	Mean	0.510	2.928	0.743	0.252	0.064	0.164	0.891	0.365	0.493	0.113	0.0151	0.0047	0.0021
	SD	0.035	0.163	0.066	0.013	0.016	0.018	0.091	0.030	0.116	0.020	0.0027	0.0009	0.0004
	n	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	10 **	31 **	51 **	9 **	-2	15 **	17 **	11 **	25 **	-7	18 **	12 *	15 *
		DN	DN	DN	DN	NS	DN	DN	DN	DN	NS	DN	DN	DN

REMARKS : † = Seminal vesicles with coagulating gland
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight and body weight relative to brain weight (%)												
		Body weight	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididy-mides	Seminal vesicles†	Prostate	Adrenal glands	Thyroids	Pituitary
Control	Mean	21846.4	490.07	106.75	50.00	14.20	30.86	165.40	71.36	84.55	26.29	2.77	0.91	0.40
	SD	2307.22	62.03	10.08	3.52	3.21	4.27	12.44	6.52	18.18	5.74	0.41	0.16	0.07
	n	24	24	24	24	24	24	24	24	23	24	24	24	24
100	Mean	22607.4	508.99	117.33	50.31	12.85	30.87	161.18	75.60	93.90	28.85	2.73	0.95	0.42
mg/kg bw/day	SD	3237.39	71.84	16.12	5.43	2.36	4.59	12.88	5.90	16.30	7.17	0.57	0.18	0.07
	n	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	3	4	10 *	1	-9	0	-3	6 *	11	10	-1	4	3
300	Mean	22190.8	527.04	123.30	50.25	12.81	31.40	170.40	75.67	83.06	24.99	3.03	0.95	0.40
mg/kg bw/day	SD	2115.16	61.86	13.10	3.66	3.03	3.55	13.09	5.88	18.34	5.71	0.39	0.21	0.10
	n	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	2	8	16 **	0	-10	2	3	6 *	-2	-5	9	3	-2
1000	Mean	19687.3	576.90	146.53	49.57	12.51	32.17	174.73	71.76	96.68	22.08	2.98	0.93	0.42
mg/kg bw/day	SD	1328.86	55.58	18.64	3.71	2.83	3.56	14.72	5.92	22.21	3.79	0.58	0.19	0.08
	n	24	24	24	24	24	24	24	24	24	24	24	24	24
	± %	-10 **	18 **	37 **	-1	-12	4	6 *	1	14 *	-16 **	8	2	5
		U	DN	U	NS	NS	NS	DN	DN	DN	U	NS	NS	NS

REMARKS : † = Seminal vesicles with coagulating gland
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 20: Summary of organ weight of parent (P) females

Group		Body weight	Organ weight (g)										
			Brain	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	Adrenal glands	Thyroids	Pituitary
Control	Mean	242.2	2.01	9.71	1.81	0.86	0.18	0.55	0.63	0.087	0.088	0.017	0.013
	SD	12.31	0.06	1.21	0.18	0.06	0.04	0.10	0.16	0.017	0.014	0.003	0.002
	n	24	24	24	24	24	24	24	24	24	24	24	23
100	Mean	248.5	1.99	10.08	1.81	0.86	0.17	0.57	0.60	0.095	0.087	0.018	0.012
mg/kg bw/day	SD	14.07	0.08	1.00	0.13	0.08	0.05	0.07	0.11	0.014	0.009	0.004	0.003
	n	24	24	24	24	24	24	24	24	24	24	23	24
	± %	3	-1	4	0	0	-7	4	-4	8	-1	7	-2
300	Mean	247.0	1.98	10.39	1.88	0.87	0.16	0.56	0.63	0.094	0.088	0.018	0.011
mg/kg bw/day	SD	15.12	0.07	0.79	0.14	0.09	0.05	0.08	0.14	0.015	0.011	0.003	0.002
	n	24	24	24	24	24	24	24	24	24	24	24	24
	± %	2	-1	7 *	4	2	-10	2	0	7	0	6	-11
1000	Mean	249.0	1.92	11.56	1.91	0.82	0.19	0.54	0.63	0.091	0.090	0.016	0.012
mg/kg bw/day	SD	16.51	0.09	1.84	0.26	0.10	0.07	0.08	0.17	0.024	0.014	0.003	0.002
	n	24	24	24	24	24	24	24	24	24	24	24	24
	± %	3	-4 **	19 **	5	-5	3	-2	-1	5	2	-5	-6
		NS	DN	U	NS	NS	NS	NS	NS	NS	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight relative to body weight (%)										
		Brain	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	Adrenal glands	Thyroids	Pituitary
Control	Mean	0.830	4.002	0.748	0.353	0.075	0.226	0.260	0.0361	0.0361	0.0070	0.0052
	SD	0.041	0.397	0.061	0.018	0.019	0.034	0.061	0.0068	0.0047	0.0014	0.0008
	n	24	24	24	24	24	24	24	24	24	24	23
100 mg/kg bw/day	Mean	0.801	4.066	0.730	0.346	0.068	0.229	0.242	0.0382	0.0350	0.0073	0.0050
	SD	0.040	0.456	0.053	0.025	0.018	0.025	0.042	0.0062	0.0039	0.0017	0.0011
	n	24	24	24	24	24	24	24	24	24	23	24
	± %	-3 *	2	-2	-2	-10	1	-7	6	-3	4	-5
300 mg/kg bw/day	Mean	0.805	4.215	0.761	0.352	0.066	0.227	0.256	0.0380	0.0356	0.0073	0.0046
	SD	0.043	0.337	0.056	0.027	0.021	0.033	0.060	0.0064	0.0044	0.0012	0.0010
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	-3 *	5	2	0	-12	0	-2	5	-1	4	-13 *
1000 mg/kg bw/day	Mean	0.772	4.640	0.766	0.328	0.075	0.216	0.251	0.0366	0.0360	0.0064	0.0048
	SD	0.037	0.633	0.082	0.035	0.029	0.029	0.064	0.0091	0.0051	0.0011	0.0007
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	-7 **	16 **	2	-7 *	0	-5	-3	2	0	-8	-9
		DN	U	NS	U	NS	NS	NS	NS	NS	NS	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight and body weight relative to brain weight (%)										
		Body weight	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	Adrenal glands	Thyroids	Pituitary
Control	Mean	12074.5	483.79	90.36	42.67	9.04	27.31	31.33	4.35	4.36	0.84	0.63
	SD	601.73	57.73	9.47	3.02	2.14	4.55	7.77	0.82	0.64	0.15	0.09
	n	24	24	24	24	24	24	24	24	24	24	23
100 mg/kg bw/day	Mean	12510.6	508.34	91.22	43.27	8.50	28.63	30.35	4.77	4.37	0.91	0.62
	SD	630.95	58.65	6.12	3.74	2.22	3.54	5.58	0.77	0.46	0.22	0.13
	n	24	24	24	24	24	24	24	24	24	23	24
	± %	4 *	5	1	1	-6	5	-3	10	0	8	-1
300 mg/kg bw/day	Mean	12451.4	524.04	94.58	43.77	8.26	28.19	31.76	4.73	4.42	0.90	0.57
	SD	657.07	41.40	7.14	3.91	2.67	4.13	7.40	0.77	0.51	0.16	0.12
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	3 *	8 **	5	3	-9	3	1	9	1	7	-10
1000 mg/kg bw/day	Mean	12987.9	602.56	99.45	42.53	9.73	28.01	32.62	4.76	4.67	0.83	0.62
	SD	603.55	86.87	11.38	4.88	3.63	4.10	8.48	1.26	0.64	0.13	0.09
	n	24	24	24	24	24	24	24	24	24	24	24
	± %	8 **	25 **	10 *	0	8	3	4	10	7	-1	-2
		DN	U	U	NS	NS	NS	NS	NS	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Necropsy

Macroscopic alterations related to the effect of the test item were not detected in male or female animals at 100, 300 or 1000 mg/kg bw/day at the necropsy.

In the parental male animals in control group, thymic hemorrhage (1/24), nutmeg-like patterned liver (1/24), renal pyelectasia (1/24, right side) smaller than normal seminal vesicle (1/24; one side) and hard tissue formation in the abdominal fatty tissue (1/24) were observed at the necropsy.

In the male animals at 100 mg/kg bw/day, hemorrhages in the thymus (1/24), congestive mucous membrane in the stomach (2/24), pyelectasia (1/24, left side) and hard tissue formation in the abdominal fatty tissue (3/24) were observed were detected at the necropsy.

At 300 mg/kg bw/day, thymic hemorrhage (3/24), brown black colored lungs (1/24), congestive mucous membrane in the stomach (1/24), and pyelectasia (4/24, right side or both sides) were noted for some male animals.

At 1000 mg/kg bw/day, hemorrhage in the thymus (1/24), congestive mucous membrane in the stomach (3/24) and pyelectasia (2/24, right side) were seen in male animals at the necropsy.

In control dams, hemorrhage in the mucous membrane in the stomach (4/21), pyelectasia (1/21, both sides), hydrometra (5/21, slight, moderate or marked) and reddish colored mesenterial lymph nodes (1/21) were observed.

At 100 mg/kg bw/day, hemorrhage in the mucous membrane in the stomach (10/23), pyelectasia (3/23, one or both sided), hydrometra (4/23, slight or moderate) were noted for dams.

In dams at 300 mg/kg bw/day, hemorrhage in the thymus (1/23) and in the mucous membrane in the stomach (8/23), pyelectasia (3/23, right or both sided) and hydrometra (4/23, marked or moderate) were observed.

At 1000 mg/kg bw/day, hemorrhage in the thymus (1/16) and in the mucous membrane in the stomach (3/16), pyelectasia (3/16, left or right side) and hydrometra (2/16, slight or marked) were detected.

In non-pregnant female animals, pyelectasia (1/1 at 100 and 300 mg/kg bw/day, both, right or both sided), ovarian cyst (1/8 at 1000 mg/kg bw/day), hydrometra (1/2 control, slight; 4/8 at 1000 mg/kg bw/day, slight or marked), hard knot at the cervix of uterus (1/1 at 300 mg/kg bw/day) and alopecia on the thorax (1/8 at 1000 mg/kg bw/day) were detected at the necropsy.

Not mated control female animal showed marked hydrometra.

Congestive mucous membrane (male animals) and hemorrhage in the stomach mucosa (female) were probably due to the local effect of the test item or treatment procedure. There was no dose response relationship in the incidence of these findings or related changes. Therefore, changes in the stomach mucosa were considered toxicologically not relevant.

Hydrometra (i.e. dilatation of uterine horns), related to the female sexual cycle, is a frequent observation in experimental rats. However, it was observed in 5/12 in the non-pregnant females and only in 15/83 pregnant females (considering control and treated groups) suggesting a relationship with infertility.

Hard knot in the fatty tissue of abdominal cavity, ovarian cyst, hard knot at the cervix and alopecia on the skin are common macroscopic findings in experimental rats of this strain with similar age. These occurred with low incidence and were considered to be toxicologically not relevant.

Pyelectasia is frequently observed in this strain of experimental rats. Histological examination did not reveal degeneration, inflammation or fibrosis. Increase in pyelectasia in the treated group as compared with controls is in relationships with increase in diuresis.

Hemorrhages in the lungs or thymus are indicative of circulatory disturbance developing during the exsanguination.

Smaller than normal seminal vesicle, nutmeg-like patterned liver and reddish colored mesenterial lymph nodes are individual findings in control animals (male or female).

Table 21: Summary of necropsy findings of parent (P) males

Organs	Observations	Frequency of observations per group			
		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
	No macroscopic findings	19/24	17/24	17/24	19/24
Thymus	Hemorrhages	1/24	1/24	3/24	1/24
Lungs	Brown-black colored	0/24	0/24	1/24	0/24
Stomach	Congestive mucous membrane	0/24	2/24	1/24	3/24
Liver	Nutmeg-like patterned	1/24	0/24	0/24	0/24
Kidneys	Pyelectasia	1/24	1/24	4/24	2/24
Seminal vesicle	Smaller than normal - left side	1/24	0/24	0/24	0/24
Abdominal cavity:	Hard fatty tissue formation	1/24	3/24	0/24	0/24
Remark:	Frequency of observations = number of animals with observations / number of animals examined.				

Table 22: Summary of necropsy findings of parent (P) females

	Organs	Observations	Frequency of observations per group			
			Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Dams		No macroscopic findings	12/21	10/23	11/23	10/16
	Thymus	Hemorrhage	0/21	0/23	1/23	1/16
	Stomach	Hemorrhages	4/21	10/23	8/23	3/16
	Kidneys	Pyelectasia	1/21	3/23	3/23	3/16
	Uterus	Hydrometra	5/21	4/23	4/23	2/16
	Mesenterial ln.	Reddish colored	1/21	0/23	0/23	0/16
Non pregnant females		No macroscopic findings	1/2	0/1	0/1	4/8
	Kidneys	Pyelectasia	0/2	1/1	1/1	0/8
	Ovaries	Cyst	0/2	0/1	0/1	1/8
	Uterus	Hydrometra Hard knot on the cervix	1/2 0/2	0/1 0/1	0/1 1/1	4/8 0/8
	Skin	Alopecia	0/2	0/1	0/1	1/8
Not mated female	Uterus	Hydrometra	1/1	/	/	/
Remark:	Frequency of observations = number of animals with observations / number of animals examined. ln. = Lymph nodes / = No data					

Histopathological findings: non neoplastic:

The investigated organs of reproductive system (testes, epididymides, prostate seminal vesicles, coagulating glands) were histologically normal and characteristic for the sexually mature organism in all parental male animals in the control and 1000 mg/kg bw/day groups.

Decreased number of developing follicles and increased number of follicular atresia were detected in female animals at 1000 mg/kg bw/day (pregnant or non-pregnant) compared with their control (on the actual level of section investigated). This finding was supported by the results of quantitative examinations of the ovaries.

The various spermatogenic cells (the spermatogonia, the spermatocytes, the spermatids and spermatozoa) representing different phases in the development and differentiation of the spermatozoons and the interstitial cells were the same in quantity and morphology in the testes of investigated control and high dose treated animals. The histological picture of epididymides, prostate, seminal vesicles, and coagulating glands was normal in all cases as well, except for one control male animal (1/24). In this animal, decreased amount of secrete in the seminal vesicle (one side) was observed. This phenomenon, without inflammatory or degenerative lesion was considered as individual disorder, without toxicological significance.

In the female animals of the control and 1000 mg/kg bw/day groups, the ovaries, uterus, cervix, vagina had a normal structure characteristic of the species, age and phase of the active sexual cycle. The cortical region of ovaries contained primary, secondary and tertiary follicles and corpora lutea, indicating the active maturation of oocytes, and ovulation. The epithelial capsule and ovarian stroma were normal in all cases as well. In addition, in non-pregnant female animals (8/8) at 1000 mg/kg bw/day decreased number of developing follicles and increased number of follicular atresia were observed along with developing follicles and corpora lutea on the actual level of section by qualitative histological examination. At the quantitative examinations, the mean number of secondary and tertiary follicles and corpora lutea slightly exceeded the control value in dams at 100 and 300 mg/kg bw/day reaching statistical significance only in the mid dose treated animals. At 1000 mg/kg bw/day, the mean number of secondary and tertiary follicles was lower and the mean number of follicular atresia was higher than in the control group in dam at the examined level of histological section. This finding was more excessive in non-pregnant female animals at 1000 mg/kg bw/day (8/8). Statistical significance was also observed in delivered female animals at 1000 mg/kg bw/day at the slightly lower mean number of primordial and primary follicles. In three female animals at 1000 mg/kg bw/day, one or both sided follicular cyst (3/24) was detected in the ovaries. The mucous membrane of uterus, cervix and vagina was normal in these female animals similar to that in the control group. The effect of high dose of test item could be considered in the development of decrease in the number of developing follicles, and the increase in the number of follicular atresia and the follicular cyst forming, in the high dose treated female animals. According the registrant, "*follicular atresia is a normal, physiological process in the ovary, to regulate the number of follicles in the developing pool and increase in follicular atresia can be observed secondary to xenobiotic administration. Since the development of small parental follicles is gonadotropin independent, an increase in atresia in these follicles is typically seen with direct-acting cytotoxic compounds, heavy metals or radiation. (Suttie, A.W., et al: Boorman's Pathology of the rat. Reference and Atlas. Second Edition. Academic Press, Elsevier, London, San Diego, Cambridge, Oxford, 2018.). In our study, the follicular atresia affected only partly the ovarian functions, however absence of corpora lutea (lack of ovulation) or total ovarian atrophy was not detectable"*.

The histological structure and the cellularity of pituitary with special attention on the cytomorphology and proportion of acidophilic and basophilic cells in the adenohypophysis were the same in the control and treated male and female animals. In some cases, dilatation of uterine horns was observed (7/24 control; 5/24 at 100 mg/kg bw/day; 4/24 at 300 g/kg bw/day; 4/24 at 1000 mg/kg bw/day).

Histopathological investigations revealed chronic progressive nephropathy in a higher incidence of male animals at 1000 mg/kg bw/day with respect to the control. Histological examination revealed the earliest stage of chronic progressive nephropathy (CPN) in a proportion of male animals in control group (5/24) and in the 1000 mg/kg bw/day group (11/24). Scattered tubular dilatation, hyaline casts, tubular basophilia, lymphocytic and histiocytic infiltrations were observed. CPN is a spontaneous renal disease of the commonly used strains of laboratory male rat. In this study, CPN was seen with a higher incidence in male animals at 1000 mg/kg bw/ comparing to the control. Therefore, it is presumed, that the high dose of test item was a predisposing factor in the pathogenesis of this renal lesions**.** Chronic progressive nephropathy was not detected in the kidneys of male animals at 100 or 300 mg/kg bw/day (24/24, both groups).

Alveolar emphysema (minimal degree) in the lungs (1/24 male control; 1/24 female control), and acute hemorrhage in the thymus (1/24 male at 100 mg/kg bw/day, 3/24 male at 300 mg/kg bw/day; 1/24 male at 1000 mg/kg bw/day; 1/23 female at 300 mg/kg bw/day and 1/16 female at 1000 mg/kg bw/day) occurred sporadically and are considered as consequence of hypoxia, dysnea and circulatory disturbance developed during exsanguinations

Hyperplasia of bronchus associated lymphoid tissue (BALT) in some control and treated animals (2/24 male and 1/24 female control; 1/24 male and 1/24 female at 1000 mg/kg bw/day) is an immuno-morphological phenomenon, without toxicological significance.

Alveolar histiocytosis accompanied with congestion in the lungs in one male animal at 300 mg/kg bw/day is a common incidental finding in elder rats and consists of small focal intra-alveolar collections of alveolar macrophages with abundant foamy (lipid-containing) cytoplasm.

Lipoma in the abdominal cavity at 100 mg/kg bw/day and the abscess in the wall of uterus at 300 mg/kg bw/day are sporadically observed in experimental rats of this strain and these findings were considered as individual disorder, without toxicological significance.

There was no morphological evidence of test item related acute or subacute injury (degeneration, inflammation, necrosis etc.) in the small and large intestines, liver, pancreas, cardiovascular system, respiratory system, immune system, hematopoietic system, skeleton, muscular system, central, or peripheral nervous system, eyes, integumentary system. The cytomorphology of endocrine glands were the same in the control and treated animals but in the absence of hormonal assays, endocrine changes cannot be excluded.

Ovary Follicle Count:

Quantitative examinations of ovaries revealed test item related decrease in the number of developing follicles and increase in the number of follicular atresia in parental female animals at 1000 mg/kg bw/day. The mean number of secondary and tertiary follicles slightly exceeded the control value in female animals at 100 and 300 mg/kg bw/day. At 1000 mg/kg bw/day, statistical significance was observed in female animals at the slightly lower mean number of primordial and primary follicles and secondary and tertiary follicles as well as at the higher mean number of follicular atresia.

Table 23: Summary of quantitative evaluation of ovaries parent (P) females

Group		Primordial and primary follicles	Secondary and tertiary follicles	Corpora lutea	Follicular atresia	Cystic degeneration	Other findings
Control	Mean	31.6	12.6	13.8	5.3	0.0	0.0
	SD	6.9	3.6	7.2	1.4	0.0	0.0
	n	24	24	24	24	24	24
100	Mean	32.1	14.5	14.0	5.3	0.0	0.0
mg/kg bw/day	SD	3.1	2.4	4.1	0.7	0.0	0.0
	n	24	24 *	24	24	24	24
300	Mean	32.2	14.9	14.9	5.4	0.0	0.0
mg/kg bw/day	SD	2.9	2.2	4.2	0.9	0.0	0.0
	n	24	24 **	24	24	24	24
1000	Mean	29.3	7.9	12.5	13.2	0.0	0.0
mg/kg bw/day	SD	3.6	2.4	6.2	6.2	0.0	0.0
	n	24 *	24 **	24	24 **	24	24
		U	DN	NS	U	-	-

Remark: Quantitative examinations were performed at the section level of ovaries
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = No data

Group		Primordial and primary follicles	Secondary and tertiary follicles	Corpora lutea	Follicular atresia	Cystic degeneration	Other findings
Control	Mean	31.2	12.3	11.9	5.2	0.0	0.0
	SD	7.2	3.8	4.4	1.4	0.0	0.0
	n	21	21	21	21	21	21
100	Mean	32.1	14.3	14.2	5.3	0.0	0.0
mg/kg bw/day	SD	3.2	2.1	4.1	0.7	0.0	0.0
	n	23	23	23	23	23	23
300	Mean	32.2	14.9	15.1	5.4	0.0	0.0
mg/kg bw/day	SD	2.9	2.3	4.2	0.9	0.0	0.0
	n	23	23	23	23	23	23
			*	*			
1000	Mean	28.7	8.1	9.8	11.4	0.0	0.0
mg/kg bw/day	SD	3.4	1.9	3.3	2.6	0.0	0.0
	n	16	16	16	16	16	16
		*	**		**		
		U	U	DN	U	-	-
1000	Mean	30.6	7.6	18.0	16.6	0.0	0.0
mg/kg bw/day	SD	4.0	3.3	7.1	1.9	0.0	0.0
NP	n	8	8	8	8	8	8

Remark: Quantitative examinations were performed at the section level of ovaries
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
NP = Non-pregnant
- = No data

Thyroid Hormone measurements:

The thyroid hormone (FT3, FT4 and TSH) levels were not adversely influenced in the parental male or female animals or in PND22 F1 offspring at any dose levels.

Slight, statistically significant difference was detected at the lower mean FT3 and FT4 concentrations in male animals at 1000 mg/kg bw/day which is not compensated by an increase in TSH level.

Table 24: Summary of thyroid hormone levels in parent (P) males

SUMMARY OF THYROID HORMONE LEVELS
PARENT (P) MALE

		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
FT3 [ng/dL]	Mean	0.34	0.32	0.34	0.26	
	SD	0.05	0.06	0.08	0.04	
	n	10	10	10	10	
	±%		-7	-1	-25 **	DN
FT4 [ng/dL]	Mean	2.87	3.25	3.14	2.34	
	SD	0.39	0.29	0.33	0.53	
	n	10	10	10	10	
	±%		13	9	-19 **	DN
TSH [µIU/mL]	Mean	0.008	0.008	0.010	0.007	
	SD	0.004	0.004	0.006	0.001	
	n	3	4	6	5	
	±%		-4	24	-21	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = No data (Values were below the limit of detection - 0.005 µIU/mL)

There were no statistically significant differences with respect to their control in the FT3, FT4 and TSH concentrations in the parental female animals or in PND 22 F1 offspring at any dose levels.

Reproductive function / performance (P0)

Oestrous cycle:

The estrous cycle was irregular in several parental female animals at 1000 mg/kg bw/day during the two last weeks of an overall of 10 weeks pre-mating period.

The examined parameters of the estrous cycle were comparable in the control and 100 and 300 mg/kg bw/day groups.

Statistical significance was noted for the lower percentage of female animals with regular cycle and for the lower mean number of days in pre-estrous at 1000 mg/kg bw/day. The number of female animals in prolonged estrous was also higher than in the control group at 1000 mg/kg bw/day.

Table 25: Summary data of oestrus cycle of parent (P) females (premating period)

Group		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
Number of animals examined	N	24	24	24	24	
Animals with regular cycles	N	20	19	15	13	*
	%	83	79	63	54	
Animals with irregular cycles (%)	N	4	5	9	11	
	%	17	21	38	46	
Number of cycles	Mean	3.7	3.3	3.0	3.1	
	SD	1.1	1.1	1.3	1.0	
	n	24	24	24	24	NS
Lenght of cycles	Mean	4.4	4.5	4.5	5.3	
	SD	1.5	2.0	1.9	2.1	
	n	22	21	17	21	NS
Days in proestrous	Mean	3.0	2.6	2.5	1.5	
	SD	1.2	1.1	1.6	1.1	
	n	24	24	24	24	** DN
Days in estrous	Mean	3.8	3.3	3.2	4.3	
	SD	1.2	1.1	1.5	1.8	
	n	24	24	24	24	NS
Days in diestrous	Mean	9.2	10.1	10.3	10.2	
	SD	2.0	2.9	2.8	2.5	
	n	24	24	24	24	NS
Animals in prolonged estrous	N	0	0	0	7	
	%	0	0	0	29	
Animals in prolonged diestrous	N	4	5	9	4	
	%	17	21	38	17	

REMARKS:
NS = Not Significant
* = $p < 0.05$; CH2
** = $p < 0.01$; CH2
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

Delivery data of dams

Delivery data of dams was not adversely affected at 100, 300 or 1000 mg/kg bw/day dose levels. The lower number of females delivering in the 1000 mg/kg bw/day group as compared to the control group was due to the lower number of pregnant females. All pregnant females delivered and there were no significant differences in most of the examined parameters with respect to the control.

The mean number of implantation sites, the mean number of post-implantation loss, the mean number of total births, mean number of viable pups and live borns and the live birth index (live pups/total birth) were comparable in all groups.

The slightly longer mean duration of pregnancy of dams at 1000 mg/kg bw/day was statistically significant (22.37 days versus 21.97 days). The value was at the upper of the historical control range (21.8-22.3 days; 13 studies).

Table 26: summary of delivery data of dams parent (P) females

Values		Group (mg/kg bw/day) Control	100	300	1000	
No. of pregnants	N	21	23	23	16	
No of dams delivered	N	21	23	23	16	
No. of implantation	Sum	265	276	283	191	
	N	21	23	23	16	
Post-implantation loss	Sum	18	24	21	13	
	N	21	22	23	16	
	%	7	9	7	7	
No. of implantation	Mean	12.6	12.0	12.3	11.9	
	SD	2.0	2.2	2.9	2.8	
	N	21	23	23	16	NS
Post-implantation loss	Mean	0.9	1.1	0.9	0.8	
	SD	1.1	1.3	0.9	0.9	
	N	21	22	23	16	NS
Type of nursing						
Dams with adequate nursing	N	19	20	21	11	
	%	90	87	91	69	
Dams with inadequate nursing	N	2	3	2	5	
	%	10	13	9	31	

Remarks:
NS = Not Significant
* = $p < 0.05$; CHI2
** = $p < 0.01$; CHI2
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

Reproductive performance:

Significantly lower reproduction indices were observed in female animals at 1000 mg/kg bw/day with respect to their control.

Mating of male animals – which did not fertilize their partners of main group – with not treated female animals provided clear evidence of reproduction ability of these male animals. This information is important since it shows that decrease in fertility of treated rats originate from alteration of reproductive function of the females.

The examined parameters of reproductive performance were not affected by the treatment with the test item in male or female animals at 100 or 300 mg/kg bw/day.

The copulatory index was higher than in the control group in all test item administered groups (100, 300 and 1000 mg/kg bw/day) as one control pair failed to mate.

The percentage of pregnant females (reproduction index) (16/24 versus 21/24) was statistically significantly lower and percentage of non-pregnant female animals (8/24 versus 2/24) was statistically significantly higher with respect to their control group at 1000 mg/kg bw/day which was associated with the test item treatment.

One control female animal died on gestation day 22 – not delivered – while all pregnant animals delivered at 100, 300 and 1000 mg/kg bw/day.

Statistical significance was observed for the slightly higher mean number of conceiving days in female animals at 300 mg/kg bw/day. Similar finding was not detected at the high dose, therefore, this difference in the mean number conceiving days at 300 mg/kg bw/day was considered to be toxicologically not relevant.

Table 27: summary of reproductive performance of dams parent (P) females

OBSERVATIONS	VALUES PER GROUP			
	Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Pairs started (n)	24	24	24	24
Estrous cycle – mean length (days)†	4.4	4.5	4.5	5.3
– frequency of irregular cycle††	4/24	5/24	9/24	11/24
Females showing evidence of copulation (n)	23	24	24	24
Females achieving pregnancy (n)	21	23	23	16

Table 28: Summary data of reproductive performance in parent (P) males

Values		Group (mg/kg bw/day) Control	100	300	1000
No. of males paired		24	24	24	24
Not mated males	N	1	0	0	0
	%	4	0	0	0
Mated males	N	23	24	24	24
	%	96	100	100	100
Males impregnated female	N	21	23	23	16
	%	91	96	96	67 **
Copulatory index	%	96	100 *	100 *	100 *
Reproduction index	%	91	96	96	67 **

Remarks:
NS = Not Significant
* = $p < 0.05$ CH2
** = $p < 0.01$ CH2

SUMMARY DATA OF REPRODUCTIVE PERFORMANCE
PARENT (P) FEMALE

Values		Control	Group (mg/kg bw/day) 100	300	1000	
No. of females paired		24	24	24	24	
Unmated females	N	1	0	0	0	
	%	4	0	0	0	
Sperm positive females	N	23	24	24	24	
	%	96	100	100	100	
Non-pregnant females	N	2	1	1	8	
	%	9	4	4	33	**
Pregnant females	N	21	23	23	16	
	%	91	96	96	67	**
Dams delivered	N	21	23	23	16	
	%	100	100	100	100	-
Pregnants with liveborn(s)	N	21	23	23	16	
	%	100	100	100	100	
Pregnants with stillborns only	N	0	0	0	0	
	%	0	0	0	0	
Pregnants not delivered	N	0	0	0	0	
	%	0	0	0	0	
Precoital interval (days)	Mean	2.1	1.9	1.6	1.8	
	SD	1.5	1.5	1.2	2.9	
	N	23	24	24	24	
Conceiving days	Mean	3.3	2.8	2.6	3.5	NS
	SD	1.5	1.5	1.2	3.3	
	N	21	23	23	16	
Copulatory index	%	96	100	*	100	*
Reproduction index	%	91	96	96	67	**
Gestation index	%	100	100	100	100	-

Remarks:
NS = Not Significant
* = $p < 0.05$ CH2
** = $p < 0.01$ CH2
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
- = no data

Reproductive function: sperm measures:

Sperm examinations did not reveal any test item related influence on the sperm cells at 1000 mg/kg bw/day. Statistical or biological significances were not detected at the mean percentage of motile sperm cells or mean percentage of immotile sperms in parental male animals at 1000 mg/kg bw/day. The total sperm count and sperms with not normal morphology (separated head and tail) were similar in the 1000 mg/kg bw/day and in the control groups.

Table 29: summary of sperm examination of parent (P) males

Group		Control	1000 mg/kg bw/day	
Number of animals examined	n	24	24	
Sperm count (x10^6/g testis)	Mean	53.66	55.58	
	SD	4.78	4.84	
	n	24	24	NS
Total number of cells examined	N	12000	12000	
Number of cells/animal examined	Mean	500	500	
	SD	0	0	
	n	24	10	
Motile sperms (%)	Mean	71.6	72.8	
	SD	2.8	1.4	
	n	24	24	
Immotile sperms (%)	Mean	28.4	27.2	
	SD	2.8	1.4	
	n	24	24	NS
Sperms with normal morphology (%)	Mean	99.6	99.7	
	SD	0.3	0.2	
	n	24	24	
Sperms with seperated head and tail (%)	Mean	0.41	0.33	
	SD	0.27	0.15	
	n	24	24	NS

Remarks: NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
T - test Versus Control

Results: P1 (second parental generation)

General toxicity (P1) F1 Cohort 1B animals

Clinical signs:

Adverse signs of systemic toxicity related to the test item were not detected at any dose level in F1 Cohort 1B animals (male or female) at 100, 300 and 1000 mg/kg bw/day at the daily clinical observations.

The behavior and physical state of all animals were normal during the entire observation period. Alopecia was observed in one control male animal (1/20) on the fore limbs and right side of the abdomen from PN 106 up to PN155. There were no clinical signs in male animals at 100, 300 or 1000 mg/kg bw/day. Female animals were also symptom-free in the control and 1000 mg/kg bw/day during the entire observation period.

Alopecia was noted for two female animals at 100 mg/kg bw/day during the pre-mating and gestation period (2/20, on the chest; on the fore limbs and hind limbs or abdomen) and for one of them during the lactation period (1/20; forelimbs, hind limbs, abdomen). In one female animal at 300 mg/kg bw/day (1/16), alopecia was detected on the right side of abdomen during the gestation period and on the fore limbs, hind limbs and right side of the abdomen during the lactation period. Alopecia on the skin is a species-specific finding, which are also observed in untreated experimental rats of this strain with similar age. These were individual findings with low incidence in animals of control, low and mid dose groups and were not related to the treatment.

Mortality:

There was no test item related mortality in F1 Cohort 1B animals in 100, 300 or 1000 mg/kg bw/day groups (male or female) during the course of the observation period. One control dam (1/19) was found dead on gestation day 22. There were no preceding clinical signs. Sanguineous vaginal orifice and 4 dead fetuses were observed at the necropsy. Histological investigation revealed metritis, pulmonary congestion and edema as individual lesions and cause of the death.

Body weight and weight changes:

The body weight was reduced in F1 Cohort 1B male animals administered with 1000 mg/kg bw/day (-11% day 154). The lower mean body weight of female animals at 1000 mg/kg bw/day during the first two weeks observation period recovered during the remaining days of observation period.

In the male animals at 100 mg/kg bw/day, the mean body weight was slightly higher than in the control group on PND70, 77, 84 and was comparable to their control on the preceding and following days. Statistical significance with respect to the control was detected at the slightly higher mean body weight gain of low dose males between PND49 and PND56.

In the male animals at 300 mg/kg bw/day, the body weight was similar to their control during pre-mating, mating and post-mating periods. Statistical significance was only noted for the slightly higher mean body weight gain between PND22-PND25 and PND39-PND42.

Statistical significance with respect to the control was detected at the lower mean body weight of F1 Cohort 1B male animals at 1000 mg/kg bw/day from PND22 up to the termination of the observation period (PND154). The body weight gain of these animals was also lower than in the control in the most cases during the entire observation period reaching statistical significances in several cases by weekly interval and also for the summarized body weight gain (between PND22 and PND154).

The mean body weight and body weight gain was comparable in the control and test item treated F1 Cohort 1B female animals at 100 and 300 mg/kg bw/day during observation periods. The mean body weight gain was slightly higher than in the control in female animals at 300 mg/kg bw/day between PND98 and PND105. This minor and transient change in body weight gain was considered to be toxicologically not relevant.

The mean body weight of F1 Cohort 1B female animals at 1000 mg/kg bw/day was statistically significantly lower than in the control from PND22 up to PND36 and it was comparable with the control from PND39 to PND112, as well as during the gestation and lactation periods. The mean body weight gain was also similar in all F1 Cohort 1B female groups (control 100, 300 and 1000 mg/kg bw/day) during the observation period. Although, sporadic statistical significance with respect to the control was noted for F1 Cohort 1B female animals at 1000 mg/kg bw/day at the lower mean body weight gain between PND25 and PND29 and at the higher mean body weight gain between PND70-PND77 and PND112-PND119. The summarized mean body weight gain of F1 Cohort 1B female animals was comparable in all groups between PND22 and PND112. There were no toxicologically significant differences between the control and test item treated groups (100, 300 and 1000 mg/kg bw/day) in the body weight or body weight gain of F1 Cohort 1B female animals during the gestation or lactation period. Statistical significance was only noted for the higher mean body weight gain of female animals at 1000 mg/kg bw/day between gestation days 0 and 7 as well as between lactation days 0 and 4.

Table 30: Summary of body weight F1 cohort 1B in males and females

SUMMARY OF BODY WEIGHT
F1 COHORT 1B MALE
Pre-mating, mating and post-mating periods

Group		Body weight (g) on post-natal days																					
		Pre-mating period															Mating period		Post-mating period †				
		22	25	29	32	36	39	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147	154
Control	Mean	53.7	69.1	92.8	113.6	141.6	161.2	178.9	221.5	262.4	295.4	321.7	343.7	360.7	376.5	389.1	401.0	410.1	421.4	428.5	433.2	443.4	451.6	461.9
	SD	4.21	5.35	7.23	9.95	13.18	16.31	20.25	29.03	30.72	36.72	34.21	36.10	40.21	42.83	43.32	40.57	42.07	42.46	42.46	43.74	45.97	46.42	51.13
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	14
100	Mean	52.8	69.0	94.2	115.8	145.2	165.3	185.5	231.8	276.8	311.5	340.7	363.7	381.6	397.1	409.9	420.3	430.2	439.8	445.6	452.4	462.0	468.5	478.6
mg/kg bw/day	SD	4.19	5.54	7.08	8.41	10.69	12.79	13.85	18.06	23.07	24.74	27.24	29.08	30.97	32.79	33.65	34.42	36.18	37.37	37.87	39.24	40.76	40.72	45.78
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	17
	± %	-2	0	1	2	3	3	4	5	5	5	6	6	6	5	5	5	5	4	4	4	4	4	4
												*	*	*										
300	Mean	51.6	68.0	93.4	113.1	142.3	161.9	182.8	229.7	273.1	305.4	332.7	353.4	371.7	389.0	399.9	410.6	418.5	428.8	436.4	438.6	447.1	455.6	465.3
mg/kg bw/day	SD	3.18	4.16	5.92	5.98	6.50	7.96	8.53	12.04	14.70	17.64	20.70	23.07	26.75	27.69	29.87	31.04	31.99	31.63	33.45	35.31	34.90	35.44	38.73
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	16
	± %	-4	-2	1	0	0	0	2	4	4	3	3	3	3	3	3	2	2	2	2	1	1	1	1
1000	Mean	50.0	62.8	86.5	105.4	132.4	150.5	168.1	209.3	250.6	280.4	302.9	322.2	338.3	352.2	362.2	368.6	374.8	380.5	385.0	390.9	400.2	407.7	409.8
mg/kg bw/day	SD	4.76	6.34	7.36	9.61	10.55	11.51	13.87	16.27	18.64	19.59	22.09	22.88	25.79	26.81	27.26	29.28	29.59	31.65	29.17	32.53	31.06	31.84	35.34
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	18	9
	± %	-7	-9	-7	-7	-6	-7	-6	-6	-4	-5	-6	-6	-6	-6	-7	-8	-9	-10	-10	-10	-10	-10	-11
		**	**	**	**	**	**	**	**	*	**	*	*	*	*	*	**	**	**	**	**	**	**	**
		DN	DN	DN	DN	U	U	U	U	U	U	DN	DN	DN	DN	DN	DN	DN	DN	DN	DN	DN	DN	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
† = Including body weight values of some animals at 300 and 1000 mg/kg bw/day, which were included in the prolonged mating from post-natal day 126.

SUMMARY OF BODY WEIGHT
F1 COHORT 1B FEMALE
Pre-mating and mating periods

Group		Body weight (g) on post-natal days																		
		22	25	29	32	36	39	42	49	56	63	70	77	84	91	98	105	112	119	126
		Pre-mating period																Mating period		
Control	Mean	51.4	64.7	87.2	103.2	123.6	135.4	148.5	163.6	181.2	193.3	203.5	211.1	217.6	226.5	229.5	232.2	235.7	225.3	-
	SD	3.96	5.02	6.07	7.01	7.49	8.27	12.91	11.28	12.09	11.97	13.71	14.98	14.84	14.34	14.43	15.03	14.24	5.03	-
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	19	3	-
100	Mean	50.9	65.1	87.5	104.9	126.2	138.2	148.6	168.6	185.8	199.1	209.5	218.0	224.0	231.6	236.1	238.0	241.4	238.7	238.7
mg/kg bw/day	SD	4.47	5.26	6.58	7.21	8.49	10.04	10.30	12.14	11.47	13.42	14.31	13.78	13.02	14.86	15.54	13.66	14.53	22.94	22.94
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	19	3	3
	± %	-1	1	0	2	2	2	0	3	3	3	3	3	3	2	3	3	2	6	-
300	Mean	50.9	64.9	86.4	103.0	123.8	135.6	145.4	167.0	184.7	197.2	208.6	216.4	223.8	229.8	232.6	237.6	240.1	243.9	239.5
mg/kg bw/day	SD	2.92	3.23	4.24	5.02	6.39	6.96	8.08	11.57	14.54	15.70	16.96	18.35	19.27	17.55	18.59	19.43	19.39	29.55	57.28
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	7	2
	± %	-1	0	-1	0	0	0	-2	2	2	2	2	3	3	1	1	2	2	8	-
1000	Mean	46.8	60.0	80.7	96.9	118.1	130.8	141.4	162.0	179.5	191.8	202.5	213.3	218.8	226.1	230.1	234.6	236.5	239.0	240.3
mg/kg bw/day	SD	5.25	5.81	6.78	8.26	8.11	8.95	9.39	9.98	9.08	10.36	10.48	11.19	10.36	10.93	10.75	11.96	12.59	14.42	17.04
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	3	3
	± %	-9	-7	-8	-6	-4	-3	-5	-1	-1	-1	-1	1	1	0	0	1	0	6	-
		**	**	**	**	*														
		DN	DN	DN	DN	DN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = No data

SUMMARY OF BODY WEIGHT
F1 COHORT 1B FEMALE
Gestation and lactation periods

Group		Body weight (g) on gestation days				Body weight (g) on lactation days	
		0	7	14	21	0	4
Control	Mean	235.9	251.4	273.9	346.3	265.6	269.5
	SD	14.79	17.52	17.64	32.37	19.25	18.65
	n	19	19	19	19	18	17
100	Mean	243.0	260.0	280.9	358.9	271.1	277.3
mg/kg bw/day	SD	13.91	16.72	18.20	27.71	18.46	18.20
	n	20	20	20	20	20	20
	± %	3	3	3	4	2	3
300	Mean	246.1	264.1	285.7	363.9	273.9	285.4
mg/kg bw/day	SD	16.09	16.65	17.97	25.68	18.97	16.36
	n	16	16	16	16	16	16
	± %	4	5	4	5	3	6 *
1000	Mean	236.3	258.6	283.7	349.5	261.7	275.8
mg/kg bw/day	SD	11.21	10.57	13.37	20.65	14.17	17.38
	n	10	10	10	10	10	10
	± %	0	3	4	1	-1	2
		NS	NS	NS	NS	NS	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Food consumption and compound intake (if feeding study):

The food consumption was not affected in F1 Cohort 1B animals at 100, 300 and 1000 mg/kg bw/day during the pre-mating and post-mating periods (male) or during the pre-mating, gestation or lactation periods (female).

Statistical significance with respect to the control was detected at the slightly higher mean daily food consumption of male animals at 100 mg/kg bw/day between PND70 and PND77 and at 1000 mg/kg bw/day between PND133 and PND147.

In the female animals, the mean daily food consumption was slightly higher than in the control at 1000 mg/kg bw/day between PND22 and PND29 as well as at 300 and 1000 mg/kg bw/day between gestation days 0 and 7. These sporadic and minor differences in the mean daily food intake of male and female animals in F1 Cohort 1A were considered to be toxicologically not relevant.

Water consumption and compound intake (if drinking water study): not specified

Ophthalmological findings: not examined

Organ weight findings including organ / body weight ratios:

The weights of kidneys (absolute, or relative to body and brain weights) were elevated in F1 Cohort 1B male and female animals at 300 or 1000 mg/kg bw/day. This was not associated with related macroscopic and histological alteration.

In the male animals at 100 mg/kg bw/day, statistical significance was detected at the slightly higher mean kidneys weights relative to brain weight.

At 300 mg/kg bw/day, statistical significance was noted for the higher mean kidneys weight (relative to body and brain weight), higher mean weights of prostate (absolute and relative to brain weight) and higher mean epididymides weight relative to brain weight in male animals.

In the male animals at 1000 mg/kg bw/day, the fasted mean body weight was significantly lower than in the control group. Statistical significance with respect to the control was detected at the lower mean brain weight and at the higher mean brain weight relative to body weight, higher mean weights of kidneys and testes (absolute and relative to body and brain weight), lower mean prostate weight, higher mean weights of epididymides and seminal vesicles (both relative to body weight).

In the female animals the mean kidney weights slightly exceeded the control at 100 mg/kg bw/day.

At 300 mg/kg bw/day, higher mean kidneys weight (absolute and relative to body and brain weights) and higher mean weight of ovaries were detected in female animals when compared to their control.

In the female animals at 1000 mg/kg bw/day, statistical significance was observed at the lower mean brain weights (absolute and relative to body weight), at the higher mean kidneys weight (absolute and relative to body and brain weights) and higher mean body weight relative to brain weight.

Histological examinations revealed no morphological changes in the renal tissue. Hematology investigations as well as clinical chemistry parameters did not reveal test item related abnormalities.

The statistically significant differences with respect to the control at several organs (brain, testes, prostate, epididymides, seminal vesicle or ovary) were judged to have little or no toxicological relevance due to the minor degree and in the lack of associated histopathological alterations.

Table 31: Summary of organ weight of F1 cohort 1B males

Group		Body weight	Organ weight (g) Brain	Kidneys	Testes	Epididy-mides	Seminal vesicles† Prostate	Prostate	Pituitary
Control	Mean	448.5	2.25	2.41	3.73	1.70	1.97	0.51	0.011
	SD	45.39	0.07	0.22	0.24	0.16	0.26	0.08	0.004
	n	20	20	20	20	20	20	20	20
100 mg/kg bw/day	Mean	465.6	2.23	2.57	3.85	1.73	2.05	0.54	0.010
	SD	43.37	0.11	0.31	0.26	0.21	0.43	0.12	0.002
	n	20	20	20	20	20	20	20	20
	± %	4	-1	6	3	2	4	7	-6
300 mg/kg bw/day	Mean	454.3	2.19	2.77	3.76	1.76	2.02	0.58	0.010
	SD	36.35	0.13	0.30	0.23	0.16	0.35	0.11	0.001
	n	20	20	20	20	20	20	20	20
	± %	1	-3	15 **	1	4	3	14 *	-6
1000 mg/kg bw/day	Mean	394.9	2.09	3.07	3.94	1.68	2.05	0.44	0.009
	SD	31.91	0.10	0.37	0.34	0.15	0.36	0.10	0.001
	n	20	20	20	20	20	20	20	20
	± %	-12 **	-7 **	27 **	6 *	-1	4	-13 *	-13
		DN	DN	DN	DN	NS	NS	DN	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$ **
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
† = Seminal vesicles with coagulating gland

Group		Organ weight relative to body weight (%) Brain	Kidneys	Testes	Epididy-mides	Seminal vesicles† Prostate	Prostate	Pituitary
Control	Mean	0.508	0.540	0.843	0.384	0.442	0.114	0.0023
	SD	0.061	0.042	0.129	0.062	0.072	0.022	0.0009
	n	20	20	20	20	20	20	20
100 mg/kg bw/day	Mean	0.481	0.552	0.835	0.374	0.439	0.117	0.0021
	SD	0.037	0.047	0.100	0.046	0.079	0.027	0.0004
	n	20	20	20	20	20	20	20
	± %	-5	2	-1	-3	-1	3	-9
300 mg/kg bw/day	Mean	0.483	0.609	0.833	0.391	0.447	0.128	0.0022
	SD	0.038	0.048	0.077	0.049	0.079	0.027	0.0003
	n	20	20	20	20	20	20	20
	± %	-5	13 **	-1	2	1	12	-7
1000 mg/kg bw/day	Mean	0.531	0.775	1.002	0.426	0.519	0.111	0.0023
	SD	0.035	0.054	0.102	0.039	0.076	0.021	0.0002
	n	20	20	20	20	20	20	20
	± %	5 *	44 **	19 **	11 *	17 **	-2	-2
		U	DN	DN	DN	DN	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
† = Seminal vesicles with coagulating gland

Group		Organ weight and body weight relative to brain weight (%)						
		Body weight	Kidneys	Testes	Epididy-mides	Seminal vesicles† Prostate	Prostate	Pituitary
Control	Mean	19929.0	107.22	165.79	75.53	87.19	22.50	0.47
	SD	2127.97	10.50	11.00	6.68	10.86	3.60	0.21
	n	20	20	20	20	20	20	20
100	Mean	20919.4	115.34	173.31	77.78	91.71	24.36	0.44
mg/kg bw/day	SD	1733.81	12.56	12.52	7.21	17.14	5.43	0.08
	n	20	20	20	20	20	20	20
	± %	5	8 *	5	3	5	8	-6
300	Mean	20816.0	126.52	172.58	80.91	92.33	26.34	0.45
mg/kg bw/day	SD	1607.49	10.18	12.80	7.84	13.91	4.67	0.06
	n	20	20	20	20	20	20	20
	± %	4	18 **	4	7 *	6	17 *	-3
1000	Mean	18890.6	146.50	188.60	80.28	97.84	20.99	0.43
mg/kg bw/day	SD	1174.71	14.48	15.36	6.92	14.86	4.01	0.05
	n	20	20	20	20	20	20	20
	± %	-5	37 **	14 **	6	12	-7	-7
		NS	DN	DN	DN	NS	DN	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
† = Seminal vesicles with coagulating gland

Table 32: Summary of organ weight of F1 cohort 1B females

Group		Body weight	Organ weight (g) Brain	Kidneys	Uterus	Ovaries	Pituitary
Control	Mean	248.9	2.06	1.58	0.62	0.096	0.015
	SD	13.12	0.10	0.07	0.16	0.013	0.003
	n	19	19	19	19	19	19
100	Mean	255.2	2.06	1.66	0.60	0.101	0.014
mg/kg bw/day	SD	15.13	0.07	0.13	0.07	0.016	0.002
	n	20	20	20	20	20	20
	± %	3	0	5 **	-3	5	-2
300	Mean	254.3	2.06	1.79	0.62	0.110	0.015
mg/kg bw/day	SD	19.53	0.09	0.18	0.10	0.023	0.002
	n	20	20	20	20	20	20
	± %	2	0	13 **	1	15 *	5
1000	Mean	248.1	1.94	1.83	0.60	0.097	0.013
mg/kg bw/day	SD	16.89	0.09	0.24	0.12	0.021	0.002
	n	20	20	20	20	20	20
	± %	0	-6 **	16 **	-2	2	-9
		NS	DN	U	NS	DN	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight relative to body weight (%) Brain	Kidneys	Uterus	Ovaries	Pituitary
Control	Mean	0.830	0.637	0.247	0.0384	0.0059
	SD	0.056	0.040	0.064	0.0048	0.0011
	n	19	19	19	19	19
100 mg/kg bw/day	Mean	0.811	0.652	0.235	0.0395	0.0056
	SD	0.047	0.045	0.032	0.0062	0.0006
	n	20	20	20	20	20
	± %	-2	2	-5	3	-5
300 mg/kg bw/day	Mean	0.812	0.703	0.246	0.0436	0.0061
	SD	0.053	0.057	0.043	0.0111	0.0010
	n	20	20	20	20	20
	± %	-2	10	0	14	3
			**			
1000 mg/kg bw/day	Mean	0.786	0.736	0.245	0.0391	0.0054
	SD	0.058	0.072	0.056	0.0080	0.0010
	n	20	20	20	20	20
	± %	-5	16	-1	2	-9
		*	**			
		DN	DN	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight and body weight relative to brain weight (%) Body weight	Kidneys	Uterus	Ovaries	Pituitary
Control	Mean	12096.4	76.82	29.90	4.66	0.71
	SD	813.68	4.43	7.84	0.73	0.14
	n	19	19	19	19	19
100 mg/kg bw/day	Mean	12378.1	80.59	29.04	4.89	0.70
	SD	734.52	6.54	3.92	0.80	0.09
	n	20	20	20	20	20
	± %	2	5	-3	5	-2
300 mg/kg bw/day	Mean	12367.6	86.80	30.29	5.37	0.75
	SD	814.82	7.80	4.53	1.26	0.11
	n	20	20	20	20	20
	± %	2	13	1	15	5
			**			
1000 mg/kg bw/day	Mean	12793.5	94.09	31.19	4.99	0.68
	SD	935.67	11.62	6.54	0.99	0.13
	n	20	20	20	20	20
	± %	6	22	4	7	-4
		*	**			
		DN	U	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Necropsy findings:

In the male animals in control group, hernia diaphragmatica (1/20), right side pyelectasia (2/20), smaller than normal seminal vesicle on the right side (1/20) and alopecia on the skin of forelimb and abdomen (1/20) were observed.

There were no macroscopic changes in the organs or tissues in male animals at 100 mg/kg bw/day.

At 300 mg/kg bw/day, right side pyelectasia (2/20) was noted for two male animals at the necropsy. Dark red small liver lobe (1/20) and right or both sided pyelectasia (5/20) were detected at the necropsy.

In dead F1 Cohort 1B dam, sanguineous vaginal orifice and four dead fetuses in the left uterine horn were observed.

In control F1 Cohort 1B dams, dark red and hard small liver lobe (1/18), and right-side pyelectasia (1/18) was seen.

In non-pregnant female animals, the organs and tissues were normal. Thymic hemorrhage (1/20) and alopecia on the skin of fore limbs, hind limbs and abdomen (1/20) were observed in single animals at 100 mg/kg bw/day.

At 300 mg/kg bw/day, right side pyelectasia (1/16) and rudimental right uterine horn (1/16) was noted for dams.

In non-pregnant female animals at 300 mg/kg bw/day, hemorrhage in the submandibular lymph node (1/4) and slight, moderate or marked hydrometra (3/4) were detected.

In dams at 1000 mg/kg bw/day, hemorrhage one or both sided pyelectasia (2/10) was observed

In non-pregnant female animals at 1000 mg/kg bw/day, hernia diaphragmatica (1/9) and marked hydrometra (2/9) were seen.

Not mated female animal at 1000 mg/kg bw/day showed moderate hydrometra (1/1).

Rudimental uterine horn was a developmental disorder in female animal at 300 mg/kg bw/day. Pyelectasia is frequently observed in this strain of experimental rats. Histological examination did not reveal degeneration, inflammation or fibrosis.

Dark liver lobe, smaller than normal seminal vesicle, alopecia on the skin, hernia diaphragmatica are common macroscopic findings in experimental rats of this strain with similar age. These occurred with low incidence and were considered to be toxicologically not relevant.

Hemorrhages in the lungs or thymus or lymph nodes are indicative of circulatory disturbance developing during the exsanguination.

Table 33: Summary of necropsy findings of F1 cohort 1B males

Organs	Observations	Frequency of observations per group			
		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
	No macroscopic findings	15/20	20/20	18/20	14/20
Diaphragm	*Hernia diaphragmatica*	1/20	0/20	0/20	0/20
Liver	Dark red small lobe	0/20	0/20	0/20	1/20
Kidneys	Pyelectasia	2/20	0/20	2/20	5/20
Seminal vesicle	Smaller than normal	1/20	0/20	0/20	0/20
Skin	Alopecia	1/20	0/20	0/20	0/20

Remark: Frequency of observations = number of animals with observations / number of animals examined.

Table 34: Summary of necropsy findings of F1 cohort 1B females

	Organs	Observations	Frequency of observations per group			
			Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Dams		No macroscopic findings	16/18	18/20	14/16	8/10
	Thymus	Hemorrhages	0/18	1/20	0/16	0/10
	Kidneys	Pyelectasia	1/18	0/20	1/16	2/10
	Liver	Dark red small lobe, hard	1/18	0/20	0/16	0/10
	Uterus	Rudimental right horn	0/18	0/20	1/16	0/10
	Skin	Alopecia	0/18	1/20	0/16	0/10
Dead pregnant	Vagina Uterus	Sanguineous orifice Four dead fetuses	1/1 1/1	/	/	/
Non pregnant female		No macroscopic findings	1/1	/	0/4	6/9
	Submandibular l.n.	Hemorrhage	0/1	/	1/4	0/9
	Diaphragm	*Hernia diaphragmatica*	0/1	/	0/4	1/9
	Uterus	Hydrometra	0/1	/	3/4	2/9
Not mated female	Uterus	Hydrometra	/	/	/	1/1

Remark: Frequency of observations = number of animals with observations / number of animals examined.
l.n. = Lymph nodes

Histopathological findings: non-neoplastic:

Histological examinations did not reveal pathologic alterations in the organs or tissues of F1 Cohort 1B male or female animals at 1000 mg/kg bw/day.

Quantitative examinations of ovaries did not reveal test item related alterations in F1 Cohort 1B female animals.

In dead female control animal, metritis – in connection with 4 dead embryos – occurred in the uterus. This finding is considered as individual disease.

In the F1 Cohort 1B male animals in the control (20/20), and 1000 mg/kg bw/day (20/20) groups the investigated organs of reproductive system (testes, epididymides, prostate seminal vesicles, coagulating glands) were histologically normal and characteristic on the sexually mature organism in all cases, including animals, which did not fertilize their partners at 300 mg/kgbw/day (4/4).

The various spermatogenic cells (spermatogonia, spermatocytes, spermatids and spermatozoa) representing different phases in the development and differentiation of the spermatozoons and the interstitial cells were the same in quantity and morphologically in the testes of investigated control and high dose treated animals. The histological picture of epididymides, prostate, seminal vesicles, and coagulating glands was normal in all cases except for one control male animal (1/20), in which decreased amount of secrete in the seminal vesicle (one side) was observed. This phenomenon, without inflammatory or degenerative lesion is considered as individual disorder, without toxicological significance.

In the F1 Cohort 1B female animals in the control (20/20) and 1000 mg/kg bw/day (20/20, including not mated female animal) groups and in non-pregnant female animals at 300 mg/kg bw/day (4/4), the ovaries, uterus, cervix, vagina had a normal structure characteristic of the species, age and phase of the active sexual cycle. The cortical region of ovaries contained primary, secondary and tertiary follicles and corpora lutea, indicating the active maturation of oocytes, and ovulation. The epithelial capsule and ovarian stroma were normal in all cases as well.

The histological structure and the cellularity of pituitary with special attention on the cytomorphology and proportion of acidophilic and basophilic cells in the adenohypophysis were the same in the control and treated male and female animals.

In two female animals at 300 mg/kg bw/day (1/16), dilatation of uterine horns was observed.

One side pyelectasia occurred in F1 Cohort 1B male and female animals: 2/20 control, 2/20 at 300 mg/kg bw/day, 5/20 at 1000 mg/kg bw/day in male animals; 1/18 control, 1/16 at 300 mg/kg bw/day, 2/10 at 1000 mg/kg bw/day in female animals.

Focal fibrosis in the Glisson's capsule of the liver is in connection with the mechanical irritation due to diaphragmatic hernia (1/20 male and 0/1 female (non-pregnant) control; 1/9 female at 1000 mg/kg bw/day).

Acute hemorrhage in the submandibular lymph nodes (1/4 female at 300 mg/kg bw/day), in the thymus (1/20 female at 100 mg/kg bw/day) and congestive liver (1/20 male at 1000 mg/kg bw/day, 1/18 female control) occurred sporadically and may be considered as consequence of hypoxia, dyspnea and circulatory disturbance developed during exsanguination procedures.

The atrophy of hair follicles in connection with focal alopecia (0/20 male control and 1/20 female at 100 mg/kg bw/day) without inflammatory lesions or fungal and parasite bodies is in connection with trophy disorder of affected skin area.

There was no morphological evidence of test item related acute or subacute injury (degeneration, inflammation, necrosis etc.) in the stomach, small and large intestines, liver, pancreas, cardiovascular system, respiratory system, immune system, hematopoietic system, skeleton, muscular system, central, or peripheral nervous system, eyes, integumentary system.

The cytomorphology of endocrine glands were the same in the control and treated animals but in the absence of hormonal assays, endocrine changes cannot be excluded.

Table 35: Summary of histopathology findings in F1 cohort 1B male and females

SUMMARY OF HISTOPATHOLGY FINDINGS
F1 COHORT 1B
MALE

Organs	Observations	Incidence of observations per group			
		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Brain	No lesion	20/20	/	/	20/20
Epididymides	**Storage of mature spermatozoa**	**20/20**	/	**4/4**	**20/20**
Kidneys	Pyelectasia	2/2	/	2/2	5/5
Liver	Focal fibrosis	1/1	/	/	0/1
	Congestion	0/1	/	/	1/1
Pituitary	No lesion	20/20	/	/	20/20
Prostate	**No lesion**	**20/20**	/	**4/4**	**20/20**
Seminal vesicle †	**Decreased amount of secrete**	**1/20**	/	**0/4**	**0/20**
Skin	**Atrophy of hair follicles**	**1/1**	/	/	/
Testes	**Active spermatogenesis**	**20/20**	/	**4/4**	**20/20**

Remark: Frequency of observations: number of animals with observation/number of animals examined
† = Seminal vesicle with coagulating gland

SUMMARY OF HISTOPATHOLOGY FINDINGS
F1 COHORT 1B
FEMALE

Organs	Observations	Incidence of observations per group				
		Control Survivor	Control Dead	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Adrenal glands	No lesion	/	1/1	/	/	/
Aorta	No lesion	/	1/1	/	/	/
Bone marrow	No lesion	/	1/1	/	/	/
Brain	No lesion	19/19	1/1	/	/	20/20
Cecum	No lesion	/	1/1	/	/	/
Colon	No lesion	/	1/1	/	/	/
Duodenum	No lesion	/	1/1	/	/	/
Eyes + optic nerve	No lesion	/	1/1	/	/	/
Esophagus	No lesion	/	1/1	/	/	/
Harderian glands	No lesion	/	1/1	/	/	/
Heart	No lesion	/	1/1	/	/	/
Ileum	No lesion	/	1/1	/	/	/
Jejunum	No lesion	/	1/1	/	/	/
Kidneys	**Pyelectasia**	**1/1**	**0/1**	/	**1/1**	**1/1**
Lachrymal glands	No lesion	/	1/1	/	/	/
Liver	**Focal fibrosis**	**1/1**	**0/1**	/	/	**1/1**
	Congestion	**1/1**	**0/1**	/	/	/
Lungs	Congestion	/	1/1	/	/	/
	Alveolar edema	/	1/1	/	/	/
Mammary gland	No lesion	/	1/1	/	/	/
Mesenteric lymph nodes	No lesion	/	1/1	/	/	/
Muscle (quadriceps)	No lesion	/	1/1	/	/	/
Ovaries: Primordial, secondary and tertiary follicles		**19/19**	**1/1**	**20/20**	**20/20**	**20/20**
	Corpora lutea	**19/19**	**1/1**	**20/20**	**20/20**	**20/20**
Pancreas	No lesion	/	1/1	/	/	/
Pituitary	No lesion	**19/19**	**1/1**	/	/	**20/20**
Rectum	No lesion	/	1/1	/	/	/
Salivary glands (subm)	No lesion	/	1/1	/	/	/
Sciatic nerve	No lesion	/	1/1	/	/	/
Skin	**Atrophy of hair follicles**	/	**0/1**	**1/1**	/	/
Spinal cord	No lesion	/	1/1	/	/	/
Spleen	No lesion	/	1/1	/	/	/
Sternum	No lesion	/	1/1	/	/	/
Stomach	No lesion	/	1/1	/	/	/
	No lesion	/	1/1	/	/	/
Subm. lymph nodes	**Acute hemorrhage**	/	**0/1**	/	**1/1**	/
Thymus	**Acute hemorrhage**	/	**0/1**	**1/1**	/	/
Thyroid + parathyroid:	No lesion	/	1/1	/	/	/
Trachea	No lesion	/	1/1	/	/	/
Urinary bladder	No lesion	/	1/1	/	/	/
Uterus	**Metritis**	**0/19**	**1/1**	**0/20**	**0/20**	**0/20**
	Dilatation	**0/19**	**0/1**	**0/20**	**2/20**	**0/20**
Vagina	**No lesion**	**19/19**	1/1	**0/20**	**2/20**	**0/20**

Remark: Frequency of observations: number of animals with observation/number of animals examined
subm. = Submandibular
/ = No data

Ovary Follicle Count:

Quantitative examinations of ovaries did not reveal test item related alterations in F1 Cohort 1B female animals. The mean number of primordial and primary follicles, secondary and tertiary follicles, corpora lutea and follicular atresia were comparable in the control, 100, 300 and 1000 mg/kg bw/day.

Table 36: Quantitative evaluation of ovaries of F1 cohort 1B females

Group		Primordial and primary follicles	Secondary and tertiary follicles	Corpora lutea	Follicular atresia	Cystic degeneration	Other findings
Control	Mean	35.8	13.3	17.1	5.3	0.0	0.0
	SD	2.8	2.4	3.5	3.5	0.0	0.0
	n	19	19	19	19	19	19
100 mg/kg bw/day	Mean	36.9	13.1	15.4	5.3	0.0	0.0
	SD	2.4	2.6	3.5	0.9	0.0	0.0
	n	20	20	20	20	20	20
300 mg/kg bw/day	Mean	36.6	14.3	18.9	5.0	0.0	0.0
	SD	2.4	2.4	5.5	1.1	0.0	0.0
	n	20	20	20	20	20	20
1000 mg/kg bw/day	Mean	36.5	13.2	16.0	6.1	0.0	0.0
	SD	3.2	2.6	5.5	2.9	0.0	0.0
	n	20	20	20	20	20	20
		NS	NS	NS	NS	-	-

Remark: Quantitative examinations were performed at the section level of ovaries
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = No data

Thyroid Hormone measurements:

At 1000 mg/kg bw/d levels of T3 as well as T4 were reduced in male animals (-12 % and -15 %, respectifvely). T4 values were also reduced in female animals at this dose level (-10 %) when compared to the control. The reduced T3 and T4 levels are consistent with findings in Cohort 1A and P0 generation male animals.

Table 37: Summary of thyroid hormone levels of F1 cohort 1B males

		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
FT3 [ng/dL]	Mean	0.29	0.28	0.25	0.26	
	SD	0.05	0.04	0.05	0.02	
	n	20	20	20	20	
	±%		-3	-12 *	-10 *	U
FT4 [ng/dL]	Mean	2.81	3.29	3.23	2.37	
	SD	0.37	0.56	0.50	0.32	
	n	20	20	20	20	
	±%		17 **	15 *	-16 **	DN
TSH [µIU/mL]		≤ LD	≤ LD	≤ LD	≤ LD	

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
LD = Limit of detection - 0.005 µIU/mL

Table 38: Summary of thyroid hormone levels of F1 cohort 1B females

		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
FT3 [ng/dL]	Mean	0.28	0.29	0.29	0.31	
	SD	0.04	0.08	0.06	0.08	
	n	19	20	20	20	
	±%		4	3	10	NS
FT4 [ng/dL]	Mean	2.22	2.54	2.38	1.98	
	SD	0.28	0.76	0.56	0.48	
	n	19	20	20	20	
	±%		14	7	-11 *	U
TSH [µIU/mL]		≤ LD	≤ LD	≤ LD	≤ LD	

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
LD = Limit of detection - 0.005 µIU/mL

Reproductive function / performance (P1)

Oestrous cycle:

Cohort 1A (representative for cohort 1B):

The examined parameters of the estrous cycle were comparable in the F1 Cohort 1A female animals in the control and 100, 300 and 1000 mg/kg bw/day groups.

The estrous cycle was irregular in several female animals in the control, 100, 300 and 1000 mg/kg bw/day groups during the two weeks observation period.

The percentage of female animals with regular cycle, mean length of cycle, mean number of days in pre-estrous, estrous, diestrous and number of female animals in prolonged diestrous were similar in all groups (control, 100, 300 and 1000 mg/kg bw/day). The number and percentage of female animals in prolonged estrous was slightly higher than in the control group at 1000 mg/kg bw/day (N=3/20, 15 % versus 0/20). Historical controls are available for 13 studies: among them 6/156 animals were in prolonged estrous (ranging from 0 to 5/12 when considering each study independently).

Table 39: Summary data of estrous cycle in F1 cohort 1A females

SUMMARY DATA OF ESTROUS CYCLE
F1 COHORT 1A FEMALE

Group		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
Number of animals examined	N	20	20	20	20	
Animals with regular cycles	N	6	4	7	8	NS
	%	30	20	35	40	
Animals with irregular cycles (%)	N	14	16	13	12	
	%	70	80	65	60	
Number of cycles	Mean	2.4	2.2	2.3	2.1	
	SD	1.0	0.8	1.1	0.9	
	n	20	20	20	20	NS
Lenght of cycles	Mean	6.4	8.5	5.1	6.0	
	SD	3.4	3.3	2.3	3.0	
	n	13	15	11	11	NS
Days in proestrous	Mean	1.3	1.5	1.5	0.9	
	SD	0.9	1.1	1.1	1.0	
	n	20	20	20	20	NS
Days in estrous	Mean	2.3	2.4	2.3	2.7	
	SD	1.0	1.2	1.3	1.7	
	n	20	20	20	20	NS
Days in diestrous	Mean	10.6	10.8	10.3	10.4	
	SD	1.8	1.8	2.0	2.4	
	n	20	20	20	20	NS
Animals in prolonged estrous	N	0	1	0	3	
	%	0	5	0	15	
Animals in prolonged diestrous	N	14	15	13	14	
	%	70	75	65	70	

REMARKS:
NS = Not Significant
* = $p < 0.05$; CH2
** = $p < 0.01$; CH2
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

Sperm measures:

Sperm examinations did not point out any test item related influence on the sperm cells at 1000 mg/kgbw/day.

Statistical or biological significances were not detected at the mean percentage of motile sperm cells or mean percentage of immotile sperms in parental male animals at 1000 mg/kg bw/day. The total sperm count and sperms with not normal morphology (separated head and tail) were similar in the 1000 mg/kg bw/day and in the control groups.

Reproductive performance:

The reproductive performance was reduced in F1 Cohort 1B animals at 300 and 1000 mg/kg bw/day (male and female) based on the lower fertility indices.

The examined parameters of reproductive performance were not affected by the treatment with the test item in male or female animals at 100 mg/kg bw/day. In this dose group, all pairs mated successfully and males fertilized respective females, therefore fertility index even exceeded the control value.

The copulatory index was lower than in the control group at 1000 mg/kg bw/day as two male animals failed to mate (male #758 and #760). Regarding male#760, corresponding female partners (female#771 and #790) did either not mate even with exchanged males (#771), or mated but did not achieve pregnancy (#790). One female partner of male #758, where no mating could be observed, was also female #771. The other female partner of male#758 (female#780) mated successfully achieving pregnancy with an exchanged partner male. Overall, the decreased copulatory index as a consequence of impaired mating behaviour by

male animals must be treated with caution, as it cannot be completely excluded that this finding originates from the corresponding female partner.

Statistical significance was observed at the lower percentage of fertile male animals (i.e. fertility index) and higher percentage of infertile male animals at 300 and 1000 mg/kg bw/day. This is due to the not achieved pregnancies of respective females.

The percentage of pregnant females (fertility index) was lower and percentage of non-pregnant female animals was higher with respect to their control group at 300 and 1000 mg/kg bw/day. This finding is consistent with observations in the P0 generation and considered test item related.

Table 40: summary of reproductive performance of F1 cohort 1B males

Values		Group (mg/kg bw/day) Control	100	300	1000
No. of males paired		20	20	20	20
Not mated males	N	0	0	0	2
	%	0	0	0	10
Mated males	N	20	20	20	18
	%	100	100	100	90
Males impregnated female	N	19	20	16	10
	%	95	100 *	80 **	56 **
Copulatory index	%	100	100	100	90 **
Reproduction index	%	95	100 *	80 **	56 **

Remarks:
NS = Not Significant
* = p < 0.05 CH2
** = p < 0.01 CH2

Table 41: summary of reproductive performance of F1 cohort 1B females

Values		Group (mg/kg bw/day) Control	100	300	1000	
No. of females paired		20	20	20	20	
Unmated females	N	0	0	0	1	
	%	0	0	0	5	
Sperm positive females	N	20	20	20	19	
	%	100	100	100	95	
Non-pregnant females	N	1	0	4	9	
	%	5	0 *	20 **	47 **	
Pregnant females	N	19	20	16	10	
	%	95	100 *	80 **	53 **	
Dams delivered	N	18	20	16	10	
	%	95	100 *	100 *	100 *	
Pregnants with liveborn(s)	N	18	20	16	10	
	%	100	100	100	100	
Pregnants with stilborns only	N	0	0	0	0	
	%	0	0	0	0	
Pregnants not delivered	N	1	0	0	0	
	%	5	0	0	0	
Precoital interval (days)	Mean	1.5	1.6	2.4	1.5	
	SD	1.4	1.4	2.0	1.5	
	N	20	20	20	19	
Conceiving days	Mean	2.4	2.6	3.6	2.1	
	SD	1.4	1.4	1.9	1.1	
	N	19	20	16 *	10	DN
Copulatory index	%	100	100	100	95 *	
Reproduction index	%	95	100 *	80 **	53 **	
Gestation index	%	95	100 *	100 *	100 *	

Remarks:
NS = Not Significant
* = $p < 0.05$ CH2
** = $p < 0.01$ CH2
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

One control female animal died on gestation day 22 – not delivered – while all pregnant animals delivered at 100, 300 and 1000 mg/kg bw/day.

Statistical significance was observed at the slightly higher mean number of conceiving days in female animals at 300 mg/kg bw/day. Similar finding was not detected at the high dose, therefore, this difference in the mean number conceiving days at 300 mg/kg bw/day was considered to be toxicologically not relevant.

Results: F1 generation

General toxicity (F1)

Clinical signs:

Pups:

There were no adverse clinical signs in the F1 offspring from post-natal day 0 to 21. However, the percentage of offspring showing signs (no milk in the stomach (16%), cold (33%), found dead (2%), missing (7%), alopecia (8%)) was higher at 1000 mg/kg bw/day compared to the control. These observations suggest inadequate nursing behaviour of the respective dams. The percentage of offspring, which were cold, did not suckle were similar in the control and 100 or 300 mg/kg bw/day groups. Some other sporadic clinical signs were also observed in the control and 100 or 300 mg/kg bw/day dose groups (cachexia, found dead, missing, wound). At 1000 mg/kg bw/day, wound, hemorrhage, exophthalmos missing or atrophied limb were noted for single pups.

Table 42: summary of clinical observations and fate of F1 offspring

Observations		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
No. of offspring examined	N	247	239	262	178
No signs	Sum	218	217	228	94
	%	88	91	87	53
No milch in the stomach	Sum	3	1	17	29
	%	1	0	6	16
Cold	Sum	25	20	24	59
	%	10	8	9	33
Cachexia	Sum	1	0	0	0
	%	0	0	0	0
Found dead	Sum	0	1	0	4
	%	0	0	0	2
Missing (Cannibalized)	Sum	3	1	4	12
	%	1	0	2	7
Skin: Alopecia	Sum	0	0	0	15
	%	0	0	0	8
Skin: Wound	Sum	1	0	2	1
	%	0	0	1	1
Skin: Hemorrhage	Sum	0	0	0	1
	%	0	0	0	1
Eye: Exophthalmos	Sum	0	0	0	1
	%	0	0	0	1
Limb: Missing	Sum	0	0	0	1
	%	0	0	0	1
Limb: Black, damaged	Sum	0	0	0	1
	%	0	0	0	1

Cohort 1A:

There were no clinical signs in male or female animals in F1 Cohort 1A generation in control, 100, 300 or 1000 mg/kg bw/day. The behavior and physical condition of all animals were normal during the entire observation period.

Mortality / viability:

Pups:

The extra uterine mortality of F1 offspring exceeded the control at 1000 mg/kg bw/day on post-natal day 0 and between postnatal days 0 and 21. The extra uterine mortality was low and comparable in the control, 100, and 300 mg/kg bw/day from birth to post-natal day 21.

Cohort 1A:

There was no mortality in F1 Cohort 1A animals in control, 100, 300 or 1000 mg/kg bw/day groups (male or female) during the course of study.

Table 43: Summary of extrauterine mortality and sex distribution of F1 offsprings

Values			Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
Number of viable pups							
on post-natal day 21	Total	N	202	208	216	144	
Survival index:		%	82	87	82	81	
	Male	N	91	108	102	68	NS
		%	45	52	47	47	
	Female	N	111	100	114	76	NS
		%	55	48	53	53	
Number of pups euthanized							
on post-natal day 4	Total	N	42	29	42	18	
		%	17	12	16	10	
	Male	N	19	12	20	3	
		%	17	10	16	4	
	Female	N	23	17	22	15	
		%	17	14	16	16	
Number of dead pups							
on post-natal day 0	Total	N	0	1	0	4	
		%	0	0	0	2	
	Male	N	0	1	0	3	
		%	0	1	0	4	
	Female	N	0	0	0	1	
		%	0	0	0	1	
between post-natal days 0-21	Total	N	3	2	4	16	
		%	1	1	2	9	
	Male	N	1	1	1	11	
		%	1	1	1	13	
	Female	N	2	1	3	5	
		%	1	1	2	5	

Remarks:
NS = Not Significant
* = $p < 0.05$ CHI2
** = $p < 0.01$ CHI2

Litter data

Values			Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day		
Number of litters		N	21	22	23	16		
Number of liveborns	Total	Mean	11.8	10.9	11.4	11.1		
		SD	2.2	2.4	2.8	2.7		
		n	21	22	23	16		NS
	Male	Mean	5.3	5.5	5.3	5.1		
		SD	2.6	1.9	1.9	1.6		
		n	21	22	23	16		
	Female	Mean	6.5	5.4	6.0	6.0		
		SD	2.6	2.2	1.8	2.1		
		n	21	22	23	16		
Number of viable pups on post-natal day 0	Total	Mean	11.8	10.8	11.4	10.9		
		SD	2.2	2.3	2.8	2.4		
		n	21	22	23	16		NS
	Male	Mean	5.3	5.5	5.3	4.9		
		SD	2.6	1.9	1.9	1.5		
		n	21	22	23	16		NS
	Female	Mean	6.5	5.4	6.0	5.9		
		SD	2.6	2.2	1.8	2.1		
		n	21	22	23	16		NS
on post-natal day 4	Total	Mean	11.7	10.8	11.8	10.1		
		SD	2.1	2.3	1.6	2.4		
		n	21	22	22	16		NS
	Male	Mean	5.3	5.5	5.6	4.4		
		SD	2.6	1.9	1.5	1.4		
		n	21	22	22	16		
	Female	Mean	6.4	5.3	6.2	5.7		
		SD	2.5	2.2	1.5	2.2		
		n	21	22	22	16		
on post-natal day 7	Total	Mean	9.7	9.5	9.8	9.0		
		SD	0.8	1.3	0.5	1.3		
		n	21	22	22	16	*	U
	Male	Mean	4.4	4.9	4.6	4.3		
		SD	1.5	1.3	0.9	1.1		
		n	21	22	22	16		
	Female	Mean	5.3	4.5	5.2	4.8		
		SD	1.6	1.4	0.7	1.4		
		n	21	22	22	16		

Remarks:
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

Litter data

Values			Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day		
Number of viable pups								
on post-natal day 14	Total	Mean	9.6	9.5	9.8	9.0		
		SD	0.9	1.3	0.5	1.3		
		n	21	22	22	16		NS
	Male	Mean	4.3	4.9	4.6	4.3		
		SD	1.5	1.3	0.9	1.1		
		n	21	22	22	16		NS
	Female	Mean	5.3	4.5	5.2	4.8		
		SD	1.6	1.4	0.7	1.4		
		n	21	22	22	16		NS
on post-natal day 21	Total	Mean	9.6	9.5	9.8	9.0		
		SD	0.9	1.3	0.5	1.3		
		n	21	22	22	16		NS
	Male	Mean	4.3	4.9	4.6	4.3		
		SD	1.5	1.3	0.9	1.1		
		n	21	22	22	16		NS
	Female	Mean	5.3	4.5	5.2	4.8		
		SD	1.6	1.4	0.7	1.4		
		n	21	22	22	16		NS
Number of pups euthanized	Total	Mean	2.0	1.3	1.9	1.1		
on post-natal day 4		SD	1.6	1.5	1.4	1.4		
		n	21	22	22	16		NS
	Male	Mean	0.9	0.5	0.9	0.2		
		SD	1.4	0.9	0.9	0.5		
		n	21	22	22	16		
	Female	Mean	1.1	0.8	1.0	0.9		
		SD	1.4	1.2	1.2	1.2		
		n	21	22	22	16		
Number of dead pups								
on post-natal day 0	Total	Mean	0.0	0.0	0.0	0.3		
		SD	0.0	0.2	0.0	0.6		
		n	21	22	22	16	*	U
	Male	Mean	0.0	0.0	0.0	0.2		
		SD	0.0	0.2	0.0	0.4		
		n	21	22	22	16		
	Female	Mean	0.0	0.0	0.0	0.1		
		SD	0.0	0.0	0.0	0.3		
		n	21	22	22	16		

Remarks:
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

Values			Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day		
Number of dead pups								
between post-natal days 0-21	Total	Mean	0.1	0.1	0.2	1.0		
		SD	0.5	0.3	0.4	1.9		
		n	21	22	23	16	*	U
	Male	Mean	0.0	0.0	0.0	0.7		
		SD	0.2	0.2	0.2	1.1		
		n	21	22	23	16		
	Female	Mean	0.1	0.0	0.1	0.3		
		SD	0.3	0.2	0.3	1.0		
		n	21	22	23	16		

Remarks:
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

Body weight and weight changes:

Pups:

Offspring of the high dose animals had a slightly reduced body weight on postnatal day 0 (-6.5 %). Body weight development between PND0 - PND21 was also slightly depressed when compared to control group offspring (- 8.6 %). Body weight of the offspring of the low and mid dose group was unremarkable and comparable to the control group.

The body weight of male pups and female pups at 100 and 300 mg/kg bw/day – evaluating separately the two genders – was similar to the control between post-natal days 0 and 21. Although, some statistical significances with respect to the control were detected at the slightly lower or higher mean body weight of male or female pups at 100 and 300 mg/kg bw/day during the first week after birth, the terminal body weight was similar to the control. Statistical significance with respect to the control was detected at the higher mean body weight of pups (male and female) at 100 mg/kg bw/day on post-natal days 4 and 7 and at the lower mean body weight of pups at 300 mg/kg bw/day on post-natal days 0 and 14. The terminal body weight (post-natal day 21) was comparable with the control in these groups, therefore, the minor differences were considered to be toxicologically not relevant. The mean body weight of offspring remained below the control at 1000 mg/kg bw/day on post-natal days 0, 4, 7, 14 and 21 being always statistically significantly lower.

The mean litter weight gain was similar in the control and at 100 and 300 groups by interval of the measurements and between post-natal days 0 and 21. The mean litter weight gain was slightly lower than in the control group at 1000 mg/kg bw/day.

Statistical significance was detected with respect to the control at the slightly higher mean pup weight gain between postnatal days 0 and 4 and at the slightly lower mean body weight gain of pups between post-natal days 7 and 14 at 100 and 300 mg/kg bw/day. The summarized body weight gain (between post-natal days 0 and 21) was comparable with the control in both of these groups. Therefore, these minor changes in body weight gain of offspring (male and female) were considered to be toxicologically not relevant at 100 and 300 mg/kg bw/day. The mean body weight gain was slightly reduced in offspring at 1000 mg/kg bw/day when compared to the control by intervals of measurement and if summarized.

Table 44: summary of body weight of F1 offspring litter weight

Group		Litter weight (g) on post-natal day 0	4	7	14	21
Control	Mean	72.7	119.9	150.3	292.4	471.8
	SD	12.2	18.5	15.2	29.4	44.8
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	67.0	115.7	153.2	289.0	467.9
	SD	12.6	19.4	21.3	37.5	59.6
	N	22	22	22	22	22
300 mg/kg bw/day	Mean	68.9	121.6	153.7	294.3	479.2
	SD	16.1	14.2	12.4	17.2	28.8
	N	23	22	22	22	22
1000 mg/kg bw/day	Mean	63.1	95.1	124.8	245.6	404.8
	SD	13.2	21.8	18.4	32.2	49.3
	N	16	16	16	16	16
			**	**	**	**
		NS	DN	DN	U	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
N = Number of litters

Table 45: summary of body weight of F1 offspring males and females

Group		Body weight (g) on post-natal day 0	4	7	14	21
Control	Mean	6.2	10.2	15.5	30.4	49.0
	SD	0.5	1.1	1.5	2.3	3.5
	n	247	246	203	202	202
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	6.2	10.7	16.2	30.6	49.5
	SD	0.5	1.3	1.6	2.3	3.8
	n	238	237	208	208	208
	N	22	22	22	22	22
			**	**		
300 mg/kg bw/day	Mean	6.0	10.3	15.7	30.0	48.8
	SD	0.5	0.9	1.3	1.9	3.3
	n	262	260	216	216	216
	N	23	22	22	22	22
		**			*	
1000 mg/kg bw/day	Mean	5.8	9.4	13.9	27.3	45.0
	SD	0.7	1.6	2.0	3.0	5.3
	n	174	162	144	144	144
	N	16	16	16	16	16
		**	**	**	**	**
		U	U	U	U	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
n = Number of offsprings
N = Number of litters

Table 46: summary of body weight of F1 offspring males

Group		Body weight (g) on post-natal day 0	4	7	14	21
Control	Mean	6.3	10.4	15.7	30.7	49.8
	SD	0.4	1.0	1.4	2.5	3.7
	n	111	111	92	91	91
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	6.3	10.9	16.4	30.7	50.0
	SD	0.5	1.2	1.6	2.2	3.8
	n	120	120	108	108	108
	N	22	22	22	22	22
			**	**		
300 mg/kg bw/day	Mean	6.2	10.4	15.8	30.2	49.3
	SD	0.5	1.0	1.3	1.9	3.2
	n	123	123	102	102	102
	N	22	22	22	22	22
		**				
1000 mg/kg bw/day	Mean	6.0	9.8	14.3	28.0	46.4
	SD	0.7	1.5	1.9	2.8	5.2
	n	79	71	68	68	68
	N	16	16	16	16	16
		**	**	**	**	**
		U	U	U	U	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
n = Number of offsprings
N = Number of litters

Table 47: summary of body weight of F1 offpsring females

Group		Body weight (g) on post-natal day 0	4	7	14	21
Control	Mean	6.0	10.1	15.4	30.1	48.5
	SD	0.4	1.1	1.5	2.2	3.2
	n	136	135	111	111	111
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	6.1	10.6	16.0	30.4	48.9
	SD	0.5	1.3	1.6	2.4	3.8
	n	118	117	100	100	100
	N	21	21	21	21	21
			**	**		
300 mg/kg bw/day	Mean	5.9	10.2	15.5	29.8	48.4
	SD	0.5	0.9	1.2	1.9	3.3
	n	139	137	114	114	114
	N	23	22	22	22	22
1000 mg/kg bw/day	Mean	5.7	9.1	13.5	26.6	43.7
	SD	0.7	1.6	2.0	3.0	5.1
	n	95	91	76	76	76
	N	16	16	16	16	16
		**	**	**	**	**
		U	U	U	U	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
n = Number of offsprings
N = Number of litters

Table 48: summary of body weight gain of F1 offspring litter weight gain

Group		Litter weight gain (g) between post-natal days 0-4	4-7	7-14	14-21	0-21
Control	Mean	47.5	50.4	142.8	179.4	412.1
	SD	8.6	8.1	17.2	18.7	40.6
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	49.2	51.2	137.1	180.5	412.6
	SD	8.7	9.6	19.0	26.8	57.4
	N	22	22	22	22	22
300 mg/kg bw/day	Mean	50.1	52.3	140.6	184.9	419.6
	SD	6.7	6.9	8.7	14.8	26.5
	N	22	22	22	22	22
1000 mg/kg bw/day	Mean	36.0	39.6	120.8	159.2	352.1
	SD	10.1	7.6	16.0	19.0	43.8
	N	16	16	16	16	16
		**	**	**	**	**
		DN	DN	U	DN	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
N = Number of litters

Table 49: summary of body weight gain of F1 offspring male and female

Group		Body weight gain (g) between post-natal days 0-4	4-7	7-14	14-21	0-21
Control	Mean	4.1	5.2	14.8	18.6	42.8
	SD	0.8	0.8	1.4	1.9	3.3
	n	246	203	202	202	202
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	4.5	5.4	14.4	18.9	43.3
	SD	0.9	0.8	1.1	2.2	3.5
	n	237	208	208	208	208
	N	22	22	22	22	22
		**		**		
300 mg/kg bw/day	Mean	4.2	5.3	14.3	18.8	42.7
	SD	0.6	0.7	1.1	2.0	3.1
	n	260	216	216	216	216
	N	23	23	23	23	23
		**		**		
1000 mg/kg bw/day	Mean	3.6	4.4	13.4	17.7	39.1
	SD	1.1	0.8	1.3	2.7	4.9
	n	162	144	144	144	144
	N	16	16	16	16	16
		**	**	**	**	**
		U	DN	U	U	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
n = Number of offsprings
N = Number of litters

Table 50: summary of body weight gain of F1 offspring males

Group		Body weight gain (g) between post-natal days 0-4	4-7	7-14	14-21	0-21
Control	Mean	6.3	10.4	15.7	30.7	49.8
	SD	0.4	1.0	1.4	2.5	3.7
	n	111	111	92	91	91
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	6.3	10.9	16.4	30.7	50.0
	SD	0.5	1.2	1.6	2.2	3.8
	n	120	120	108	108	108
	N	22	22	22	22	22
			**	**		
300 mg/kg bw/day	Mean	6.2	10.4	15.8	30.2	49.3
	SD	0.5	1.0	1.3	1.9	3.2
	n	123	123	102	102	102
	N	22	22	22	22	22
		**				
1000 mg/kg bw/day	Mean	3.7	4.5	13.7	18.4	40.4
	SD	1.1	0.7	1.2	2.7	4.9
	n	71	68	68	68	68
	N	16	16	16	16	16
		**	**	**	**	**
		U	U	U	U	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
n = Number of offsprings
N = Number of litters

Table 51: summary of body weight gain of F1 offspring females

Group		Body weight gain (g) between post-natal days 0-4	4-7	7-14	14-21	0-21
Control	Mean	4.1	5.2	14.7	18.3	42.4
	SD	0.9	0.8	1.4	1.9	3.0
	n	135	111	111	111	111
	N	21	21	21	21	21
100 mg/kg bw/day	Mean	4.5	5.5	14.4	18.5	42.8
	SD	0.9	1.1	1.2	2.0	3.5
	n	117	104	100	100	100
	N	21	21	21	21	21
		**	*	*		
300 mg/kg bw/day	Mean	4.3	5.4	14.3	18.6	42.4
	SD	0.7	1.0	1.2	2.0	3.1
	n	138	116	114	114	114
	N	23	23	23	23	23
		*		**		
1000 mg/kg bw/day	Mean	3.4	4.3	13.1	17.0	38.0
	SD	1.1	0.8	1.3	2.4	4.7
	n	91	76	76	76	76
	N	16	16	16	16	16
		**	**	**	**	**
		U	U	DN	DN	U

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
n = Number of offsprings
N = Number of litters

Cohort 1A:

The body weight development was reduced in F1 Cohort 1A male animals administered with 1000 mg/kg bw/day. The lower mean body weight of female animals at 1000 mg/kg bw/day during the first half of the observation period recovered during the second half of the observation period.

The mean body weight was comparable to their control in F1 Cohort 1A male animals at 100 and 300 mg/kg bw/day during the entire observation period. Statistically significant difference with respect to the control was detected at the lower mean body weight gain of male animals at 100 mg/kg bw/day between PN42 and PN49 and at 300 mg/kg bw/day between PN32 and PN36. However, these minor differences in the mean body weight gain had no influence on the mean body weight of these male animals.

In F1 Cohort 1A male animals at 1000 mg/kg bw/day, the mean body weight was significantly lower than in the control during the entire observation period (from PND22 up to and including PND90) (-12% day 90). The body weight gain of these animals was also lower than in the control during the entire observation period reaching statistical significances in several cases by weekly interval and also for the summarized body weight gain (between PND22 and PND90).

The mean body weight and body weight gain was comparable in the control and test item treated F1 Cohort 1A female animals at 100 and 300 mg/kg bw/day during observation periods. Statistical significance at the slightly higher mean body weight of female animals at 100 mg/kg bw/day was considered to be toxicologically not relevant.

The mean body weight of F1 Cohort 1A female animals at 1000 mg/kg bw/day was statistically significantly lower than in the control from PND22 up to PND42 (-6% day 42) and it was comparable with the control between PN49 and 90 (-3% day 90). The mean body weight gain was similar in all F1 Cohort 1A female groups (control 100, 300 and 1000 mg/kg bw/day) during the observation period. Although, statistical significance with respect to the control was noted for F1 Cohort 1A female animals at 1000 mg/kg bw/day at the lower mean body weight gain between PND22 and PND29 and at the higher mean body weight gain

between PND42 and PND49. The summarized mean body weight gains of F1 Cohort 1A female animals were comparable in all groups between PND22 and PND90.

Table 52: summary of body weight of F1 cohort 1A males

Group		Body weight (g) on post-natal days													
		22	25	29	32	36	39	42	49	56	63	70	77	84	90
Control	Mean	52.6	68.0	93.2	114.2	144.5	164.6	185.1	233.0	278.1	313.7	341.0	363.7	384.1	399.6
	SD	4.35	4.93	6.65	8.24	10.35	14.25	14.76	16.84	18.50	20.64	22.00	24.01	26.55	29.62
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
100	Mean	52.4	68.2	93.4	114.2	142.6	163.6	183.0	227.3	271.5	305.3	333.1	355.8	374.6	391.8
mg/kg bw/day	SD	4.47	6.13	7.77	8.82	11.14	12.63	13.80	16.14	16.56	18.74	20.93	22.55	23.88	24.89
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	0	0	0	0	-1	-1	-1	-2	-2	-3	-2	-2	-2	-2
300	Mean	51.4	67.5	92.5	113.4	140.9	162.0	181.1	226.0	271.2	305.3	334.0	356.4	374.6	389.2
mg/kg bw/day	SD	3.99	5.76	7.53	9.11	11.39	13.82	15.10	17.49	23.34	28.10	33.50	37.50	40.03	42.49
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-2	-1	-1	-1	-2	-2	-2	-3	-2	-3	-2	-2	-2	-3
1000	Mean	48.4	61.6	85.0	104.1	129.5	149.3	166.7	207.9	250.3	279.3	303.9	321.7	338.8	352.1
mg/kg bw/day	SD	4.83	5.20	7.72	9.68	11.05	11.31	12.31	15.17	15.37	18.24	19.08	20.54	21.96	22.34
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-8	-9	-9	-9	-10	-9	-10	-11	-10	-11	-11	-12	-12	-12
		**	**	**	**	**	**	**	**	**	**	**	**	**	**
		DN	DN	DN	DN	DN	DN	DN	DN	DN	DN	DN	U	U	U

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 53: summary of body weight F1 of cohort 1A females

Group		Body weight (g) on post-natal days													
		22	25	29	32	36	39	42	49	56	63	70	77	84	90
Control	Mean	51.4	65.0	86.1	102.6	122.0	134.3	144.5	162.1	179.4	192.5	202.3	210.0	224.8	232.8
	SD	3.82	4.79	5.84	6.74	7.61	8.12	9.19	9.90	10.24	11.67	13.33	12.81	14.79	14.51
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
100	Mean	50.9	65.2	87.2	104.9	126.6	140.9	149.9	169.6	186.6	199.9	210.9	219.0	232.4	238.6
mg/kg bw/day	SD	4.11	4.47	6.32	6.60	8.70	7.25	8.18	10.11	11.81	14.22	14.98	14.58	14.92	14.55
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-1	0	1	2	4	5	4	5	4	4	4	4	3	3
									*						
300	Mean	49.5	64.0	85.6	102.9	123.9	136.5	146.9	165.2	183.8	196.7	207.2	215.6	230.7	236.9
mg/kg bw/day	SD	3.56	4.68	5.76	7.20	9.34	10.57	11.45	13.82	15.12	16.03	18.07	18.91	19.75	21.89
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-4	-1	-1	0	2	2	2	2	2	2	2	3	3	2
1000	Mean	46.6	58.7	78.9	94.7	113.7	126.0	136.0	156.7	174.6	188.5	198.8	207.3	220.6	224.8
mg/kg bw/day	SD	5.48	6.89	7.98	9.36	9.04	9.81	10.36	10.57	9.16	10.04	10.47	11.42	13.28	15.68
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-9	-10	-8	-8	-7	-6	-6	-3	-3	-2	-2	-1	-2	-3
		**	**	**	**	**	**	**							
		DN	DN	DN	DN	DN	DN	DN	DN	NS	NS	NS	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 54: summary of body weight gain of F1 cohort 1A males

Group		Body weight gain (g) between post-natal days													
		22-25	25-29	29-32	32-36	36-39	39-42	42-49	49-56	56-63	63-70	70-77	77-84	84-90	22-90
Control	Mean	15.4	25.2	21.0	30.3	20.1	20.6	47.9	45.1	35.7	27.3	22.7	20.4	15.5	347.0
	SD	1.68	2.26	2.29	3.30	5.69	6.16	5.45	3.89	4.08	4.74	5.30	4.39	5.44	27.57
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
100 mg/kg bw/day	Mean	15.8	25.2	20.8	28.4	21.0	19.4	44.3	44.2	33.9	27.8	22.7	18.8	17.2	339.4
	SD	2.22	2.10	1.71	3.43	2.50	2.39	4.44	5.08	4.61	4.14	3.44	4.35	4.94	23.82
	n	20	20	20	20	20	20	20 *	20	20	20	20	20	20	20
300 mg/kg bw/day	Mean	16.2	25.0	20.9	27.5	21.1	19.1	45.0	45.2	34.2	28.7	22.4	18.2	14.6	337.8
	SD	3.31	3.55	2.08	2.80	3.10	2.47	7.37	9.44	6.11	6.68	5.84	3.32	7.50	40.38
	n	20	20	20	20 *	20	20	20	20	20	20	20	20	20	20
1000 mg/kg bw/day	Mean	13.2	23.4	19.1	25.4	19.8	17.4	41.3	42.4	29.1	24.6	17.8	17.1	13.3	303.7
	SD	2.48	4.43	4.35	4.01	2.86	3.87	4.14	3.65	4.89	4.85	4.94	4.06	3.88	19.57
	n	20 **	20	20	20 *	20	20 *	20 **	20	20 **	20	20 **	20 *	20	20 **
		U	NS	NS	DN	NS	U	U	NS	DN	NS	DN	DN	NS	U

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 55: summary of body weight gain of F1 cohort 1A females

Group		Body weight gain (g) between post-natal days													
		22-25	25-29	29-32	32-36	36-39	39-42	42-49	49-56	56-63	63-70	70-77	77-84	84-90	22-90
Control	Mean	13.6	21.2	16.4	19.4	12.3	10.2	17.6	17.3	13.1	9.8	7.7	14.8	8.0	181.4
	SD	1.54	1.99	2.02	3.53	3.81	3.11	4.13	3.03	3.11	3.62	4.16	7.77	7.01	14.35
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
100 mg/kg bw/day	Mean	14.3	22.1	17.7	21.7	14.3	9.0	19.7	17.0	13.4	11.0	8.1	13.4	6.2	187.7
	SD	1.62	2.34	2.14	4.35	3.80	3.26	3.96	4.36	4.80	4.14	3.54	6.06	5.83	12.80
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
300 mg/kg bw/day	Mean	14.5	21.6	17.3	21.0	12.6	10.4	18.3	18.7	12.9	10.6	8.4	15.2	6.2	187.4
	SD	2.07	1.89	2.12	3.90	2.23	2.62	5.25	4.63	4.40	3.41	4.49	7.57	6.34	20.20
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
1000 mg/kg bw/day	Mean	12.2	20.1	15.8	19.1	12.3	10.0	20.7	17.9	13.9	10.4	8.5	13.3	4.2	178.2
	SD	2.74	2.03	2.47	2.75	2.80	2.87	2.60	3.59	3.93	4.83	5.10	7.31	8.50	14.95
	n	20 *	20	20	20	20	20	20 **	20	20	20	20	20	20	20
		U	NS	NS	NS	NS	NS	U	NS	NS	NS	NS	NS	NS	NS

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Food consumption and compound intake (if feeding study):

Pups: not applicable

Cohort 1A:

The food consumption was not affected in F1 Cohort 1A male or female animals at 100, 300 and 1000 mg/kg bw/day.

The mean daily food consumption of F1 Cohort 1A male or female animals was similar in the control and test item treated groups (100, 300 and 1000 mg/kg bw/day) during the observation period (between PND22 and PND90).

Food efficiency: not examined

Water consumption and compound intake (if drinking water study): not specified

Table 56: summary of food consumption of F1 cohort 1A males

Group		Daily mean food consumption (g / animal /day) between post-natal days									
		22-29	29-36	36-42	42-49	49-56	56-63	63-70	70-77	77-84	84-90
Control	Mean	10.4	16.0	16.6	20.6	21.9	22.2	21.6	22.7	21.2	21.4
	SD	0.82	1.08	1.46	1.10	0.92	1.03	1.23	2.86	1.42	1.72
	n	8	8	8	8	8	8	8	8	8	8
100 mg/kg bw/day	Mean	10.3	15.6	16.5	20.0	21.0	21.1	21.3	20.7	20.1	21.0
	SD	1.02	0.47	1.14	0.74	0.72	0.90	1.56	1.35	1.37	1.59
	n	10	10	10	10	10	10	10	10	10	10
	± %	0	-3	-1	-3	-4 *	-5	-1	-9 *	-5	-2
300 mg/kg bw/day	Mean	9.8	15.3	16.5	19.9	20.7	21.2	21.7	20.7	19.7	20.9
	SD	1.50	0.89	1.59	1.21	1.69	1.37	1.51	2.32	1.72	1.56
	n	9	9	9	9	9	9	9	9	9	9
	± %	-5	-5	-1	-4	-6	-4	1	-9	-7 *	-2
1000 mg/kg bw/day	Mean	9.6	18.3	16.2	18.7	20.4	20.4	20.8	18.7	19.2	19.6
	SD	1.08	13.69	4.03	0.86	0.74	0.85	1.39	1.38	0.96	2.59
	n	10	10	10	10	10	10	10	10	10	10
	± %	-8	14 *	-3 *	-9 **	-7 **	-8 *	-4	-18 **	-9 **	-8
		NS	U	U	DN	U	DN	NS	DN	DN	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 57: summary of food consumption of F1 cohort 1A females

Group		Daily mean food consumption (g / animal /day) between post-natal days									
		22-29	29-36	36-42	42-49	49-56	56-63	63-70	70-77	77-84	84-90
Control	Mean	9.5	13.5	12.7	14.5	14.2	14.0	14.2	14.7	14.6	14.5
	SD	1.35	1.32	0.75	0.88	0.91	0.95	1.53	1.04	0.97	0.87
	n	8	8	8	8	8	8	8	8	8	8
100 mg/kg bw/day	Mean	10.0	14.7	13.7	14.6	14.7	14.7	15.1	15.3	14.8	15.4
	SD	0.90	2.08	1.15	1.06	1.64	1.92	1.64	1.58	1.19	1.54
	n	10	10	10	10	10	10	10	10	10	10
	± %	5	9	8	1	3	5	6	4	1	6
300 mg/kg bw/day	Mean	9.9	13.4	13.1	14.3	14.3	14.6	15.9	15.6	15.0	14.6
	SD	0.81	1.21	0.83	0.95	1.32	1.99	2.55	2.65	1.14	1.46
	n	9	9	9	9	9	9	9	9	9	9
	± %	3	0	3	-1	1	4	12	6	2	1
1000 mg/kg bw/day	Mean	9.1	12.8	12.1	14.2	14.1	14.8	14.3	13.9	14.6	15.3
	SD	0.86	1.39	1.30	1.07	0.82	2.49	0.90	0.67	1.70	1.20
	n	11	11	11	11	11	11	11	11	11	11
	± %	-5	-5	-4	-2	0	6	1	-6	0	6
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Ophthalmological findings: not examined

Haematological findings:

Pups: not examined

Cohort 1A:

There were no test item related adverse changes in the examined hematological parameters in F1 Cohort 1A male or female animals at 100, 300 or 1000 mg/kg bw/day.

In the male animals at 100 mg/kg bw/day, statistical significances were detected at the slightly higher mean percentage of neutrophil granulocytes (NEU) and reticulocytes (RET) and at the slightly shorter mean prothrombin time (PT) when compared to the control.

At 300 mg/kg bw/day, higher mean percentage of neutrophil granulocytes and monocytes (MONO) and lower mean percentage of lymphocytes (LYM) were observed in male animals when compared to the control.

At 1000 mg/kg bw/day, statistical significances were detected at the slightly higher mean percentage of neutrophil granulocytes, lower mean percentage of lymphocytes, eosinophil granulocytes (EOS) In the female animals at 100 mg/kg bw/day, the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were slightly lower than in the control group.

All examined parameters were comparable with the control in female animals at 300 mg/kg bw/day.

Statistical significance was detected with respect to the control at the slightly higher mean percentage of monocytes (MONO), at the lower mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and at the slightly shorter mean prothrombin time (PT) in F1 Cohort1A female animals at 1000 mg/kg bw/day.

The changes noted in these hematology or blood coagulation parameters were considered to have little or no toxicological relevance. Slight elevation in NEU in male animals were mainly due to the relative low control value. Higher mean percentage of monocytes in female animals were not accompanied by related signs of inflammation, therefore were considered to be toxicologically not relevant. The mean values of LYM, EOS, MCHC, RET, PT, MCH and MCHC were well within the historical control range in male or female animals, where relevant.

Clinical biochemistry findings:

Pups: not examined

Cohort 1A:

Pathologic alterations were not detected at the evaluation of clinical chemistry parameters in F1 Cohort 1A male or female animals at 100, 300 or 1000 mg/kg bw/day.

The examined clinical chemistry parameters were comparable in male animals in the control and 100 and 300 mg/kg bw/day groups.

In the male animals at 1000 mg/kg bw/day, statistical significance was noted for the slightly lower mean concentration of total protein (TPROT).

In the F1 Cohort 1A female animals, statistically significant difference with respect to the control was detected at the lower mean activity of alanine aminotransferase (ALT) at 100 mg/kg bw/day. All examined parameters were comparable with the control in the female animals at 300 mg/kg bw/day. In the female animals at 1000 mg/kg bw/day, higher mean activity of alanine aminotransferase, higher mean concentrations of total bilirubin (TBIL) and cholesterol (CHOL) were observed when compared to the control.

The statistically significant changes of some clinical chemistry parameters were considered to be of little or no biological significance as the mean values correlated well with the historical control values (total protein, ALT, TBIL, CHOL) or the profile of change has no biological significance (ALT in low dose female animals). Significantly elevated activity of ALT and aspartate aminotransferase (AST) in one female animal at 1000 mg/kg bw/day (no. 728) was considered to be individual alteration. There were no supporting histological findings in this female animal. Therefore changes in enzyme activities might be indicative of functional alteration.

Urinalysis findings:

Pups: not examined

Cohort 1A:

There were no test item related adverse changes in the examined urine parameters in F1 Cohort 1A animals (male or female) at 100, 300 or 1000 mg/kg bw/day.

Most of the examined urine parameters were comparable in the control and 100, 300 or 1000 mg/kg bw/day groups (male and female).

Slightly but statistically significantly higher volume of the urine with respect to their control was detected in male animals at 1000 mg/kg bw/day.

In the female animals, statistical significance was noted for the slightly lower pH of urine at 300 and 1000 mg/kg bw/day and the sediment was positive in four rats (4/10) at 1000 mg/kg bw/day due the moderate amount of amorphous crystals.

Table 58: summary of urinalysis of F1 cohort 1A males

Group		Volume (mL)	Color	Clarity	pH	Glucose	Nitrite	Protein	Ketone	Urobili-nogen	Bilirubin	Blood (Ery/µL)	Spec. Gravity	Leu (Leu/µL)	Sediment
Control	Mean	6.2	Norm	Clear	6.0	Neg	Neg	Pos	Neg	Norm	Neg	Neg	1027.5	Neg	Neg
	SD	7.6		or	0.5				or				7.9		or
	n	10		Cloudy	10				Pos				10		Pos
100 mg/kg bw/day	Mean	3.6	Norm	Clear	5.2	Neg	Neg	Pos	Neg	Norm	Neg	Neg	1029.5	Neg	Neg
	SD	2.1			0.4				or				1.6		or
	n	10			10				Pos				10		Pos
					**										
300 mg/kg bw/day	Mean	7.7	Norm	Clear	5.3	Neg	Neg	Pos	Neg	Norm	Neg	Neg	1027.0	Neg	Neg
	SD	6.2			0.5				or				5.4		
	n	10			10				Pos				10		
					**										
1000 mg/kg bw/day	Mean	8.0	Norm	Clear	5.0	Neg	Neg	Pos	Pos	Norm	Neg	Neg	1030.0	Neg	Neg
	SD	2.8		or	0.0								0.0		or
	n	10		Cloudy	10								10		Pos
		*			**										
		U			DN								NS		

REMARKS : NS = Not Significant
* = p < 0.05
** = p < 0.01
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 59: summary of urinalysis of F1 cohort 1A females

Group		Volume (mL)	Color	Clarity	pH	Glucose	Nitrite	Protein	Ketone	Urobili-nogen	Bilirubin	Blood (Ery/µL)	Spec. Gravity	Leu (Leu/µL)	Sediment
Control	Mean	7.2	Norm	Clear	5.9	Neg	Neg	Neg	Neg	Norm	Neg	Neg	1024.5	Neg	Neg
	SD	8.4		or	0.6			or	or				8.3		
	n	10		Cloudy	10			Pos	Pos				10		
100 mg/kg bw/day	Mean	8.3	Norm	Clear	5.7	Neg	Neg	Neg	Neg	Norm	Neg	Neg	1026.0	Neg	Neg
	SD	11.2		or	0.8			or	or				8.4		
	n	10		Cloudy	10			Pos	Pos				10		
300 mg/kg bw/day	Mean	4.5	Norm	Clear	5.3	Neg	Neg	Pos	Neg	Norm	Neg	Neg	1028.5	Neg	Neg
	SD	3.7		or	0.5				or				4.7		
	n	10		Cloudy	10 *				Pos				10		
1000 mg/kg bw/day	Mean	8.1	Norm	Clear	5.1	Neg	Neg	Neg	Neg	Norm	Neg	Neg	1027.5	Neg	Neg
	SD	8.1		or	0.3			or	or				5.4		or
	n	10		Cloudy	10 **			Pos	Pos				10		Pos
		NS			DN								NS		

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Sexual maturation:

The sexual maturity was not adversely affected in F1 Cohort 1B male or female animals at 100, 300 or 1000 mg/kg bw/day.

The balano-preputional separation was completed in all F1 Cohort 1B male animals – control, 100, 300 and 1000 mg/kg bw/day – on post-natal day 35, although, the mean body weight was slightly lower with respect to the control in male animals at 1000 mg/kg bw/day on PND35.

In the F1 Cohort 1B female animals, there were no differences between the control and test item treated groups in mean days of vaginal patency or in the mean body weight on the day of vaginal patency.

Table 60: summary of sexual maturity of F1 cohort 1B males

		Group (mg/kg bw/day) Control	100	300	1000	
Balano-preputial separation on postnatal day 35						
- No. of animals examined	N	20	20	20	20	
- No. of animals with positive response	Sum	20	20	20	20	-
	%	100	100	100	100	
- No. of animals with negative response	Sum	0	0	0	0	-
	%	0	0	0	0	
Body weight (g) on the day of balano-preputional separation	Mean	134.1	136.8	135.0	125.4	
	SD	12.6	10.5	6.2	10.7	
	n	20	20	20	20	** U

Remark: NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = no data

Table 61: summary of sexual maturity of F1 cohort 1B females

		Control		Group (mg/kg bw/day) 100		300		1000		
No. of animals examined (N)		20		20		20		20		
Animals with positive response (vaginal patency)		n	%	n	%	n	%	n	%	
	PN28	1	5	0	0	0	0	0	0	
	PN29	2	10	2	10	0	0	0	0	
	PN30	2	10	2	10	2	10	1	5	
	PN31	4	20	4	20	2	10	1	5	
	PN32	10	50	7	35	10	50	4	20	
	PN33	13	65	15	75	16	80	8	40	
	PN34	15	75	16	80	18	90	15	75	
	PN35	18	90	19	95	20	100	17	85	
	PN36	19	95	20	100	/	/	19	95	
	PN37	19	95	/	/	/	/	20	100	
	PN38	20	100	/	/	/	/	/	/	
Body weight (g) on the day of vaginal patency	Mean	106.6		108.4		105.8		105.2		
	SD	11.8		11.9		10.1		15.9		
	N	20		20		20		20		NS
Post-natal day of vaginal patency	Mean	32.9		32.8		32.6		33.8		
	SD	2.5		1.8		1.5		1.6		
	N	20		20		20		20		NS

Remarks:
n = Summarized number of animals with positive response on the day of the examination
% = Summarized percentage of animals with positive response on the day of the examination
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
PN = Post-natal day

Concerning the F1 offspring's development (surface righting reflex, pinna detachment, eye opening) no clear test item influence was observed.

There were no toxicologically relevant differences in the offspring's development between the control and 100 or 300 mg/kg bw/day groups. Some statistical significance indicates higher percentage of pups with positive response or lower percentage of pups with negative response at 100 mg/kg bw/day (pinna detachment, eye opening) or at 300 mg/kg bw/day (eye opening).

At 1000 mg/kg bw/day, the percentage of pups with positive response was lower and the percentage of pups with negative response was higher than in the control group at surface righting reflex, pinna detachment and eye opening.

Table 62: Summary of developmental of F1 offsprings

SUMMARY OF DEVELOPMENT OF F1 OFFSPRINGS

Parameters		GROUPS (mg/kg bw/day) Control	100	300	1000	
Surface righting reflex on post-natal day 0						
- No. of offspring examined	N	247	238	262	174	
- No. of offspring with positive response	Sum	187	188	191	112	
	%	76	79	73	64	NS
- No. of offspring with negative response	Sum	60	50	71	62	
	%	24	21	27	36	NS
Pinna detachment on post-natal day 2						
- No. of offspring examined	N	247	237	260	168	
- No. of offspring with positive response	Sum	117	140	127	63	
	%	47	59	49	38	NS
- No. of offspring with negative response	Sum	130	97	133	105	
	%	53	41	51	62	NS
Absolute anogenital distance on post-natal day 4						
Male	Mean	6.1	6.0	6.0	5.8	
	SD	0.45	0.48	0.37	0.51	
	n	111	120	123	71 **	U
Female	Mean	3.6	3.6	3.5	3.4	
	SD	0.50	0.48	0.50	0.59	
	n	135	118	137	92 **	DN
Normalized anogenital distance on post-natal day 4						
Male	Mean	2.8	2.7	2.7	2.7	
	SD	0.20	0.20	0.17	0.21	
	n	111	120 *	123	71	DN
Female	Mean	1.7	1.7	1.6	1.6	
	SD	0.21	0.20	0.21	0.25	
	n	135	118	137	92	NS
Nipple retention on post-natal day 13						
Male	Mean	0.0	0.0	0.0	0.0	
	SD	0.00	0.00	0.00	0.00	
	n	92	108	102	68	-
Eye opening on post-natal day 14						
- No. of offspring examined	N	202	208	217	144	
- No. of offspring with positive response	Sum	118	144	148	44	
	%	58	69	68	31 **	CHI2
- No. of offspring with negative response	Sum	84	64	69	100	
	%	42	31	32	69 **	CHI2

Remarks
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
CHI2 = CHI2 test
- = No data

Cohort 1A:

The sexual maturity was not adversely affected in F1 Cohort 1A male or female animals at 100, 300 or 1000 mg/kg bw/day.

The balano-preputional separation was completed in all F1 Cohort 1A male animals – control, 100, 300 and 1000 mg/kg bw/day – on post-natal day 35, although, the mean body weight was slightly lower with respect to the control in male animals at 1000 mg/kg bw/day on PND35 as described above.

In the F1 Cohort 1A female animals, statistical significance was noted for the longer period of vaginal patency at 1000 mg/kg bw/day and at the longer period of appearance of the first cornified vaginal smear at 100 and 1000 mg/kg bw/day. The interval between days of vaginal patency and first cornified smear were similar in all groups (control, 100, 300 and 1000 mg/kg bw/day).

Table 63: Summary of sexual maturity in F1 cohort 1A males and females

SUMMARY OF SEXUAL MATURITY
F1 COHORT 1A MALE

		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
Balano-preputial separation on postnatal day 35						
No. of animals examined	N	20	20	20	20	
Animals with positive response	Sum	20	20	20	20	
	%	100	100	100	100	
Animals with negative response	Sum	0	0	0	0	
	%	0	0	0	0	
Body weight (g)	Mean	136.3	135.3	134.8	122.3	
on the day of balano-preputional	SD	10.0	10.7	11.1	10.1	
separation	n	20	20	20	20 **	DN

Remark: NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

SUMMARY OF SEXUAL MATURITY
F1 COHORT 1A FEMALE

		Control		100 mg/kg bw/day		300 mg/kg bw/day		1000 mg/kg bw/day		
No. of animals examined (N)		20		20		20		20		
Animals with positive response (vaginal patency)		n	%	n	%	n	%	n	%	
	PN29	0	0	1	5	0	0	0	0	
	PN30	3	15	2	10	1	5	0	0	
	PN31	7	35	4	20	2	10	2	10	
	PN32	14	70	7	35	10	50	4	20	
	PN33	18	90	15	75	17	85	9	45	
	PN34	20	100	15	75	19	95	13	65	
	PN35	/	/	18	90	20	100	18	90	
	PN36	/	/	19	95	/	/	20	100	
	PN37	/	/	20	100	/	/	/	/	
Body weight (g)	Mean	101.6		109.1		105.7		104.4		
on the day of vaginal patency	SD	9.3		13.3		9.1		15.7		
	N	20		20		20		20		NS
Post-natal day of	Mean	31.9		33.0 *		32.6		33.7		
vaginal patency	SD	1.2		2.0		1.1		1.5		
	N	20		20		20		20 **		DN
Post-natal day of the	Mean	32.3		34.2		33.5		34.3		
first cornified smear	SD	1.1		3.5		2.2		1.7		
	N	20		20 *		20		20 **		U
Interval between days of	Mean	0.4		1.3		0.9		0.6		
vaginal patency and	SD	0.7		2.6		1.7		0.9		
first cornified smear (day)	N	20		20		20		20		NS

Remarks:
n = Summarized number of animals with positive response on the day of the examination
% = Summarized percentage of animals with positive response on the day of the examination
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
PN = Post-natal day

Anogenital distance (AGD):

Pups:

The anogenital distances were not adversely affected by the test item in male or female offspring at 100, 300 and 1000 mg/kg bw/day.

Statistical significance was detected at the shorter absolute anogenital distance of male and female pups at 1000 mg/kg bw/day. However, the normalized anogenital distances were comparable with the control both in male and female offspring at 1000 mg/kg bw/day. Slightly shorter normalized anogenital distance at 100 mg/kg bw/day was judged to be toxicologically not relevant due to the minor degree and in the lack of dose response relationship.

Cohort 1A: not applicable

Nipple retention in male pups:

Pups:

Nipples/areoles were not visible in any of the examined male offspring in the control or 100, 300 or 1000 mg/kg bw/day groups on post-natal day 13.

Cohort 1A: not applicable

Organ weight findings including organ / body weight ratios:

Pups:

There were no test item related changes in the weights of examined organs (absolute and relative to body and brain weights) in male and female F1 offspring (necropsy at weaning). The examined organ weights were comparable in selected male offspring at the weaning.

Statistical significances with respect to the control were detected at the slightly higher mean thymus weights (absolute and relative to brain weight) at 100 mg/kg bw/day and at the lower mean body weight and brain weight at 1000 mg/kg bw/day in female pups. The minor changes of thymus weights were considered to be independent from the treatment as similar findings was not detected at the higher doses. The lower mean brain weight was probably related to the lower mean body weight as it exceeded the control value – no statistical significance – if related to the body weight.

Cohort 1A:

The weights of the examined organs were not adversely affected in F1 Cohort 1A male and female animals at 100, 300 or 1000 mg/kg bw/day.

Slight elevation in the weights of liver and kidneys at 300 mg/kg bw/day (female) and at 1000 mg/kg bw/day (male and female). There were no supporting histopathological alterations in the liver or kidneys.

Slight reduction of thymus weights (absolute and relative to body and brain weights) in F1 Cohort 1A male animals at 1000 mg/kg bw/day dose might be related to the test item influence. Nevertheless, there were no related histopathological findings and changes in immune system. Moreover, absolute as well as relative weight values range within the historical control values.

In the male animals at 100 mg/kg bw/day, statistical significance with respect to the control was detected at the slightly lower mean thymus weight and higher mean testes weight relative to body and brain weights.

At 300 mg/kg bw/day, the mean thymus weight was slightly lower and the kidney weight relative to body weight was slightly higher than in the control group in F1 Cohort 1A male animals.

In the male animals at 1000 mg/kg bw/day, the mean fasted body weight was significantly lower than in the control group resulting in lower mean body weight relative to brain weight, lower mean weights of some organ and higher mean weights of some organ referred to body weight.

Statistical significance was detected at the lower mean brain weight, higher mean brain weight relative to body weight, higher mean liver and kidneys weight (absolute and relative to the body and brain weights, both), at the lower mean weights of heart, thymus (absolute and relative to the body and brain weights), prostate and pituitary in male animals at 1000 mg/kg bw/day when compared to the control. The weights of testes (relative to body and brain weights), epididymides (relative to body weight) and adrenal glands

(relative to body and brain weight) exceeded the control value in male animals administered with the high dose.

In the female animals at 100 mg/kg bw/day, statistical significance with respect to the control was observed at the slightly higher mean body weight relative to brain weight and lower mean brain weight relative to body weight, at the higher mean weights of liver and thyroid glands both relative to brain weight. In female animals at 300 mg/kg bw/day, the body weight relative to brain weight was higher, the brain weights (absolute and relative to body weight) were lower than in the control group. Higher mean weights of liver and kidneys (absolute and relative to body and brain weights), thyroid glands (absolute and relative to brain weight), adrenal gland relative to brain weight.

At 1000 mg/kg bw/day, statistical significances with respect to the control were observed at the higher mean body weight relative to brain weight, at the lower mean brain weights (absolute and relative to body weight), at the higher mean weights of liver and kidneys (absolute and relative to body and brain weights), adrenal gland relative to body and brain weights and thyroid glands relative to brain weight in the F1 Cohort 1A female animals.

The statistically significant differences with respect to the control at several organs (brain, heart, prostate, testes, epididymides, adrenal glands, thyroid glands or pituitary) were judged to have little or no toxicological relevance due to the minor degree and in the lack of associated histopathological alterations.

Table 64: summary of organ weight of F1 cohort 1A males

Group		Body weight	Organ weight (g) Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididy-mides	Seminal vesicles†	Prostate	Adrenal glands	Thyroids	Pituitary
Control	Mean	386.9	2.19	10.81	2.49	1.05	0.52	0.74	3.51	1.43	1.54	0.41	0.064	0.017	0.009
	SD	26.90	0.07	1.39	0.24	0.08	0.10	0.10	0.23	0.13	0.36	0.07	0.015	0.003	0.002
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
100 mg/kg bw/day	Mean	379.2	2.19	10.26	2.45	1.01	0.46	0.69	3.67	1.46	1.43	0.40	0.070	0.018	0.009
	SD	22.97	0.10	0.83	0.18	0.06	0.08	0.12	0.30	0.15	0.28	0.10	0.009	0.003	0.002
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-2	0	-5	-2	-4	-11 *	-6	5	2	-7	-4	9	5	5
300 mg/kg bw/day	Mean	376.4	2.14	10.55	2.55	0.99	0.45	0.67	3.55	1.45	1.47	0.39	0.067	0.016	0.009
	SD	41.31	0.09	1.74	0.29	0.11	0.09	0.12	0.24	0.14	0.26	0.08	0.014	0.003	0.002
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-3	-2	-2	3	-5	-13 *	-9	1	1	-5	-6	5	-7	-3
1000 mg/kg bw/day	Mean	334.9	2.05	11.36	2.92	0.93	0.37	0.65	3.85	1.36	1.50	0.33	0.071	0.016	0.007
	SD	24.24	0.10	1.21	0.28	0.08	0.08	0.10	1.19	0.12	0.24	0.07	0.009	0.003	0.001
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-13 **	-7 **	5	17 **	-11 **	-28 **	-11	10	-5	-3	-20 **	11	-4	-18 *
		U	DN	NS	DN	DN	DN	NS	NS	NS	NS	DN	NS	NS	DN

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight relative to body weight (%) Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididy-mides	Seminal vesicles†	Prostate	Adrenal glands	Thyroids	Pituitary
Control	Mean	0.569	2.787	0.645	0.272	0.134	0.190	0.910	0.371	0.400	0.107	0.0166	0.0044	0.0022
	SD	0.043	0.199	0.053	0.016	0.025	0.018	0.079	0.035	0.104	0.019	0.0039	0.0008	0.0004
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
100 mg/kg bw/day	Mean	0.579	2.705	0.647	0.267	0.121	0.182	0.968	0.385	0.378	0.104	0.0185	0.0047	0.0024
	SD	0.043	0.144	0.037	0.014	0.019	0.030	0.075	0.037	0.075	0.024	0.0021	0.0006	0.0006
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	2	-3	0	-2	-9	-4	6 *	4	-5	-2	12	7	8
300 mg/kg bw/day	Mean	0.573	2.792	0.679	0.264	0.119	0.177	0.951	0.388	0.390	0.103	0.0180	0.0042	0.0023
	SD	0.051	0.220	0.044	0.017	0.021	0.020	0.090	0.044	0.063	0.018	0.0038	0.0008	0.0005
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	1	0	5 *	-3	-11	-7	4	4	-2	-4	9	-4	1
1000 mg/kg bw/day	Mean	0.613	3.388	0.871	0.279	0.111	0.195	1.153	0.406	0.447	0.098	0.0213	0.0049	0.0021
	SD	0.034	0.204	0.057	0.019	0.023	0.024	0.350	0.034	0.065	0.018	0.0030	0.0009	0.0005
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	8 **	22 **	35 **	3	-17 *	3	27 **	9 **	12	-8	29 **	11	-5
		DN	DN	DN	NS	DN	NS	U	DN	NS	NS	U	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight and body weight relative to brain weight (%)												
		Body weight	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididy-mides	Seminal vesicles†	Prostate	Adrenal glands	Thyroids	Pituitary
Control	Mean	17681.9	493.91	113.78	47.97	23.61	33.68	160.21	65.37	70.15	18.83	2.93	0.77	0.40
	SD	1352.65	62.24	10.36	3.77	4.54	4.67	9.36	5.62	15.93	3.46	0.68	0.14	0.09
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
100	Mean	17352.9	469.35	112.26	46.23	21.06	31.59	167.72	66.77	65.39	18.14	3.21	0.82	0.42
mg/kg bw/day	SD	1271.80	42.17	10.02	3.33	3.84	5.43	14.11	7.13	12.72	4.70	0.41	0.12	0.10
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-2	-5	-1	-4	-11	-6	5 *	2	-7	-4	9	6	5
300	Mean	17579.9	492.56	119.27	46.42	20.99	31.27	166.13	67.76	68.33	18.04	3.15	0.74	0.40
mg/kg bw/day	SD	1555.96	72.88	10.79	4.59	4.31	4.96	10.33	6.28	10.88	3.45	0.65	0.14	0.08
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-1	0	5	-3	-11	-7	4	4	-3	-4	8	-5	-1
1000	Mean	16365.6	554.98	142.44	45.56	18.23	31.90	188.41	66.34	73.17	16.09	3.48	0.80	0.35
mg/kg bw/day	SD	890.94	52.37	11.06	3.08	4.13	4.23	58.58	5.59	11.66	3.07	0.40	0.13	0.07
	n	20	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-7 **	12 **	25 **	-5	-23 **	-5	18 **	1	4	-15	19 **	3	-13
		DN	DN	DN	NS	DN	NS	U	NS	NS	NS	U	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Table 65: summary of organ weight of F1 cohort 1A females

Group		Body weight	Organ weight (g)									
			Brain	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	Adrenal glands	Thyroids	Pituitary
Control	Mean	218.5	2.06	6.40	1.49	0.68	0.40	0.49	0.58	0.093	0.072	0.015	0.011
	SD	13.10	0.06	0.81	0.14	0.05	0.06	0.06	0.13	0.015	0.010	0.003	0.002
	n	20	20	20	20	20	20	20	20	20	20	20	20
100	Mean	225.6	2.03	6.90	1.54	0.70	0.40	0.49	0.59	0.098	0.074	0.016	0.011
mg/kg bw/day	SD	12.97	0.10	0.92	0.11	0.06	0.07	0.07	0.10	0.016	0.011	0.003	0.003
	n	20	20	20	20	20	20	20	20	20	20	20	20
	± %	3	-2	8	4	3	1	0	2	5	3	12	2
300	Mean	223.0	1.99	7.21	1.60	0.68	0.40	0.50	0.58	0.096	0.079	0.017	0.012
mg/kg bw/day	SD	19.84	0.09	0.78	0.17	0.05	0.06	0.08	0.12	0.018	0.012	0.002	0.003
	n	20	20	20	20	20	20	20	20	20	20	20	20
	± %	2	-4 *	13 **	8 *	0	-1	3	1	4	9	15 *	10
1000	Mean	211.8	1.88	7.41	1.64	0.65	0.40	0.46	0.54	0.089	0.077	0.016	0.010
mg/kg bw/day	SD	15.17	0.10	0.70	0.13	0.05	0.06	0.05	0.11	0.017	0.011	0.003	0.003
	n	20	20	20	20	20	20	20	20	20	20	20	20
	± %	-3	-9 **	16 **	10 **	-5	-1	-7	-6	-4	6	12	-6
		NS	DN	DN	DN	NS	NS	NS	NS	NS	NS	DN	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight relative to body weight (%)										
		Brain	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	Adrenal glands	Thyroids	Pituitary
Control	Mean	0.947	2.927	0.679	0.313	0.183	0.224	0.265	0.0427	0.0330	0.0067	0.0050
	SD	0.059	0.305	0.041	0.023	0.030	0.021	0.062	0.0079	0.0039	0.0015	0.0009
	n	20	20	20	20	20	20	20	20	20	20	20
100	Mean	0.900	3.055	0.683	0.311	0.178	0.217	0.262	0.0433	0.0330	0.0073	0.0050
mg/kg bw/day	SD	0.050	0.350	0.045	0.016	0.029	0.028	0.052	0.0073	0.0045	0.0015	0.0013
	n	20	20	20	20	20	20	20	20	20	20	20
	± %	-5 *	4	1	-1	-3	-3	-1	2	0	8	-1
300	Mean	0.897	3.232	0.720	0.306	0.178	0.226	0.264	0.0432	0.0355	0.0076	0.0055
mg/kg bw/day	SD	0.073	0.215	0.072	0.018	0.023	0.032	0.063	0.0078	0.0052	0.0011	0.0014
	n	20	20	20	20	20	20	20	20	20	20	20
	± %	-5 *	10 **	6 *	-2	-3	0	0	1	8	13	9
1000	Mean	0.892	3.499	0.775	0.308	0.187	0.216	0.258	0.0422	0.0363	0.0078	0.0049
mg/kg bw/day	SD	0.066	0.227	0.044	0.028	0.024	0.027	0.054	0.0079	0.0055	0.0016	0.0013
	n	20	20	20	20	20	20	20	20	20	20	20
	± %	-6 *	20 **	14 **	-2	2	-4	-3	-1	10 *	16	-3
		DN	DN	U	NS	NS	NS	NS	NS	DN	NS	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Group		Organ weight and body weight relative to brain weight (%)										
		Body weight	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	Adrenal glands	Thyroids	Pituitary
Control	Mean	10595.1	310.74	72.09	33.18	19.32	23.79	27.98	4.50	3.49	0.71	0.53
	SD	636.95	42.89	7.44	2.95	2.78	2.76	6.25	0.77	0.46	0.15	0.10
	n	20	20	20	20	20	20	20	20	20	20	20
100	Mean	11144.2	340.50	76.05	34.65	19.80	24.24	29.14	4.81	3.68	0.81	0.56
mg/kg bw/day	SD	593.55	42.99	5.58	2.84	3.17	3.69	5.46	0.76	0.56	0.18	0.15
	n	20	20	20	20	20	20	20	20	20	20	20
	± %	5	10	6	4	2	2	4	7	5	15	4
		*	*								*	
300	Mean	11218.0	362.55	80.50	34.27	19.98	25.31	29.41	4.83	3.97	0.85	0.61
mg/kg bw/day	SD	914.04	37.51	7.76	2.25	3.20	4.06	6.20	0.89	0.54	0.12	0.13
	n	20	20	20	20	20	20	20	20	20	20	20
	± %	6	17	12	3	3	6	5	7	14	19	14
		*	**	**						*	*	
1000	Mean	11265.9	393.80	87.25	34.60	21.04	24.28	29.09	4.75	4.09	0.87	0.55
mg/kg bw/day	SD	755.09	31.63	6.90	2.80	3.31	3.07	6.39	0.92	0.66	0.17	0.14
	n	20	20	20	20	20	20	20	20	20	20	20
	± %	6	27	21	4	9	2	4	5	17	23	3
		**	**	**						**	**	
		DN	DN	DN	NS	NS	NS	NS	NS	DN	DN	NS

REMARKS : ±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Necropsy findings:

Pups:

Specific macroscopic alterations were not found in F1 offspring subjected to gross pathological examination before the weaning or at the weaning.

Some common sporadic necropsy findings were detected in pups necropsied before the weaning: wound around anus (1/23 male at 300 mg/kg bw/day), partially cannibalized (2/17 male and 4/22 female at 100 mg/kg bw/day; 1/24 female at 300 mg/kg bw/day; 1/7 male at 1000 mg/kg bw/day).

The organs and tissues were free of morphological changes in dead or stillborn offspring – empty stomach or autolysis in visceral organs were only detected. At weaning, one or both side pyelectasia was noted for several male and female pups at each dose level except for female pups at 1000 mg/kg bw/day. In the lack of dose response relationship and inflammatory or other pathological changes pyelectasia was considered to be a species specific alteration and not related to the test item.

Some individual findings without relation to the test item treatment were also detected as follows:
- red granulous spot (1/70 male at 100 mg/kg bw/day) or hemorrhage (1/62 male at 300 mg/kg bw/day) in the stomach;

- scar on the tail (1/70 female control);

- alopecia (3/28 male and 3/35 female at 1000 mg/kg bw/day);

- exophthalmos (1/35 male at 1000 mg/kg bw/day);

- damage of forelimb (1/35 female at 1000 mg/kg bw/day);

Cohort 1A:

Macroscopic alterations related to the effect of the test item were not detected in F1 Cohort 1A male or female animals at 100, 300 or 1000 mg/kg bw/day at the necropsy.

Hemorrhage in the stomach (1/20), hernia diaphragmatica (1/20) and renal pyelectasia (4/20, right side, each) were observed in the control male animals.

In the male animals at 100 mg/kg bw/day, right side pyelectasia was detected in some animals (3/20).

At 300 mg/kg bw/day, hemorrhage in the stomach (1/20) and right side pyelectasia (1/20) were noted for single male animals.

In the male animals at 1000 mg/kg bw/day, hemorrhage in the lungs (1/20) and stomach (1/20) and pyelectasia (8/20, right or both sided) were seen at the necropsy.

In control female animals necropsy observations revealed the following findings: right or both sided pyelectasia (3/20); slight, moderate or marked hydrometra (5/20); soft formation in the left horn of uterus (1/20) and ovarian cyst (1/20).

One side pyelectasia (1/20) and slight, moderate or marked hydrometra (8/20) were noted for some female animals at 100 mg/kg bw/day.

At 300 mg/kg bw/day, pyelectasia (2/20, right or both sided), hydrometra (3/20, slight, moderate or marked) and ovarian cyst (1/20) were observed in female animals.

In female animals at 1000 mg/kg bw/day, thymic hemorrhage (1/20) one or both sided pyelectasia (5/20) and slight or moderate hydrometra (3/20) were observed.

These macroscopic findings are common in experimental rats of this strain and age. Pyelectasia is frequently observed in this strain of experimental rats. Histological examination did not reveal degeneration, inflammation or fibrosis. Therefore, this finding was considered as slight individual lesion without toxicological significance. Hydrometra (i.e. dilatation of uterine horns), related to the female sexual cycle, is a frequent observation in experimental rats. In the lack of related inflammatory or other pathological signs, it was judged to be toxicologically not relevant and not test item related as no dose response was noted.

Hemorrhage in the thymus and lungs were related to exsanguination procedure. Hemorrhage in the stomach mucosa was probably related to the treatment procedure. Hernia diaphragmatica, ovarian cyst and soft formation in the uterine horn are also species-specific changes occurring in not treated animals.

Table 66: summary of necropsy findings of F1 offspring

Offspring necropsied before the weaning

Organs	Observations	Group (mg/kg bw/day) Control Male	Control Female	100 Male	100 Female	300 Male	300 Female	1000 Male	1000 Female
	Number of animals examined	20	22	17	22	23	24	7	16
	No macroscopic findings	19/20	22/22	15/17	19/22	21/23	21/24	6/7	15/16
	Wound around anus	0/20	0/22	0/17	0/22	1/23	0/24	0/7	0/16
	Stillborn	1/20	0/22	1/17	3/22	2/23	2/24	1/7	0/16
	Found dead	0/20	0/22	3/17	2/22	1/23	0/24	2/7	0/16
	Partially cannibalized	0/20	0/22	2/17	4/22	0/23	1/24	0/7	0/16
	Missing hindlimb	0/20	0/22	0/17	0/22	0/23	0/24	0/7	1/16
	Autolysis	0/20	0/22	0/17	0/22	0/23	0/24	1/7	1/16

Offspring necropsied at weaning

Organs	Observations	Group (mg/kg bw/day) Control Male	Control Female	100 Male	100 Female	300 Male	300 Female	1000 Male	1000 Female
	Number of animals examined	51	70	70	58	62	74	28	35
	No macroscopic findings	48/51	66/70	63/70	51/58	52/62	69/74	23/28	30/35
Kidneys	Pyelectasia	3/51	3/70	6/70	7/58	9/62	5/74	2/28	0/35
Stomach	Red granulous spot	0/51	0/70	0/70	1/58	0/62	0/74	0/28	0/35
	Hemorrhage	0/51	0/70	0/70	0/58	1/62	0/74	0/28	0/35
Tail	Wound at the end	0/51	1/70	0/70	0/58	0/62	0/74	0/28	0/35
Skin	Alopecia	0/51	0/70	0/70	0/58	0/62	0/74	3/28	3/35
Eye	Exophthalmos	0/51	0/70	0/70	0/58	0/62	0/74	0/28	1/35
Forelimb	Damaged	0/51	0/70	0/70	0/58	0/62	0/74	0/28	1/35

Remark: Frequency of observations = number of animals with observations / number of animals examined.

Table 67: summary of necropsy findings of F1 cohort 1A males

Organs	Observations	Frequency of observations per group Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
	No macroscopic findings	15/20	17/20	18/20	10/20
Lungs	Hemorrhages	0/20	0/20	0/20	1/20
Stomach	Hemorrhages	1/20	0/20	1/20	1/20
Liver	*Hernia diaphragmatica*	1/20	0/20	0/20	0/20
Kidneys	Pyelectasia	4/20	3/20	1/20	8/20

Remark: Frequency of observations = number of animals with observations / number of animals examined.

Table 68: summary of necropsy findings of F1 cohort 1A females

Organs	Observations	Frequency of observations per group Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
	No macroscopic findings	12/20	12/20	16/20	11/20
Thymus	Hemorrhages	0/20	0/20	0/20	1/20
Kidneys	Pyelectasia	3/20	1/20	2/20	5/20
Uterus	Hydrometra	5/20	8/20	3/20	3/20
	Soft formation in the left horn	1/20	0/20	0/20	0/20
Ovaries	Cyst - left side	0/20	0/20	1/20	0/20

Frequency of observations = number of animals with observations / number of animals examined.

Histopathological findings: no effects observed

Pups:

Histological investigation did not reveal test item related pathologic changes in the examined organs in F1 offspring.

Renal pyelectasia was observed in examined offspring – with macroscopic findings: 3/51 male and 3/70 female control; 6/70 male and 7/58 female at 100 mg/kg bw/day; 9/62 male and 5/74 female at 300 mg/kg bw/day; 2/28 male and 0/35 female at 1000 mg/kg bw/day. Pyelectasia without signs of inflammation or other pathological findings is considered as a species-specific alteration. There was no dose relevancy in the incidence therefore renal pyelectasia was judged to be toxicologically not relevant in this study.

Congestion in the stomach mucosa was detected in two offspring (1/58 female (red granulous spot) at 100 mg/kg bw/day and 1/62 male (hemorrhage) at 300 mg/kg bw/day) as individual alteration. This finding was not seen in offspring in the high dose group therefore test item effect was excluded.

Cohort 1A:

Histological examinations did not reveal pathologic alterations in the organs or tissues of F1 Cohort 1A male or female animals at 1000 mg/kg bw/day.

The investigated organs of reproductive system (testes, epididymides, prostate seminal vesicles, coagulating glands) were histologically normal and characteristic for the sexually mature organism in all F1 Cohort 1A male animals in the control and 1000 mg/kg bw/day groups.

The various spermatogenic cells (the spermatogonia, the spermatocytes, the spermatids and spermatozoa) representing different phases in the development and differentiation of the spermatozoons and the interstitial cells were the same in quantity and morphologically in the testes of investigated control and high dose treated animals. The histological picture of epididymides, prostate, seminal vesicles, and coagulating glands was normal in all cases as well.

In the F1 Cohort 1A female animals of the control and 1000 mg/kg bw/day groups, the ovaries, uterus, cervix, vagina had a normal structure characteristic of the species, age and phase of the active sexual cycle. The cortical region of ovaries contained primary, secondary and tertiary follicles and corpora lutea, indicating the active maturation of oocytes, and ovulation. The epithelial capsule and ovarian stroma were normal in all cases as well.

The histological structure and the cellularity of pituitary with special attention on the cytomorphology and proportion of acidophilic and basophilic cells in the adenohypophysis were the same in the control and treated male and female animals. However such an investigation is not sufficient to conclude in an absence of effect on the hypophysis function.

In some cases, dilatation of uterine horns was observed (5/20 control; 8/8 at 100 mg/kg bw/day; 3/3 at 300 g/kg bw/day; 3/20 at 1000 mg/kg bw/day). In one control female animal (1/20) adenoma was observed in the uterine horn as an individual disease.

One or both sided pyelectasia was seen in several F1 Cohort 1A male and female animals: 4/20 male and 3/20 female control; 4/20 male and 4/20 female at 100 mg/kg bw/day; 1/20 male and 2/20 female at 300 mg/kg bw/day; 8/20 male and 5/20 female at 1000 mg/kg bw/day. Pyelectasia without other histopathological lesions (for example degeneration, inflammation, fibrosis etc.) is considered as an individual disorder without toxicological significance.

Alveolar emphysema (minimal or moderate degree) in the lungs (2/20 male 1/20 female control; 1/20 male and 2/20 female at 1000 mg/kg bw/day) and acute hemorrhage in the lungs (1/20 male at 1000 mg/kg bw/day) and in the thymus (1/20 female at 1000 mg/kg bw/day) occurred sporadically and are considered as consequence of hypoxia, dyspnea and circulatory disturbance developed during exsanguinations. Hyperplasia of bronchus associated lymphoid tissue (BALT) in some control and treated animals (1/20 male

and 1/20 female control; 2/20 female at 1000 mg/kg bw/day) is an immuno-morphological phenomenon, without toxicological significance.

Erosion and congestion in the mucous membrane of stomach was observed in some male animal (1/20 control, 0/20 at 100 mg/kg bw/day, 1/20 at 300 mg/kg bw/day, 1/20 at 1000 mg/kg bw/day) presumably due to the gavage administration of the vehicle or test item.

The focal interstitial fibrosis in the Glisson's capsule of the liver in one control male animal (1/20) was in connection with the mechanical irritation due to diaphragmatic hernia. There was no morphological evidence of test item related acute or subacute injury (degeneration, inflammation, necrosis etc.) in the small and large intestines, liver, pancreas, cardiovascular system, respiratory system, immune system, hematopoietic system, skeleton, muscular system, central, or peripheral nervous system, eyes, integumentary system. The cytomorphology of endocrine glands were the same in the control and treated animals.

Table 69: summary of histopathological findings of F1 cohort 1A males

Organs	Observations	Incidence of observations per group			
		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Adrenal glands	No lesion	20/20	/	/	20/20
Aorta	No lesion	20/20	/	/	20/20
Bone marrow	No lesion	20/20	/	/	20/20
Brain	No lesion	20/20	/	/	20/20
Cecum	No lesion	20/20	/	/	20/20
Colon	No lesion	20/20	/	/	20/20
Duodenum	No lesion	20/20	/	/	20/20
Eyes + optic nerve	No lesion	20/20	/	/	20/20
Epididymides	**Storage of mature spermatozoa**	**20/20**	/	/	**20/20**
Esophagus	No lesion	20/20	/	/	20/20
Harderian glands	No lesion	20/20	/	/	20/20
Heart	No lesion	20/20	/	/	20/20
Ileum	No lesion	20/20	/	/	20/20
Jejunum	No lesion	20/20	/	/	20/20
Kidneys	**Pyelectasia**	**4/20**	**3/3**	**1/1**	**8/20**
Lachrymal glands	No lesion	20/20	/	/	20/20
Liver	**Interstitial fibrosis**	**1/20**	/	/	**0/20**
Lungs	**Alveolar emphysema**	**2/20**	/	/	**1/20**
	Acute hemorrhage	**0/20**	/	/	**1/20**
	Hyperplasia of BALT	**1/20**	/	/	**0/20**
Mesenteric lymph nodes	No lesion	20/20	/	/	20/20
Muscle (quadriceps)	No lesion	20/20	/	/	20/20
Pancreas	No lesion	20/20	/	/	20/20
Pituitary	No lesion	20/20	/	/	20/20
Prostate	**No lesion**	**20/20**	/	/	**20/20**
Rectum	No lesion	20/20	/	/	20/20
Salivary glands (subm.)	No lesion	20/20	/	/	20/20
Sciatic nerve	No lesion	20/20	/	/	20/20
Seminal vesicle ††	**No lesion**	**20/20**	/	/	**20/20**
Skin	No lesion	20/20	/	/	20/20
Spinal cord	No lesion	20/20	/	/	20/20
Spleen	No lesion	20/20	/	/	20/20
Sternum	No lesion	20/20	/	/	20/20
Stomach	**Congestion**	**1/20**	/	**1/1**	**1/20**
	Erosion	**1/20**	/	**1/1**	**1/20**
Subm. lymph nodes	No lesion	20/20	/	/	20/20
Thymus	No lesion	20/20	/	/	20/20
Thyroid + parathyroid	No lesion	20/20	/	/	20/20
Testes	**Active spermatogenesis**	**20/20**	/	/	**20/20**
Trachea	No lesion	20/20	/	/	20/20
Urinary bladder	No lesion	20/20	/	/	20/20

Remark: Frequency of observations: number of animals with observation/number of animals examined
† = Seminal vesicle with coagulating gland
subm. = Submandibular

Table 70: summary of histopathological findings of F1 cohort 1A females

Organs	Observations	Incidence of observations per group			
		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Adrenal glands	No lesion	20/20	/	/	20/20
Aorta	No lesion	20/20	/	/	20/20
Bone marrow	No lesion	20/20	/	/	20/20
Brain	No lesion	20/20	/	/	20/20
Cecum	No lesion	20/20	/	/	20/20
Colon	No lesion	20/20	/	/	20/20
Duodenum	No lesion	20/20	/	/	20/20
Eyes + optic nerve	No lesion	20/20	/	/	20/20
Esophagus	No lesion	20/20	/	/	20/20
Harderian glands	No lesion	20/20	/	/	20/20
Heart	No lesion	20/20	/	/	20/20
Ileum	No lesion	20/20	/	/	20/20
Jejunum	No lesion	20/20	/	/	20/20
Kidneys	**Pyelectasia**	**3/20**	/	**2/2**	**5/20**
Lachrymal glands	No lesion	20/20	/	/	20/20
Liver	No lesion	20/20	/	/	20/20
Lungs	**Alveolar emphysema**	**1/20**	/	/	**2/20**
	Hyperplasia of BALT	**1/20**	/	/	**2/20**
Mammary gland	No lesion	20/20	/	/	20/20
Mesenteric lymph nodes	No lesion	20/20	/	/	20/20
Muscle (quadriceps)	No lesion	20/20	/	/	20/20
Ovaries:	**Primordial, secondary and tertiary follicles**	**20/20**	**20/20**	**20/20**	**20/20**
	Corpora lutea	**20/20**	**20/20**	**20/20**	**20/20**
Pancreas	No lesion	20/20	/	/	20/20
Pituitary	No lesion	20/20	/	/	20/20
Rectum	No lesion	20/20	/	/	20/20
Salivary glands (subm)	No lesion	20/20	/	/	20/20
Sciatic nerve	No lesion	20/20	/	/	20/20
Skin	No lesion	20/20	/	/	20/20
Spinal cord	No lesion	20/20	/	/	20/20
Spleen	No lesion	20/20	/	/	20/20
Sternum	No lesion	20/20	/	/	20/20
Stomach	No lesion	20/20	/	/	20/20
Subm. lymph nodes	No lesion	20/20	/	/	20/20
Thymus	**Acute hemorrhage**	**0/20**	/	/	**1/20**
Thyroid + parathyroid:	No lesion	20/20	/	/	20/20
Trachea	No lesion	20/20	/	/	20/20
Urinary bladder	No lesion	20/20	/	/	20/20
Uterus	**Dilatation**	**5/20**	**8/8**	**3/3**	**3/20**
	Adenoma	**1/20**	**0/8**	**0/3**	**0/20**
Vagina	**No lesion**	20/20	/	/	20/20

Remark: Frequency of observations: number of animals with observation/number of animals examined
subm. = Submandibular
/ = No data

Sperm parameters:

Cohort 1A:

Sperm examinations did not point out any test item related influence on the sperm cells at 1000 mg/kg bw/day.

Statistical or biological significances were not detected at the mean percentage of motile sperm cells or mean percentage of immotile sperms in animals of 1000 mg/kg bw/day. The total sperm count and sperms with not normal morphology (separated head and tail) were similar in the 1000 mg/kg bw/day and in the control groups.

Table 71: summary of sperm examinations of F1 cohort 1A males

Group		Control	1000 mg/kg bw/day	
Number of animals examined	n	20	19	
Sperm count (x10⁶/g testis)	Mean	56.68	58.98	
	SD	7.44	5.58	
	n	20	20	NS
Total number of cells examined	N	10000	9500	
Number of cells/animal examined	Mean	500	500	
	SD	0	0	
	n	20	19	
Motile sperms (%)	Mean	67.9	69.7	
	SD	4.0	2.1	
	n	20	19	
Immotile sperms (%)	Mean	32.1	30.3	
	SD	4.0	2.1	
	n	20	19	NS
Sperms with normal morphology (%)	Mean	99.5	99.7	
	SD	0.3	0.2	
	n	20	19	
Sperms with seperated head and tail (%)	Mean	0.54	0.34	
	SD	0.27	0.18	
	n	20	19	**

Remarks: NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
T - test Versus Control

Ovary Follicle Count:

Quantitative examinations of ovaries did not reveal test item related changes in the number of developing follicles, corpora lutea or in the number of follicular atresia at the examined level of section of F1 Cohort 1A female animals at 1000 mg/kg bw/day.

The mean number of primordial and primary follicles were slightly higher than in the control group in F1 Cohort 1A female animals at 100, 300 and 1000 mg/kg bw/day. The mean number of secondary and tertiary follicles, corpora lutea and follicular atresia was similar in the control and at 100, 300 and 1000 mg/kg bw/day.

Table 72: Quantitative evaluation of ovaries of F1 cohort 1A females

Group		Primordial and primary follicles	Secondary and tertiary follicles	Corpora lutea	Follicular atresia	Cystic degeneration	Other findings
Control	Mean	34.1	13.3	18.0	5.6	0.0	0.0
	SD	2.6	2.5	6.7	1.0	0.0	0.0
	n	20	20	20	20	20	20
100 mg/kg bw/day	Mean	35.7	12.1	21.7	5.3	0.0	0.0
	SD	2.8	2.5	5.5	1.1	0.0	0.0
	n	20	20	20	20	20	20
		*					
300 mg/kg bw/day	Mean	36.6	13.3	21.4	5.1	0.0	0.0
	SD	2.3	2.3	5.9	1.0	0.0	0.0
	n	20	20	20	20	20	20
		**					
1000 mg/kg bw/day	Mean	36.2	14.1	19.2	5.1	0.0	0.0
	SD	2.6	2.5	4.6	1.2	0.0	0.0
	n	20	20	20	20	20	20
		*					
		DN	NS	NS	NS	-	-

Remark: Quantitative examinations were performed at the section level of ovaries
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = No data

Thyroid Hormone measurements:

F1 Pups (PND22):

Mean values of thyroid hormones (T3 and T4) from pooled samples per litter of thyroid hormones were not changed when compared to the control.

Cohort 1A (at necropsy, approx. PND90):

T3 and T4 levels were statistically significantly reduced in males animals of the high dose group (-22 and -25 %) when compared to the control. This finding is consistent with other cohorts (1B and P0 generation).

Table 73: Summary of thyroid hormone levels of F1 cohort 1A males

		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
FT3 [ng/dL]	Mean	0.35	0.32	0.33	0.28	
	SD	0.04	0.05	0.04	0.05	
	n	10	10	10	10	
	±%		-10	-5	-22 **	DN
FT4 [ng/dL]	Mean	3.54	3.87	4.14	2.64	
	SD	0.43	0.70	0.58	0.32	
	n	10	10	10	10	
	±%		9	17 *	-25 **	DN
TSH [µIU/mL]	Mean	-	-	-	0.006	
	SD	-	-	-	-	
	n	-	-	-	1	
	±%					NS

±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = No data (Values were below the limit of detection - 0.005 µIU/mL)

Table 74: Summary of thyroid hormone levels of F1 cohort 1A females

		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	
FT3	Mean	0.35	0.37	0.36	0.36	
[ng/dL]	SD	0.05	0.05	0.06	0.72	
	n	10	10	10	10	
	±%		6	6	6	NS
FT4	Mean	2.75	3.09	3.42	3.43	
[ng/dL]	SD	0.75	0.50	0.78	0.72	
	n	10	10	10	10	
	±%		12	12	12	NS
TSH	Mean	-	-	-	-	
[µIU/mL]	SD	-	-	-	-	
	n	-	-	-	-	

±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = No data (Values were below the limit of detection - 0.005 µIU/mL)

The was no histopathological findings in corresponding organs of the hypothalamus-pituitary-thyroid- axis.

A determination of spleenic subpopulation analysis was considered not required.

Developmental neurotoxicity (F1)

Behaviour (functional findings): no effects observed

Developmental immunotoxicity (F1)

Developmental immunotoxicity: no effects observed

Results: F2 generation

General toxicity (F2)

Clinical signs:

The percentage of offspring showing signs seems to be higher at 1000 mg/kg bw/d (no milk in the stomach, cold).

The percentage of dead F2 offspring was higher (+12%) than in the control group at 1000 mg/kg bw/day.

Some other clinical signs were detected in single animals as follows: damaged tail in the control and 1000 mg/kg bw/day; darker than normal eye at 300 mg/kg bw/day.

Table 75: summary of clinical observations of F2 offspring

		Control	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
No. of offspring examined	N	197	225	193	113
No signs	Sum	155	210	171	68
	%	79	93	89	60
No milch in the stomach	Sum	9	6	5	12
	%	5	3	3	11
Cold	Sum	33	8	15	30
	%	17	4	8	27
Pale	Sum	2	1	0	1
	%	1	0	0	1
Found dead	Sum	1	0	1	14
	%	1	0	1	12
Missing (Cannibalized)	Sum	4	5	2	4
	%	2	2	1	4
Tail: Damaged	Sum	1	0	0	1
	%	1	0	0	1
Eye: Darker than normal	Sum	0	0	1	0
	%	0	0	1	0
Skin: Wounds - by bite - on the back	Sum	0	1	0	0
	%	0	0	0	0

#% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
G = Gestation day
CHI2 = Chi^2 test

Mortality / Sex Distribution / Survival of Offspring :

The extra uterine mortality of F2 offspring was higher than in the control group at 1000 mg/kg bw/day on post-natal day 0 and between postnatal days 0 and 4.

The extra uterine mortality was low and comparable in the control, 100, and 300 mg/kg bw/day from birth to post-natal day 4.

Statistical significance with respect to the control was noted for the higher mean number of dead pups on postnatal day 0 and between postnatal days 0 and 4 at 1000 mg/kg bw/day.

Table 76: summary of extrauterine mortality of F2 offspring

Values			Control	100 mg/kg bw/day		300 mg/kg bw/day	1000 mg/kg bw/day
Number of litters		N	18	20		16	10
Number of liveborns	Total	N	197	225		192	113
	Male	N	110	97		93	55
		%	56	43		48	49
	Female	N	87	128		99	58
		%	44	57		52	51
Number of viable pups on lactation day 0	Total	N	196	225		192	99
		%	99	100		100	88
	Male	N	109	97	*	93	47
		%	56	43		48	47
	Female	N	87	128	*	99	52
		%	44	57		52	53
on lactation day 4 Survival index:	Total	N	192	220		189	96
		%	97	98		98	85
	Male	N	107	95	*	92	46
		%	56	43		49	48
	Female	N	85	125	*	97	50
		%	44	57		51	52
Number of dead pups on lactation day 0	Total	N	0	0		0	14
		%	0	0		0	12
	Male	N	0	0		0	8
		%	0	0		0	15
	Female	N	0	0		0	6
		%	0	0		0	10
between lactation days 0-4	Total	N	5	5		3	17
		%	3	2		2	15
	Male	N	3	2		1	9
		%	3	2		1	16
	Female	N	2	3		2	8
		%	2	2		2	14

NS = Not Significant
* = $p < 0.05$ CHI2
** = $p < 0.01$ CHI2

Litter data

Values			Control	100 mg/kg bw/day		300 mg/kg bw/day	1000 mg/kg bw/day	
Number of litters		N	18	20		16	10	
Number of liveborns	Total	Mean	10.9	11.3		12.0	11.3	
		SD	3.8	2.7		2.4	1.5	
		n	18	20		16	10	NS
	Male	Mean	6.1	4.9		5.8	5.5	
		SD	2.6	2.2		1.3	1.8	
		n	18	20		16	10	
	Female	Mean	4.8	6.4		6.2	5.8	
		SD	2.6	2.2		2.6	0.9	
		n	18	20		16	10	
Number of viable pups								
On lactation day 0	Total	Mean	11.5	11.3		12.0	9.9	
		SD	2.9	2.7		2.4	2.6	
		n	17	20		16	10	NS
	Male	Mean	6.4	4.9		5.8	4.7	
		SD	2.3	2.2		1.3	2.2	
		n	17	20	*	16	10	DN
	Female	Mean	5.1	6.4		6.2	5.2	
		SD	2.3	2.2		2.6	1.5	
		n	17	20		16	10	NS
On lactation day 4	Total	Mean	11.3	11.0		11.8	9.6	
		SD	2.7	2.6		2.4	2.5	
		n	17	20		16	10	
	Male	Mean	6.3	4.8		5.8	4.6	
		SD	2.3	2.2		1.3	2.2	
		n	17	20		16	10	
	Female	Mean	5.0	6.3		6.1	5.0	
		SD	2.3	2.3		2.6	1.6	
		n	17	20		16	10	
Number of dead pups								
On lactation day 0	Total	Mean	0.0	0.0		0.0	1.4	
		SD	0.0	0.0		0.0	1.6	
		n	17	20		16	10	** DN
	Male	Mean	0.0	0.0		0.0	0.8	
		SD	0.0	0.0		0.0	0.8	
		n	17	20		16	10	
	Female	Mean	0.0	0.0		0.0	0.6	
		SD	0.0	0.0		0.0	1.3	
		n	17	20		16	10	
Between lactation days 0-4	Total	Mean	0.3	0.3		0.2	1.7	
		SD	0.6	0.4		0.4	1.6	
		n	18	20		16	10	** U
	Male	Mean	0.2	0.1		0.1	0.9	
		SD	0.4	0.3		0.3	0.7	
		n	18	20		16	10	
	Female	Mean	0.1	0.2		0.1	0.8	
		SD	0.3	0.4		0.3	1.3	
		n	17	20		16	10	

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

The sex distribution of F2 offspring were not affected by the test item on post-natal days 0 and 4. The survival index was slightly lower at 1000 mg/kg bw/day with respect to the control at 1000 mg/kg bw/day on PND4.
The mean number of live births per litter, and mean number of viable pups per litter were comparable in the control and 100, 300 and 1000 mg/kg bw/day groups on post-natal days 0 and 4.

The sex distribution (mean percentage of male and female pups per litter) was comparable in the control and 300 and 1000 mg/kg bw/day groups on post-natal days 0 and 4. Statistical significance with respect to the control was detected at the lower mean percentage of male pups and higher mean percentage of female pups at 100 mg/kg bw/day on post-natal days 0 and 4. Similar findings was not observed at the higher dose groups, therefore this difference was considered to be indicative of biological variation and not related to the treatment.

Body weight and weight changes:

The body weight development of the F2 offspring was slightly reduced at 1000 mg/kg bw/day. Statistical significance with respect to the control was detected at the higher mean body weight of pups (male and female) at 100 mg/kg bw/day on post-natal day 0 and at the lower mean body weight of pups at 300 and 1000 mg/kg bw/day on post-natal days 0 and 4.

The mean pup weight gain was slightly lower than in the control between PND0 and PND4 at 1000 mg/kg bw/day. The mean litter weight and litter weight gain were similar in the control and in 100 and 300 mg/kg bw/day groups between PND0 and PND4.

The mean litter weight on PND4 and the mean litter weight gain between PND0 and PND4 were slightly lower than in the control at 1000 mg/kg bw/day.

Table 77: Summary of body weight and body weight gain of F2 offsprings

Group		Body weight (g) on post-natal day 0	Body weight (g) on post-natal day 4	Body weight (g) gain between post-natal days 0-4
Control	Mean	6.0	9.9	3.9
	SD	0.5	1.1	0.9
	n	196	192	192
100 mg/kg bw/day	Mean	6.1	9.9	3.9
	SD	0.5	1.2	0.9
	n	225	220	220
		*		
300 mg/kg bw/day	Mean	5.8	9.6	3.7
	SD	0.5	1.2	1.0
	n	192	189	189
		**	**	
1000 mg/kg bw/day	Mean	5.7	9.1	3.4
	SD	0.5	1.2	1.0
	n	99	96	96
		**	**	**
		DN	DN	DN

Remarks:
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
n = Number of offsprings

Table 78: Summary of litter weight and body weight gain of F2 offsprings

Group		Litter weight (g) on post-natal day 0	4	Body weight (g) gain between post-natal days 0-4
Control	Mean	68.7	111.5	44.1
	SD	16.3	23.5	10.0
	N	17	17	17
100 mg/kg bw/day	Mean	68.1	109.4	42.7
	SD	16.6	24.1	9.8
	N	20	20	20
300 mg/kg bw/day	Mean	69.5	112.8	44.3
	SD	12.3	19.3	9.2
	N	16	16	16
1000 mg/kg bw/day	Mean	56.2	87.7	33.0
	SD	14.1	18.7	6.9
	N	10	10	10
			*	**
		NS	DN	DN

Remarks:
* = p < 0.05
** = p < 0.01
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test
N = Number of litters

F2 offspring's development

The survival index was slightly lower at 1000 mg/kg bw/day with respect to the control.

The mean number of live births per litter, and mean number of viable pups per litter were comparable in the control and 100, 300 and 1000 mg/kg bw/day groups on post-natal days 0 and 4.

The sex distribution (mean percentage of male and female pups per litter) was comparable in the control and 300 and 1000 mg/kg bw/day groups on post-natal days 0 and 4. Statistical significance with respect to the control was detected at the lower mean percentage of male pups and higher mean percentage of female pups at 100 mg/kg bw/day on post-natal days 0 and 4. Similar findings was not observed at the higher dose groups, therefore this difference was considered to be indicative of biological variation and not related to the treatment

The F2 offspring's development (surface righting reflex, pinna detachment, anogenital distance) was undisturbed at 100, 300 and 1000 mg/kg bw/day.

There were no toxicologically relevant differences in the offspring's development between the control and 100, 300 or 1000 mg/kg bw/day groups.

Statistical significance with respect to the control was detected for the lower percentage of pups with positive response and higher percentage of pups with negative response in pinna detachment at 300 mg/kg bw/day. Similar finding was not detected at the higher dose, therefore it was considered as indicative of biological variation and not related to the test item.

The absolute anogenital distance was slightly shorter than in the control group in male pups at 1000 mg/kg bw/day but the normalized anogenital distance was similar in the control and 1000 mg/kg bw/day male pups.

Statistical significance was detected at the slightly longer normalized anogenital distance of male pups at 100 and 300 mg/kg bw/day. This minor difference with respect to the control was judged to be toxicologically not relevant.

Nipple retention in male pups: not examined

Table 80: summary of F2 offspring's development

Parameters		GROUPS (mg/kg bw/day) Control	100	300	1000	
Surface righting reflex on post-natal day 0						
- No. of offspring examined	N	196	225	192	99	
- No. of offspring with positive response	Sum	118	139	101	56	
	%	60	62	53	57	NS
- No. of offspring with negative response	Sum	78	86	91	43	
	%	40	38	47	43	NS
Pinna detachment on post-natal day 2						
- No. of offspring examined	N	193	221	190	97	
- No. of offspring with positive response	Sum	63	78	26	34	
	%	33	35	14 **	35	CHI2
- No. of offspring with negative response	Sum	130	143	164	63	
	%	67	65	86 **	65	CHI2
Absolute anogenital distance (mm) on post-natal day 4						
Male	Mean	5.9	6.0	6.0	5.7	DN
	SD	0.5	0.5	0.5	0.5	
	n	107	95	92	46 *	
Female	Mean	3.6	3.7	3.7	3.5	NS
	SD	0.5	0.5	0.5	0.5	
	n	85	125	97	50	
Normalized anogenital distance (mm) on post-natal day 4						
Male	Mean	2.7	2.8 *	2.8 *	2.7	DN
	SD	0.2	0.2	0.2	0.2	
	n	107	95	92	46	
Female	Mean	1.7	1.7	1.8	1.7	NS
	SD	0.2	0.2	0.2	0.2	
	n	85	125	97	50	

Remarks
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
G = Gestation day
CHI2 = Chi^2 test

Organ weight findings including organ / body weight ratios:

There were no test item related changes in the weights of examined organs (absolute and relative to body and brain weights) in male or female F2 offspring at 100, 300 and 1000 mg/kg bw/day.

The weights of examined organs (brain, spleen, thymus; absolute and relative to body and brain weights) were comparable in selected male and female F2 offspring.

Necropsy findings:

Specific macroscopic alterations were not found in F2 offspring subjected to gross pathological examination after PND4.

In dead F2 offspring, undernourishment, underdevelopment, empty stomach were detected at 300 mg/kg bw/day (1% each). At 1000 mg/kg bw/day, empty stomach (9/14 or 64%), autolysis (2/14=14%) yellow foamy intestinal content (1/14=7%) or gas filled intestines (1/14=7%) were observed.

In surviving offspring, there were no macroscopic findings in the control (192/192) and 300 mg/kg bw/day (191/191) at the necropsy. Undernourishment, underdevelopment and empty stomach (1/220) and wounds on the back by bite (1/220) were observed at 100 mg/kg bw/day. At 1000 mg/kg bw/day, undernourishment, underdevelopment (2/95) and damaged tail (1/95) were detected. These findings were considered to be toxicologically not relevant as these occurred with low incidence and were not related to doses.

Table 81:summary of necropsy findings of F2 offsprings

Observations		Control		100 mg/kg bw/day	300 mg/kg bw/day		1000 mg/kg bw/day	
		Survivors	Dead		Survivors	Dead	Survivors	Dead
No. of offspring examined	N	192	1	220	191	1	95	14
No macroscopic findings	Sum	192	1	218	190	1	92	3
	%	100	100	99	99	100	97	21
Underdeveloped, undernourished	Sum	0	0	1	1	1	2	0
	%	0	0	0	1	100	2	0
Skin: Wounds on the back	Sum	0	0	1	0	0	0	0
	%	0	0	0	0	0	0	0
No milk in the stomach	Sum	0	0	0	1	1	0	9
	%	0	0	0	1	100	0	64
Autolysis	Sum	0	0	0	0	0	0	2
	%	0	0	0	0	0	0	14
Tail: Damaged	Sum	0	0	0	0	0	1	0
	%	0	0	0	0	0	1	0
Intestines: Yellow foamy content	Sum	0	0	0	0	0	0	1
	%	0	0	0	0	0	0	7
Stomach, intestines: Gas filled	Sum	0	0	0	0	0	0	1
	%	0	0	0	0	0	0	7

Histopathological findings: not examined

Other effects:

Developmental neurotoxicity (F2)

Behaviour (functional findings): not examined

Developmental immunotoxicity (F2)

Developmental immunotoxicity: not examined

1.2.2 Developmental toxicity studies

1.2.2.1 [Anonymous, 2013]

Study reference:

Anonymous, 2013

Detailed study summary and results:

Test type

Prenatal Developmental Toxicity Study (2013)

OECD TG 414

GLP compliant

Test substance

- *Tert*.-Butylperoxy- 2-ethylhexanoate (TBPEH)
- CAS 3006-82-4

- EC 221-110-7
- Manufacturer : United Initiators GmbH and Co KG
- State: Liquid
- Purity : confidential (see annex II)
- Batch number : 247412384
- Stability : stable at storage conditions for at least 3 months
- Storage conditions: < 10°C
- Expiry date : 24.12.2012

Vehicle : Helianthy Annui Oleum Raffinatum / Sunflower oil

Test animals

- Pregnant female Hsd. Brl. Han: WISTAR rats , SPF
- Source : Toxi-Coop Zrt. 1103 Budapest, Cserkesz u. 90. Hungary
- 45 males, 150 feamles to achieve at least 24 sperm-positive females/dose group
- *Age and weight at study initiation :*

Age at study initiation: Females: Young adult and nulliparous females, 10-11 weeks of age at start of the mating period. Males: experienced males 35-37 weeks of age at start of the mating period

- *Acclimatation time :* 20 days for females
- *Animal health :* only healthy animals were used for the test. The breeder certified the healthy status.
- *Cage type :* Type II polypropylene/polycarbonate
- *Light :* 12 hours daily, from 6:00 to 6 pm
- *Temperature :* 21-22°C
- *Relative humidity :* 36 -46%
- *Ventilation :* 8-12 air exchanges by central air-condition system.
- *Food and water supply :* ssniff SM R/M-Z+H (Autoclave complete feed for rats and mice (breeding and maintenance" produced by ssniff Spezialdiaten GmbH, D-59494, Gaermany, *ad libitum* ; and tap water from municipal supply, as for human consumption from 500 mL bottle and *ad libitum*. The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. An analytical certificate of the contents of the standard diet for the batch used was provided. The drinking water was periodically analysed and was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

Administration/exposure

- *Gavage* – oral (gavage)
- *Duration of Exposure :* GD 5 to GD 19; Once daily from GD 6 to GD 19, examination on GD 20

- *Doses tested:* 200, 400 and 1000 TBPEH mg/kg bw/day.
- *rationale for dose level selection :* The doses have been chosen by the Sponsor on the basis of a previous study (GLP OECD 421 Reproduction/Developmental Toxicity Screening Study of *Tert*.-Butylperoxy- 2-ethylhexanoate (TBPEH) (CAS 3006-82-4) in the Wistar Rat).
- *rationale for animal assignment:* The sperm positive females were allocated to each experimental group on each mating day in such a way that the group averages of the body weight were as similar as possible on the first day of gestation. If possible, females paired with the same male were allocated to different groups on the same mating day
- *control group and treatment :*

  A constant treatment volume of 2 mL dose preparation/kg body weight was administered in all groups. The individual volume of the treatment was based on the most recent individual body weight of the animals (which was determined at least every 3 days).

  The test item was administered in a single dose by oral gavage (stomach tube) on a 7 days/week basis every day at similar time.

  Control group : yes, concurrent vehicle for 24 sperm positive females

Group No.	Dose (mg/kg bw/day)	Concentration (mg/mL)	Number of Sperm Positive Females
1	0	0	24
2	200	100	24
3	400	200	24
4	1000	500	24

- *historical control data if available:* Historical contral data are available and were used for evaluation of study results.
- *vehicle* vehicle: sunflower oil,
- *justification of choice of vehicle* The test item is not soluble and not stable in water therefore sunflower was used for preparing formulations appropriate for oral administration. Oleum helianthy /sunflower oil is a suitable vehicle to facilitate formulation analysis for the test item, Concentration in vehicle: 100, 200, 500 mg/mL; Amount of vehicle: 2 mL/kg bw

Description of test design:

- *details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy)*

  - Impregnation procedure: cohoused
  - If cohoused:
  - Mating : M/F ratio per cage: 1/3

  - Length of cohabitation: the females were paired to males in the mornings for two to four hours until the number of sperm positive females per goup achieved at least 24.

  - Further matings after two unsuccessful attempts: no
  - Verification of same strain and source of both sexes: yes
  - Proof of pregnancy: vaginal plug and/or sperm in vaginal smear referred to as day 0 of pregnancy

  Vaginal smears were prepared from each emale, stained with 1% aqueous methylene blue solution and examined for presence of sperm and for estrus cycle. The day of mating was regarding as day 0 of pregnancy (vaginal pluf) and /or sperm in the vaginal smear). Sperm positive females were separated and caged in groups of 2 to 3 animals; caging of the females individually was avoided if

possible.

Randomization : the sperm positive females were allocated to each experimental group on each mating day in such a way that the group averages of the body weight were as similar as possible on the first day of gestation; il possible, females paired with the same male were allocated to different groups on the same mating day.

Observations

Maternal examinations:

Mortality checked

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: General clinical observations were made once a day, after treatment at approximately the same time considering the peak period of anticipated effects after dosing. When signs of toxicity were observed, animals were observed more frequently. Individual observation included the check of behavior and general condition. Duration and severity of the clinical signs were recorded.

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: Observations for signs of morbidity and mortality were made twice daily, at the beginning of the working period and in the afternoon.

BODY WEIGHT: Yes

- Body weight of positive females was measured on gestation days 0, 3, 5, 8, 11, 14, 17 and 20 (accuracy of 1 g). The corrected body weight was calculated for the 20^{th} day of pregnancy (body weight on day 20 minus the weight of the gravid uterus).

FOOD CONSUMPTION: Yes
- Time schedule for examinations: Food consumption was measured between gestation days 0 to 3, 3 to 5, 5 to 8, 8 to 11, 11 to 14, 14 to 17 and 17 to 20 by re-weighing the non-consumed diet (accuracy: 1g)

POST-MORTEM EXAMINATIONS: Yes
- Sacrifice on gestation day 20 by caesarian section
- Organs examined: the uterus with cervix and the left ovary were removed and weighed. The right ovary was placed into a Petri dish after removal. After removing the uterus gross pathology of dams' viscera was performed. The number of corpora lutea in each ovary and implantation sites in each uterine horn, live fetuses, early and late embryonic death and fetal death were counted. Animals, in which unambiguous implantation sites, but not fetuses have been found, were considered as pregnant.

EXAMINATION OF PLACENTAL SIGNS:
The placentas were weighted and examined externally. All sperm positive animals were examined for vaginal bleeding (placental sign of gestation) on the 13th gestational day. If negative on day 13, the examination was repeated on day 14 of gestation.

Ovaries and uterine content:

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea in each ovary: Yes
- Number of implantations sites in each horn: Yes (animals, in which unambiguous implantation sites, but not fetuses have been found, were considered as pregnant)

- Number of live fetuses : Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes

- Number of dead fetuses : Yes

Fetal examinations:

Fetuses were removed from the opened uterus. Euthanasia of the fetuses was performed by hypothermia**.** The fetuses were sunk in a Petri-dish filled up with water. Spontaneous movement of fetuses was observed as a viability assessment. The fetuses were washed with tap water and randomly laid on a filter paper. Bleeding from the umbilical cord after it was cut was observed also as a sign of viability. Each live fetus and its placenta was weighed individually (fetuses accuracy 0.01 g, placentas accuracy 0.001g), and subjected to external examination. The gender of the fetuses was determined according to the anogenital distance.

-Weight of fetuses: Yes

- External examinations: Yes: all per litter
- Soft tissue examinations: Yes: half per litter
- Skeletal examinations: Yes: half per litter. Fixation in isopropanol then cartilage-bone staining by KOH-Alizarin red-S method, and the skeletons were examined by means of a dissecting microscope. All abnormalities found during he fetal examinations were recorded.
- Head examinations: Yes: half per litter, by Wilson's free-hand razor blade method

-Visceral examination : Yes: half per litter, bodies micro dissected by means of a dissecting microscope. The abdominal region of those subjected to skeletal examination was opened, the viscera and skin of fetuses wrer removed and the cadavers were fixed in alcian-blue-acetic-ethanol mixture.

Statistical evaluation:

Data were individually recorded on data sheets, transferred, and complied by computer or compiled manually.

The statistical evaluation of data was performed with the program package SPSS PC+4.0.

The homogeneity of variance between groups was checked by Bartlett's homogeneity of variance test. Where no significant heterogeneity was detected a one-way analysis of variance (ANOVA) was carried out. If the obtained result was significant Duncan's Multiple Range test was used to access the significance of inter-group differences. If significance was the result of the Bartlett's test, the Kruskal-Wallis analysis of variance was used and the inter-group comparisons were performed using Mann-Whitney U-test.

Dams or litters were excluded from the data evaluation in cases of:

- Any disease or death of the dam unrelated to the treatment (total exclusion)

- Non pregnant females or dams with 3 or less implantations independent of their viability (total exclusion)

Although these animals were excluded from the data evaluation the final report contains all data of these animals, too.

A male/female fetus was considered as retarded in body weight, when its weight is below the average minus twofold standard deviation of the control male/female fetuses.

Results :

In total, there were 21, 19, 23 and 19 evaluated litters using the control, 200, 400 and 1000 mg/kg groups, respectively.

Results : maternal animals

General toxicity (maternal animals)

Clinical signs:

Description (incidence and severity):

Alopecia was found sporadically without a dose response in the females. Salivation was recorded in association with the treatment in nine of 23 females in the 400 mg/kg bw/day group and in all of the dams of the 1000 mg/kg bw/day dose group, directly after treatment. This was attributed to be an effect of the treatment, however as non-adverse.

Summary of clinical signs and necropsy findings of dams

(sum, %)

DESCRIPTION	DOSES: No. of animals	control 21	200 mg/kg bw/day 19	400 mg/kg bw/day 23	1000 mg/kg bw/day 19
CLINICAL SYMPTOMS					
- none	N	21	17	14	0
	%	100	89	61	0
- alopecia	N	0	2	0	1
	%	0	11	0	5
- salivation	N	0	0	9	19
	%	0	0	39	100
NECROPSY FINDINGS					
- no macroscopic alterations	N	21	19	23	19
	%	100	100	100	100

Mortality:

One pregnant female in the control group died in the course of the study on gestational day 20. The death was considered to be due to the intrauterine autolyzing of dead embryos and fetuses. This dam had no clinical signs before death but lost weight.

Dose groups	Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
No. of sperm positive females	24	24	24	24
Died or moribund	1	0	0	0
No. of pregnant females with live fetuses	21 88%	19 79%	23 96 %	19 79 %
No. of females with no implantation but corpora lutea (100% preimplantation loss)	0	0	0	1
No. of females with no implantation and no corpora lutea	2	5	1	4
No. of dams with total intrauterine death	1	0	0	0
No. of pregnant females with viable fetuses but with 3 or less than 3 implantations	0	0	0	0
No. of evaluated dams and litters	21	19	23	19
No. of evaluated dams with malformed fetuses	1 5 %	0 0 %	0 0%	2 11 %

Body weight and weight changes:

There was no indication of an effect of the test item on body weight development of the dams in the 200 and 400 mg/kg bw/day dose groups. The body weight gain was statistically significant ($p<0.01$) reduced on the first three days of treatment in the 1000 mg/kg bw/day group. which was in the range of historical control data, might be a consequence of the statistically significant reduction of the food consumption between gestation day 5 and 11 and lower mean body weight gain of the dams between gestation day 5 and 8. Between gestational days 8 and 11 it turned to an increased body weight gain with a statistical significance ($p<0.05$).

There were no dose related differences in the corrected body weight and corrected body weight gain of the dams in the experimental groups.

Placental weight was similar in all experimental groups. There was a statistically significant increase indicated in the relative placental weight in the 1000 mg/kg bw/day dose group, however it was below the historical control level, according to registrants.

Summary of body weight and body weight gain of dams

(mean, SD)

Body weight (g)

TIME Gestational days		DOSE GROUPS Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
0	MEAN	200.6	202.4	199.6	201.8
	SD	14.62	18.05	16.23	14.93
	n	21	19	23	19
3	MEAN	212.4	214.8	213.4	215.1
	SD	15.04	18.21	16.46	15.66
	n	21	19	23	19
5	MEAN	219.0	221.9	221.0	221.3
	SD	15.01	17.44	17.61	15.19
	n	21	19	23	19
8	MEAN	225.9	230.1	228.9	223.5
	SD	17.01	17.24	18.47	14.85
	n	21	19	23	19
11	MEAN	237.8	243.3	242.5	238.4
	SD	15.93	18.43	18.86	15.24
	n	21	19	23	19
14	MEAN	251.0	255.4	255.7	252.9
	SD	16.36	19.41	18.99	15.07
	n	21	19	23	19
17	MEAN	274.0	276.9	279.2	275.6
	SD	16.17	23.19	20.13	16.66
	n	21	19	23	19
20	MEAN	308.4	309.2	312.1	308.6
	SD	19.89	27.85	22.50	23.18
	n	21	19	23	19

Remarks: * = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

(mean, SD)

Body weight gain (g)

TIME Gestational days		DOSE GROUPS Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day		
0-3	MEAN	11.8	12.4	13.8	13.3		
	SD	3.48	3.82	3.30	2.64		
	n	21	19	23	19		
3-5	MEAN	6.7	7.2	7.6	6.2		
	SD	1.46	2.39	3.09	2.72		
	n	21	19	23	19		
5-8	MEAN	6.8	8.1	7.9	2.2		
	SD	4.25	2.75	3.12	3.19		
	n	21	19	23	19	**	DN
8-11	MEAN	11.9	13.3	13.6	14.8		
	SD	3.13	3.25	3.45	4.18		
	n	21	19	23	19	*	DN
11-14	MEAN	13.2	12.1	13.2	14.6		
	SD	3.24	3.52	3.32	3.93		
	n	21	19	23	19		
14-17	MEAN	23.0	21.5	23.5	22.7		
	SD	6.90	6.15	4.82	4.41		
	n	21	19	23	19		
17-20	MEAN	34.4	32.3	32.9	33.0		
	SD	8.43	5.56	6.05	10.21		
	n	21	19	23	19		
0-20	MEAN	107.8	106.8	112.5	106.8		
	SD	14.48	15.06	14.66	15.37		
	n	21	19	23	19		

Remarks: * = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Food consumption and compound intake (if feeding study):

There was no indication of an effect of the test item on the food consumption of the dams in the 200 and 400 mg/kg bw/day dose groups. There was a statistically significantly ($p<0.01$) reduced food consumption on the first six days of treatment in the 1000 mg/kg bw/day dose group related to the treatment with the test item. Statistical significant increases ($p<0.05$) were indicated in two occasions (once before the treatment period and once at the beginning of it) in the food consumption of the animals in the 200 mg/kg bw/day dose group which are not associated with the test item.

Summary of food consumption data of dams

(mean, SD)

TIME Gestational days		Control	DOSE GROUPS 200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day	
0-3	MEAN	18.3	18.9	18.7	18.7	
	SD	1.24	1.86	1.73	2.02	
	n	21	19	23	19	
3-5	MEAN	20.6	21.9	21.1	21.5	
	SD	1.65	1.27	1.39	1.89	
	n	21	19 *	23	19	DN
5-8	MEAN	19.4	20.7	20.0	15.1	
	SD	1.53	1.48	1.75	1.64	
	n	21	19 *	23	19 **	DN
8-11	MEAN	20.7	21.2	21.3	17.9	
	SD	1.62	1.33	1.72	1.34	
	n	21	19	23	19 **	DN
11-14	MEAN	21.8	22.2	22.3	21.0	
	SD	1.76	1.57	1.40	1.92	
	n	21	19	23	19	
14-17	MEAN	22.2	22.8	23.6	22.7	
	SD	2.34	1.92	1.59	2.82	
	n	21	19	23	19	
17-20	MEAN	22.6	22.3	22.8	22.0	
	SD	3.26	2.25	2.48	2.19	
	n	21	19	23	19	

Remarks: n = number of dams
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Gross pathological findings: There were no macroscopic alterations recorded for the dams during necropsy.

Results : fetuses

Fetal body weight changes:

The mean fetal weight was similar in the control, 200 and 400 mg/kg bw/day groups. There was a slight but statistically significant ($p<0.01$) reduction in the mean body weight of the male and female fetuses in the 1000 mg/kg bw/day group. Although a statistical significance in the fetal weight in the 1000 mg/kg bw/day group was noted, the value was in the range of the historical control data (no range provided), and therefore considered to be non-adverse, according to the registrants.

Placental weight was similar in all experimental groups. There was a statistically significant increase indicated in the relative placental weight in the 1000 mg/kg bw/day dose group ($p<0.05$), however it was below the historical control level.

Litter means of fetal, and placental weight

(mean, SD)

		DOSE GROUPS Control			200 mg/kg bw/day			400 mg/kg bw/day			1000 mg/kg bw/day		
		M+F	M	F	M+F	M	F	M+F	M	F	M+F	M	F
Fetal weight (g)	MEAN	3.5	3.6	3.4	3.6	3.7	3.4	3.5	3.7	3.4	3.3	3.4	3.2
	SD	0.22	0.22	0.26	0.18	0.20	0.23	0.23	0.18	0.37	0.18	0.24	0.18
	n	21	20	21	19	19	19	23	23	22	19	19	19
											**	**	**
											DN	DN	U
Placental weight (g)	MEAN	654.9	671.4	640.1	667.0	662.0	667.7	664.1	656.9	665.2	665.8	667.0	656.1
	SD	75.02	78.65	76.08	67.08	61.66	80.66	62.35	60.58	64.96	59.88	69.07	62.16
	n	21	20	21	19	19	19	23	23	22	19	19	19
Relative placental weight (mg/g)	MEAN	186.9	185.5	187.6	188.6	179.7	195.3	190.0	180.5	197.1	204.3	198.9	206.4
	SD	17.63	19.13	20.60	21.39	18.19	28.02	22.87	16.17	34.65	17.88	21.22	17.35
	n	21	20	21	19	19	19	23	23	22	19	19	19
											*	*	*
											DN	DN	U

Remarks: * = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
M = Male
F = Female

Intrauterine mortality, viable fetuses and their sex-distributiin

There was no dose related significant difference in the intrauterine mortality of the conceptuses, the number of implantations, viable fetuses and their sex distribution. The number of late embryonic death increased slightly but statistically significantly ($p<0.05$) in the 400 mg/kg bw/day dose group and without a statistical significance in the 1000 mg/kg bw/day group. No dose response was indicated and the values are in the range of the historical control data (no range provided), according to the registrants. There was no statistical significance indicated in the mean percentage value of the late embryonic death in the experimental groups.

Summary of intrauterine mortality, viable fetuses and their distribution

(mean, SD)

GROUPS:		Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
NUMBER OF DAMS:		21	19	23	19
Corpora Lutea	Mean:	13.1	12.8	13.4	12.8
	SD:	1.37	1.75	1.34	1.72
Preimplantation Loss %	Mean:	10.3	14.1	11.0	12.6
	SD:	16.76	14.01	9.46	13.12
Implantation	Mean:	11.7	11.0	11.8	11.2
	SD:	2.43	2.47	1.03	2.06
Early Embryonic Death %	Mean:	7.8	8.7	9.4	6.4
	SD:	11.58	7.96	9.76	7.41
Late Embryonic Death %	Mean:	0.4	0.5	2.7	2.4
	SD:	1.68	2.29	5.73	4.23
Dead Fetuses %	Mean:	0.0	0.0	0.4	0.4
	SD:	0.00	0.00	1.90	1.91
Postimplantation Loss %	Mean:	8.2	9.2	12.4	9.2
	SD:	11.70	7.67	12.68	8.04
Total Intrauterine Mortality %	Mean:	16.9	21.9	22.0	20.7
	SD:	19.82	15.20	13.82	13.47
Viable fetuses	Mean:	10.9	10.0	10.4	10.1
	SD:	2.73	2.47	1.95	1.97
Male fetuses %	Mean:	41.8	50.8	49.8	50.9
	SD:	15.52	13.93	21.41	11.51
Female fetuses %	Mean:	58.2	49.2	50.2	49.1
	SD:	15.52	13.93	21.41	11.51

Remarks: * = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

(sum, %)

GROUPS:		Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
NUMBER OF DAMS:		21	19	23	19
Corpora Lutea	Sum:	275	243	308	243
Preimplantation Loss	Sum:	29	34	36	31
(Data compared to no. of corpora lutea)	%:	11	14	12	13
Implantation	Sum:	246	209	272	212
Early Embryonic Death	Sum:	17	18	25	14
(Data compared to no. of implantations)	%:	7	9	9	7
Late Embryonic Death	Sum:	1	1	7 *	5
(Data compared to no. of implantations)	%:	0	0	3	2
Dead Fetuses	Sum:	0	0	1	1
(Data compared to no. of implantations)	%:	0	0	0	0
Postimplantation Loss	Sum:	18	19	33	20
(Data compared to no. of implantations)	%:	7	9	12	9
Total Intrauterine Mortality	Sum:	47	53	69	51
(Data compared to no. of corpora lutea)	%:	17	22	22	21
Viable fetuses	Sum:	228	190	239	192
Male fetuses	Sum:	97	98	114	98
(Data compared to no. of viable fetuses)	%:	43	52	48	51
Female fetuses	Sum:	131	92	125	94
(Data compared to no. of viable fetuses)	%:	57	48	52	49

Remarks:
* = $p < 0.05$; CH2
** = $p < 0.01$; CH2

Changes in sex ratio: no effects observed

Changes in litter size and weights: no effects observed

Changes in postnatal survival: not examined

External malformations:

The number of evaluated fetuses was 228, 190, 239 and 192 at external and 114, 96, 120 and 97 at visceral examination in the control, 200, 400 and 1000 mg/kg bw/day groups, respectively.

- Malformations

Umbilical hernia was found in one fetus as a malformation at external and visceral examination in the 1000 mg/kg bw/day dose group.

Types of external abnormalities

(Sum., %)

		DOSE GROUPS Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
Number of Dams	N	21	19	23	19
Number of Fetuses examined	N	228	190	239	192
Number of Fetuses with abnormalities	N	10	3	7	12
	%	4	2	3	6
Variation	N	10	3	7	11
	%	4	2	3	6
Malformation	N	0	0	0	1
	%	0	0	0	1
Fetal variations					
- Retarded in body weight	N	9	3	7	11
	%	4	2	3	6
- Haemorrhages (head, neck)	N	1	0	0	0
	%	0	0	0	0
Fetal malformations					
- Umbilical hernia	N	0	0	0	1
	%	0	0	0	1

Remarks:
* = $p < 0.05$; CH2
** = $p < 0.01$; CH2

Visceral abnormalities

There was a tendency but not statistical increased incidence of visceral variations in the test item treated groups. Visceral variations such as bilateral hydroureter (0, 1, 0, 1 in each group, respectively) or hydroureter with dilated renal pelvis (1, 0, 0, 2, in each group, respectively)) occurred with a very low incidence without significant difference among the experimental groups (200 and 400 mg/kg bw/d), including control. The incidence of the fetuses with visceral variations increased significantly ($p<0.01$) in 4 animals in the 1000 mg/kg bw/day dose group.

(Sum., %)

		DOSE GROUPS			
		Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
Number of Dams	N	21	19	23	19
Number of Fetuses examined	N	114	96	120	97
Number of Fetuses with abnormalities	N	1	1	0	4 *
	%	1	1	0	4
Variation	N	1	1	0	3
	%	1	1	0	3
Malformation	N	0	0	0	1
	%	0	0	0	1
Fetal variations					
- Hydroureter (bilateral)	N	0	1	0	1
	%	0	1	0	1
- Hydroureter and dilated renal pelvis unilateral	N	1	0	0	2
	%	1	0	0	2
Fetal malformations					
- Umbilical hernia	N	0	0	0	1
	%	0	0	0	1

Remarks:
* = $p < 0.05$; CH2
** = $p < 0.01$; CH2

Skeletal malformations:

The number of examined fetuses was 114, 94, 119 and 95 in the control, 200, 400 and 1000 mg/kg bw/day respectively.

There was no test item related effect indicated at skeletal examination of the fetuses in the 200 and 400 mg/kg bw/day dose group. The incidence of skeletal abnormalities (malformations and variations) increased with a statistical significance ($p<0.01$) due to the increase in the variations ($p<0.01$) in the 1000 mg/kg bw/day dose group.

- Malformation

Malformations were recorded such as a bipartite thoracic centrum and dumb-bell shaped cartilage of thoracic centrum in the control and in the 1000 mg/kg bw/day dose group with an incidence of 2 and 1 respectively without a relationship with the test item.

- Variation

Incomplete ossification of the skull, bipartite supraoccipital, incompletely ossified or misaligned sternebrae, wavy ribs, dumb-bell shaped or bipartite vertebral centra, incomplete or asymmetric ossification of sacral arches and asymmetric or incomplete ossification of metacarpal or metatarsal, were evaluated as variations during the skeletal examination. There was a slightly but statistically significant ($p<0.01$) increase in the incidence of fetuses with incomplete ossification of the skull-bones and metacarpal/metatarsal in the 1000 mg/kg bw/day dose group.

Types of skeletal abnormalities

(sum, %)

		DOSE GROUPS Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day
Number of Dams	N	21	19	23	19
Number of Fetuses examined	N	114	94	119	95
Number of Fetuses with abnormalities	N	9	5	10	31 **
	%	8	5	8	33
Variation	N	7	5	10	30 **
	%	6	5	8	32
Malformation	N	2	0	0	1
	%	2	0	0	1
Fetal variations					
Skull					
- incomplete ossification (three bones or more)	N	0	0	1	12 **
	%	0	0	1	13
- incomplete ossification marked (one bone or more)	N	2	0	0	6
	%	2	0	0	6
- incomplete ossification marked (three bones or more)	N	0	0	2	7 **
	%	0	0	2	7
Supra occipital					
- bipartite ossification	N	0	0	0	2
	%	0	0	0	2
Sternebra					
- 3 or less ossified	N	1	0	1	1
	%	1	0	1	1
- misaligned	N	0	0	1	0
	%	0	0	1	0
Ribs					
- wavy	N	1	0	3	0
	%	1	0	3	0
Vertebrae					
Vertebral centra					
Thoracic and lumbar					
- dumb-bell shaped or dumb-bell shaped and/or asymmetric more than 3	N	1	0	0	0
	%	1	0	0	0
Thoracic					
- bipartite or bipartite and asymmetric	N	1	3	4	0
	%	1	3	3	0
Sacral					
-slightly misshapen (asymmetric)	N	0	0	0	1
	%	0	0	0	1
Vertebral arches					
- SII smaller (right)	N	0	0	0	1
	%	0	0	0	1
-from SII not ossified	N	0	0	0	2
	%	0	0	0	2
Forelimbs and hindlimbs					
Metacarpal or metatarsal					
- less than 3 ossified	N	0	2	1	6 **
	%	0	2	1	6
- asymmetric ossification	N	1	0	0	3
	%	1	0	0	3
Fetal malformations					
Thoracic centra					
- bipartite and cartilage dumb-bell shaped	N	2	0	0	1
	%	2	0	0	1

Remarks:
* = p < 0.05; CH2
** = p < 0.01; CH2

Summary table for results of external, visceral and skeletal examinations

(percentile litter means and SD)

		DOSE GROUPS				
		Control	200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day	
EXTERNAL EXAMINATION						
Litters examined	N	21	19	23	19	
Fetuses examined	N	228	190	239	192	
Fetuses with abnormalities	Mean	3.7	2.4	3.9	6.6	
	SD	6.30	5.86	9.52	9.35	
Variation	Mean	3.7	2.4	3.9	6.0	
	SD	6.30	5.86	9.52	9.40	
Malformation	Mean	0.0	0.0	0.0	0.6	
	SD	0.00	0.00	0.00	2.55	
Retarded in body weight	Mean	3.4	2.4	3.9	6.0	
	SD	6.29	5.86	9.52	9.40	
VISCERAL EXAMINATION						
Litters examined	N	21	19	23	19	
Fetuses examined	N	114	96	120	97	
Fetuses with abnormalities	Mean	0.0	0.9	0.0	4.5	
	SD	0.00	3.82	0.00	11.17	
Variation	Mean	0.0	0.9	0.0	3.4	
	SD	0.00	3.82	0.00	10.55	
Malformation	Mean	0.0	0.0	0.0	1.1	
	SD	0.00	0.00	0.00	4.59	
SKELETAL EXAMINATION						
Litters examined	N	21	19	23	19	
Fetuses examined	N	114	94	119	95	
Fetuses with abnormalities	Mean	8.0	5.4	8.9	33.7 **	U
	SD	13.49	9.56	16.01	31.83	
Variation	Mean	6.6	5.4	8.9	32.6 **	U
	SD	12.73	9.56	16.01	31.94	
Malformation	Mean	1.4	0.0	0.0	1.1	
	SD	6.23	0.00	0.00	4.59	

Remarks: * = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

(sum, %)

		Control	DOSE GROUPS 200 mg/kg bw/day	400 mg/kg bw/day	1000 mg/kg bw/day	
EXTERNAL EXAMINATION						
Litters examined	N	21	19	23	19	
Fetuses examined	N	228	190	239	192	
Fetuses	N	10	3	7	12	
with abnormalities	%	4	2	3	6	
Variation	N	10	3	7	11	
	%	4	2	3	6	
Malformation	N	0	0	0	1	
	%	0	0	0	1	
Retarded in body weight	N	9	3	7	11	
	%	4	2	3	6	
VISCERAL EXAMINATION						
Litters examined	N	21	19	23	19	
Fetuses examined	N	114	96	120	97	
Fetuses	N	0	1	0	4	*
with abnormalities	%	0	1	0	4	
Variation	N	0	1	0	3	
	%	0	1	0	3	
Malformation	N	0	0	0	1	
	%	0	0	0	1	
SKELETAL EXAMINATION						
Litters examined	N	21	19	23	19	
Fetuses examined	N	114	94	119	95	
Fetuses	N	9	5	10	31	**
with abnormalities	%	8	5	8	33	
Variation	N	7	5	10	30	**
	%	6	5	8	32	
Malformation	N	2	0	0	1	
	%	2	0	0	1	

Remarks:
* = p < 0.05; CH2
** = p < 0.01; CH2

1.2.2.2 [Anonymous, 2018]

Study reference:

Anonymous, 2018

Detailed study summary and results:

Test type

Prenatal Developmental Toxicity Study (2018)

OECD TG 414

GLP compliant

Test substance

- *Tert*.-Butylperoxy- 2-ethylhexanoate (TBPEH)
- CAS 3006-82-4
- EC 221-110-7
- Manufacturer : United Initiators GmbH and Co KG
- State: Liquid
- Purity : confidential (see Annex II)
- Batch number : 000055055
- Stability : stable at storage conditions for at least 3 months
- Storage conditions: < = 20°C in closed systems (avoid contact with humidity)
- Expiry date : April 2018

Test animals

- inseminated New Zealand White rabbits
- Source : S & K-LAP Kft., Csàszàr ùt 135, 2173 Kartal, Hungary
- 26-27 animals/dose group
- Age and weight at study initiation:

Age at study initiation: young, healthy and breeding mature rabbits. Females were nulliparous before first insemination at study initiation

Weight at study initiation (insemination): 3566-4484 g

- *Acclimatation period :* 8 days for the first transport and 7 days for the second transport
- *Animal health :* only animals in an acceptable health condition were used for the test
- *Housing :* Animals were housed individually in metal cages
- *Light :* 12 hours daily, from 6:00 a.m to 6:00 p.m
- *Temperature :* 15-21°C
- *Relative humidity :* 29 - 62%
- *Ventilation :* 8-12 air exchanges by central air-condition system.
- The temperature and relative humididty were checked and recorded one daily during the study. Any devaiations were documented.
- *Food and water sypply :* the animals received S & K-LAP separating rabbit diet produced by Cargill Takamany Zrt., 5300 Karcag, Madarasi ùt 0399, Hungary, *ad libitum*.Contents of S & K-LAP separating rabbit diet are presented in the study report. The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. The supplier provided an analytical certificate of the standard diet for the batch used. Animals received tap water bottles *ad libitum.* The drinking water was periodically analysed and is considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

Administration/exposure

- *Gavage* – oral (gavage)
- *Duration of Exposure :* 21 days. Period GD 6 to GD 27
- Frequency of treatment : The test item was administered in a single dose by oral gavage (stomach tube) on a 7 days/week basis every day at similar time. Control animals were treated concurrently with the vehicle only. Animals were not treated on the day of gross pathology.
- Doses tested: 30, 100 and 300 mg TBPEH /kg bw/day
- *rationale for dose level selection :* The dose setting was based on findings obtained in the non GLP preliminary study. In this dose range finding study the administration of TBPEH caused 100% mortality at 1000 mg/kg bw/day, no death but a single event of severe and reversible clinical signs in the 300 mg/kg bw/day dose group as well as increased early embryonic death at the dose level of 100 mg/kg bw/day.

Group No.	Dose (mg/kg bw/day)	Concentration (mg/mL)	Number of Inseminated Females
1	0	0	26
2	30	15	26
3	100	50	26
4	300	150	27

- *Rationale for animal assignment:* random
- *control group and treatment*

  Control group : yes, concurrent vehicle
- *historical control data if available :* yes
- Treatment volume: A constant treatment volume of 2 mL dose preparation/kg body weight was administered in all groups. The individual volume of the treatment was based on the most recent individual body weight of the animals (which was determined at least every three days).
- *vehicle*: sunflower oil
- *justification of choice of vehicle (if other than water) :* The test item is not soluble in water therefore sunflower oil was used for preparing formulations appropriate for oral administration. Oleum helianthy/sunflower oil is a suitable vehicle to facilitate formulation analysis for the test item. Concentration in vehicle: 15, 50 and 150 mg/mL.
- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation*

Analytical verification of doses or concentrations: yes

Details on analytical verification of doses or concentrations:

The suitability of the chosen vehicle for the test item was analytically proven. TPBEH was proved to be stable in sunflower oil formulations at ~2 and ~500 mg/mL concentration levels at least for 24 hours at room temperature and at least 3 days in refrigerator (5 ± 3°C) according to the partial analytical method validation at Toxi-Coop Zrt. Recovery of TBPEH from sunflower oil formulations at two concentration levels (~2 and ~500 mg/mL) was 104 % and 98 %. The dosing solutions were stored according to these results. Analytical control of dosing solutions (control of test item concentration) was performed in the Analytical Laboratory of Test Facility twice during the study. The mean of the test item concentrations of the test item in the dosing

formulations varied in the acceptable range between 94 and 108 % of nominal concentrations at both analytical occasions confirming proper dosing.

Description of test design:

- *details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy)*

- Impregnation procedure: artificial insemination
Day of insemination was regarded as day 0 of gestation. Synchronization of the cycle was completed 48 hours prior to insemination by administering PMSG (gonadotropin) hormone subcutaneously into the neck region. The insemination procedure was performed at the test facility by the breeder.

-Procedure : the inseminated females were treated from gestational day 6 to 27.

-Randomization : Females were randomly assigned to dose groups on the basis of their body weight on the day of insemination in such a way that the group averages of the body weight were as similar as possible on the first day (day 0) of gestation.

Observations

Maternal examinations:

CAGE SIDE OBSERVATIONS: Yes

Check for Mortality, Morbidity and Abortion
- Time schedule: An inspection for signs of morbidity, mortality and abortion were made twice a day (at the same time as the clinical observation and at the end of work period).

Moribund or animals obviously in pain or showing signs of severe and enduring distress were euthanized. The animals were subjected to necropsy to determine the cause of moribund state of death. Implantation sites or corpora lutea were counted. Females showing signs for abortion or premature delivery before gestation day 25 were euthanized before scheduled necropsy. Animals with abortion/premature delivery after gestation day 25 were necropsied at scheduled Caesarian section. These animals were subjected throughout macroscopic examination. Sample soft tissues showing gross pathological changes, which could not be diagnosed macroscopically, were fixed in 4% neutral formaldehyde solution. Histological processing and microscopic examination of the retained tissues will be only perfomed if requested by the Sponsor.

DETAILED CLINICAL OBSERVATIONS: Yes

- General clinical examination were made once a day, after treatment at approximately the same time, considering the peak period of anticipated effects after dosing. When signs of toxicity were observed, animals were observed more frequently. Individual observation included the chek of behavior and general condition. Duration and severity of the clinical signes were recorded.

BODY WEIGHT: Yes
- Time schedule for examinations: Individual body weight was recorded on gestation days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 28 (accuracy 1 g).
The corrected body weight was calculated for the 28th day of pregnancy (body weight on day 28 minus the weight of the gravid uterus).

FOOD CONSUMPTION AND COMPOUND INTAKE: Yes
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/animal/day: Yes; The food consumption was measured between gestation days 0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27 and 27 to 28 by re-weighing the non-consumed diet

(accuracy 1 g).

WATER CONSUMPTION AND COMPOUND INTAKE: Yes, visual inspection

POST-MORTEM EXAMINATIONS: Yes
- Sacrifice on gestation day 28 by Caeasarian section on each doe. Euthanasia of the animals was executed by lethal injection of Euthaminal 40% or Release® administered intravenously.
- Organs examined: The organs of neck, thorax and abdomen of the does were examined macroscopically. Organs pathological changes which could not be diagnosed macroscopically were fixed in 4 % neutral formaldehyde solution. Corresponding organs from control animals were kept for comparison. Histological examination on organs was not performed.

The ovaries and uterus were removed and the uterus (including cervix) of the pregnant females was weighed (accuracy 1 gram). Uterus of each female was examined for early, late embryonic and fetal death and for the number of live fetuses. Euthanasia of the animals was executed by lethal injection of Euthaminal 40% or Release®.

Ovaries and uterine content:

The ovaries and uterine content was examined after termination: Yes
Live fetuses (accuracy 0.1 g) and their placentas (accuracy 0.01 g) were weighed individually (litter mean was calculated). The crown-rump length of fetuses was measured (accuracy 1 millimeter) (litter mean was calculated). Fetuses and their placentas were examined externally.

Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes

Fetal examinations:

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

The viscera and skin of fetuses were moved and the cadaver was fixed in alcian-blue-acetic – ethanol mixture. After fixation in isopropanol the skeletons were stained by KOH-Alizarin red-S method and examined by means of a dissecting microscope. All abnormalities found during the fetal exminations were recorded.
- Skeletal examinations: Yes: all per litter.

- Head examinations: Yes: half per litter. Head of about 50% of each litter was removed and fixed in modified Sanomiya solution and was washed in 90% isopropanol for Wilson-sections. Examination of the heads was done by Wilson’s free-hand razor blade method

-Visceral examination: included also the determination of the gender.

Data evaluation

Statistics:

Data were individually recorded on data sheets, transferred, and compiled by computer or compiled manually.
The statistical evaluation of data was performed with the program package SPSS PC+4.0. The homogeneity of variance between groups was checked by Bartlett’s homogeneity of variance test.

Where no significant heterogeneity is detected a one-way analysis of variance (ANOVA) is carried out. If the obtained result is significant Duncan's Multiple Range test was used to assess the significance of intergroup differences. If significance is the result of the Bartlett's test, the Kruskal-Wallis analysis of variance was used and the inter-group comparisons were performed using Mann-Whitney U-test.

Does or litters were excluded from the data evaluation in cases of:

- A disease or death of the doe unrelated to the treatment (total exclusion)

- Non pregnant females i.e. females with no implantation and no corpora lutea (total exclusion)

- Body weight, body weight gain, food consumption, clinical signs and necropsy findings of females with no implantation but corpora lutea i.e. total preimplantation loss (only the intrauterine parameters were evaluated, partial exclusion)

- Circumstances unrelated to the test item which are considered to be reason for exclusion, at the discretion of the Study Director

Although these animals were excluded from the data evaluation the study report contains all data.

A male/female fetus was considered as retarded in body weight/crown-rump length, when its weight/length was below the average minus twofold standard deviation of the control male/female fetuses.

Historical control data: yes

Does or litters listed below were excluded from the data evaluation:

A disease or death of the doe unrelated to the treatment (total exclusion):

- Control: No.: 40124 (disease -weakness, lying, moribund state, yellowish, greyish purulent plexuses in the lungs, pur in the trachea, nutmeg like pattern in the liver), 40097 (technical reason, died), 40078 (disease (diarrhea, reduced activity, died, inflammation of the intestines, greyish discoloration of the lungs)

- 100 mg/kg bw/day: 40119 (technical reason, died), 40028 (technical reason, died), 60043 (disease revealed at necropsy (ca. 10 cm diameter white round formation full with pur), live litter)

- 300 mg/kg bw/day: 60048 (technical reason, died), 40186 (technical reason, died)

Non pregnant females i.e. females with no implantation and no corpora lutea (total exclusion):
- 30 mg/kg bw/day: 60027

- 100 mg/kg bw/day: 40019, 40070, 60089

Results

Results: maternal animals

General toxicity (maternal animals)

Disposition of females

The number of inseminated females was 26 in the control, 30 and 100 mg/kg bw/d as well as 27 in the 300 mg/kg bw/day group. There was one female each in the control and 30 mg/kg bw/day and 3 in the 100 mg/kg bw/day group without any implantation (corpora lutea was present in the control animal).

Number of abortions, Mortality:

The occurrence of abortions was 0, 1, 2 and 8 in the control, 30, 100 and 300 mg/kg bw/day groups, respectively

Early delivery (g.d. 27 or 28 (before or in the morning of scheduled necropsy)) occurred in case of 2 does both in the control and 100 mg/kg bw/day groups.

There were three additional moribund animals in the high dose which were euthanized before scheduled necropsy. The number of animals died due to a technical reason (misgavage) was one in the control, and two each in the 100 and 300 mg/kg bw/day dose group. One female died (diarrhea, inflammation of the intestines, and lungs) as well as one was moribund and euthanized in the control group (lying unmoved, weak, purulent inflammation of the lungs and trachea and abnormal pattern of the liver was recorded at necropsy). One female with a litter of live fetuses was excluded from the evaluation because of a large, purulent abscess in the abdomen at 100 mg/kg bw/day. In total, on gestation day 28 there were 22, 24, 20 and 17 does with implantation sites (including the animals aborted from g. d. 25) and 20, 23, 16 and 10 evaluated litters in the control, 30, 100 and 300 mg/kg bw/day group respectively. See table above.

Pregnancy data of females, mortality, malformations

(sum, %)

Dose groups	Control		30 mg/kg bw/day		100 mg/kg bw/day		300 mg/kg bw/day	
Number of inseminated females	26		26		26		27	
Number of females with no implantation but corpora lutea (total preimplantation loss)	1		1		0		0	
Number of females with no implantation and no corpora lutea	0		1		3		0	
Number and percent of pregnant females (females with implantation)	22	85%	24	92%	22	85%	27	100%
Number of pregnant females with total post implantation loss	0		0		0		4	
Number of does aborted or delivered early (between days 25 to 28), necropsied at scheduled necropsy	2		1		3		3	
Number of females with implantation sites at scheduled necropsy	22		24		20		17	
Number and percent of does aborted before gestation day 25, euthanized	0		0		1		5 (1 moribund)	
Number of does died due to toxicity	0		0		0		0	
Number of pregnant females moribund due to toxicity (euthanized)	0		0		0		4 (1 aborted)	
Number of pregnant females died due to a technical reason	1		0		2		2	
Number of pregnant females with an intercurrent disease	1 moribund, 1 died		0		0		0	
Number of litters with viable fetuses at scheduled Caesarean section	20		23		17		10	
Number of does with live fetuses with an intercurrent disease (excluded)	0		0		1		0	
Number of evaluated litters	20		23		16		10	
Number and percent of evaluated litters with malformed fetuses	11	55%	8	35%	7	44%	6	60%

Clinical signs, mortality:

The increase of gastro-intestinal tract related observations like absence or decreased amount (observed in all groups but increased significantly in the high dose) or weak consistency of faeces (observed also in one doe each in the 30 and 100 mg/kg bw/day dose group however only in one occasion and judged to be not adverse) was attributed to the treatment at 300 mg/kg bw/day.

Bleeding from the vagina (associated to abortion or postimplantation loss), was attributed to the treatment at 300 mg/kg bw/day. Blood in the bedding was recorded for one rabbit each in the 30 and 100 mg/kg bw/day groups, both aborted. Orange discoloration in the bedding was observed in all groups and it was significantly more frequent in the 300 mg/kg bw/day dose group which was attributed to the treatment however judged as likely not adverse since the reddish colour of the urine of rabbits is considered to be normal according to specialist literature. “Red urine is a descriptive term for the condition where a rabbit's urine varies in color from the normal pale yellow to dark yellow, carrot orange, brown, or bright red” (John E. Harkness, Patricia V. Turner, Susan VandeWoude, Colette L. Wheler Harkness and Wagner's Biology and Medicine of Rabbits and Rodents, 1988). Based on this, red discoloration of the bedding in case of one animal at 30 mg/kg bw/day was not attributed to the treatment.

“Lying or/and weakness and reduced activity was recorded for all moribund animals (four, one also aborted) after significant weight loss. Also, noisy breath was observed in one of these animals.

Sneezing was recorded in the groups without a dose relationship.

Summary of clinical signs, and necropsy findings of does

(sum, %)

DESCRIPTION	DOSES: No. of animals:	control 22	30 24	100 mg/kg bw/day 20	300 25
MORTALITY, MORBIDITY					
- died due to toxicity	N	0	0	0	0
	%	0	0	0	0
- moribund due to toxicity	N	0	0	0	4
	%	0	0	0	16
CLINICAL SYMPTOMS					
- none	N	3	5	0	0
	%	14	21	0	0
- no faeces	N	7	9	7	19
	%	32	38	35	76
- minimal faeces	N	0	0	0	1
	%	0	0	0	4
- less faeces	N	17	17	16	19
	%	77	71	80	76
- weak faeces	N	0	1	1	6
	%	0	4	5	24
- slightly weak faeces	N	0	0	0	1
	%	0	0	0	4
- orange discoloration in the bedding	N	2	4	3	12
	%	9	17	15	48
- red discoloration in the bedding	N	0	1	0	0
	%	0	4	0	0
- blood in the bedding	N	0	1	1	0
	%	0	4	5	0
- bleeding from the vagina	N	0	2	2	6
	%	0	8	10	24
- abortion	N	0	1	2	8
	%	0	4	10	32
- early delivery (g.d. 27 or 28)	N	2	0	2	0
	%	9	0	10	0
- reddish discoloration at snout region	N	1	0	0	0
	%	5	0	0	0
- sneezing	N	4	0	2	2
	%	18	0	10	8
- noisy breath	N	0	0	0	1
	%	0	0	0	4
- lying or/and weak	N	0	0	0	5
	%	0	0	0	20
-reduced activity	N	0	0	0	5
	%	0	0	0	20

(sum, %)

DESCRIPTION	DOSES: No. of animals:	control 22	30 24	100 mg/kg bw/day 20	300 25
NECROPSY FINDINGS					
- no macroscopic alterations	N	16	18	17	10
	%	73	75	85	40
- pinhead sized haemorrhages in the lungs	N	2	2	0	1
	%	9	8	0	4
- pinhead sized or point like haemorrhages in the lungs and brownish discoloration	N	2	2	0	0
	%	9	8	0	0
- dark discoloration (slight) of the lungs and dark points	N	0	0	0	1
	%	0	0	0	4
- reddish mottled lungs	N	0	1	1	1
	%	0	4	5	4
- some pinhead sized dark points and bright bulgs in the lungs	N	0	0	0	1
	%	0	0	0	4
-reddish mottled lungs and pea sized darker and brighter bulgs	N	0	0	0	1
	%	0	0	0	4
-brownish discoloration extended size in the lungs or 1-2 cm brownish areas	N	0	0	0	2
	%	0	0	0	8
- stomach fuller than usual or filled to distension and/or dry content	N	0	1	2	10
	%	0	4	10	40
- black points in the stomach wall	N	0	0	0	2
	%	0	0	0	8
- abdomen filled with bloody fluid	N	0	1	0	0
	%	0	4	0	0
- pea sized pinch in the spleen	N	0	1	0	0
	%	0	4	0	0
-region of vaginal orifice tainted with blood	N	0	0	1	3
	%	0	0	5	12
-pale liver	N	2	0	0	1
	%	9	0	0	4
-gall bladder filled to distention with reddish fluid or markedly filled and 1-2 cm diameter brownish spots	N	0	0	0	2
	%	0	0	0	8
-empty intestines except appendix	N	0	0	0	2
	%	0	0	0	8
-intestines filled with gas	N	0	0	0	1
	%	0	0	0	4
-anal region tainted with weak faeces	N	0	0	0	1
	%	0	0	0	4

Body weight and weight changes:

The mean body weight of the animals in the high dose group was lower from after the first treatment in the whole in-life phase ($p<0.01$ from g.d. 9 to 21 and $p<0.05$ on g.d. 24) at 300 mg/kg bw/day.

The average of body weight gain was negative in all groups on the first three days of treatment, but dose relationship was indicated only in the 300 mg/kg bw/day dose group. Weight loss was observed up to day 18 (statistical significance: $p<0.01$ from g.d. 6 to 12 and 15 to 18) in the 300 mg/kg bw/day dose group. From GD 12 to 15 also weight loss was indicated without a statistical significance. From g.d. 15 to 18 a statistically significantly lower body weight gain ($p<0.05$) was observed in the 100 mg/kg bw/day dose group. From gestation day 18 there were no dose related reductions observed in the groups.

The corrected body weight gain was negative in all groups and more expressed in the high dose than the other groups (not statistically significant) where no dose relationship was observed.

Summary of body weight and body weight gain of does

(mean, SD)

Body weight (g)

TIME Gestational days		DOSE GROUPS (mg/kg bw/day) Control	30	100	300	
0	MEAN	4155.8	4158.4	4175.6	4174.0	
	SD	187.57	167.61	190.46	171.88	
	n	22	24	20	25	NS
3	MEAN	4217.8	4210.2	4240.7	4257.8	
	SD	167.65	162.40	175.27	160.23	
	n	22	24	20	25	NS
6	MEAN	4301.5	4268.1	4313.0	4346.4	
	SD	167.70	154.57	178.48	181.39	
	n	22	24	20	25	NS
9	MEAN	4296.5	4256.4	4308.0	4107.6	
	SD	210.23	206.39	210.65	173.63	
	n	22	24	20	25 **	DN
12	MEAN	4344.1	4298.4	4333.4	4021.8	
	SD	244.25	225.92	225.81	228.48	
	n	22	24	20	25 **	DN
15	MEAN	4381.9	4381.2	4395.9	3991.6	
	SD	216.19	211.12	260.32	321.13	
	n	22	24	20	25 **	DN
18	MEAN	4465.5	4452.3	4419.8	3920.6	
	SD	203.40	213.52	274.27	339.16	
	n	22	24	20	24 **	DN
21	MEAN	4476.2	4458.7	4430.1	3995.5	
	SD	204.76	220.20	329.91	419.14	
	n	22	24	20	21 **	U
24	MEAN	4487.3	4506.6	4510.2	4191.6	
	SD	211.18	245.25	305.98	415.39	
	n	22	24	19	17 *	U
27	MEAN	4500.9	4583.9	4612.4	4327.7	
	SD	213.05	233.41	274.29	357.90	
	n	22	23	16	14	NS
28	MEAN	4530.1	4583.0	4631.4	4358.3	
	SD	226.01	242.79	289.67	383.14	
	n	20	23	16	14	NS

REMARK: NS= not significant

(mean, SD)

Body weight gain (g)

TIME Gestational days		DOSE GROUPS (mg/kg bw/day) Control	30	100	300	
0-3	MEAN	62.0	51.8	65.1	83.8	
	SD	61.65	63.19	80.06	68.93	
	n	22	24	20	25	NS
3-6	MEAN	83.8	57.9	72.3	88.6	
	SD	46.63	83.57	71.71	60.86	
	n	22	24	20	25	NS
6-9	MEAN	-5.0	-11.7	-5.0	-238.8	
	SD	122.65	115.90	88.44	140.85	
	n	22	24	20	25 **	DN
9-12	MEAN	47.5	42.0	25.4	-85.8	
	SD	97.70	122.59	96.64	162.53	
	n	22	24	20	25 **	U
12-15	MEAN	37.8	82.8	62.5	-30.1	
	SD	79.92	112.41	123.57	158.96	
	n	22	24	20	25	NS
15-18	MEAN	83.5	71.1	23.9	-101.3	
	SD	96.70	88.97	80.76	160.25	
	n	22	24	20 *	24 **	U
18-21	MEAN	10.7	6.3	10.3	3.5	
	SD	68.66	72.96	141.61	208.11	
	n	22	24	20	21	NS
21-24	MEAN	11.1	47.9	42.5	68.9	
	SD	57.99	68.56	82.81	178.80	
	n	22	24	19	17	NS
24-27	MEAN	13.6	54.8	43.9	15.4	
	SD	101.74	53.07	83.38	79.40	
	n	22	23	16	14	NS
27-28	MEAN	22.7	-0.9	19.0	30.6	
	SD	59.45	39.55	53.08	67.06	
	n	20	23	16	14	NS
0-28	MEAN	390.6	423.2	401.3	221.0	
	SD	213.68	257.20	267.40	419.19	
	n	20	23	16	14	NS

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test
- = no data

Summary of gravid uterine weight corrected by body weight and body weight gain of does

		DOSE GROUPS (mg/kg bw/day) Control	30	100	300	
Gravid uterine weight (g)	MEAN	528.8	497.6	534.9	459.4	
	SD	105.96	123.93	120.33	82.20	
	n	20	23	16	10	NS
Corrected body weight (g)	MEAN	4001.4	4085.4	4096.4	3962.2	
	SD	212.41	212.65	287.26	240.19	
	n	20	23	16	10	NS
Corrected body weight gain (g)	MEAN	-284.2	-183.2	-259.2	-353.0	
	SD	182.40	206.66	215.19	279.53	
	n	20	23	16	10	NS

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Food consumption and compound intake (if feeding study):

Significantly reduced food consumption was observed from start of the treatment up to gestation day 21 in the 300 mg/kg bw/day dose group ($p<0.01$ from g.d. 6 to 18 and $p<0.05$ from g.d. 18 to 21). The majority of the animals which aborted, were moribund or had total post-implantation loss at 300 mg/kg bw/day had minimal or zero food consumption sporadically during the in-life phase. Between gestation day 9 and 12 a slight reduction ($p<0.05$) was seen also at 100 mg/kg bw/day.

Summary of food consumption data of does

(mean, SD)

TIME Gestational days		DOSE GROUPS (mg/kg bw/day) Control	30	100	300	
0-3	MEAN	212.7	217.4	216.0	218.3	
	SD	28.02	24.83	29.28	19.31	
	n	22	24	20	24	NS
3-6	MEAN	224.9	228.1	231.9	234.7	
	SD	18.65	32.79	20.66	32.29	
	n	19	23	20	24	NS
6-9	MEAN	123.8	123.9	110.5	43.1	
	SD	57.68	61.43	42.09	37.61	
	n	21	24	18	25 **	DN
9-12	MEAN	165.6	150.0	131.0	52.2	
	SD	45.86	57.89	54.59	44.04	
	n	21	23	20 *	25 **	DN
12-15	MEAN	89.6	106.9	90.2	43.3	
	SD	35.86	53.72	42.24	43.40	
	n	21	24	20	24 **	DN
15-18	MEAN	106.7	115.4	91.9	32.5	
	SD	38.08	51.42	51.59	43.13	
	n	20	24	20	24 **	DN
18-21	MEAN	112.8	110.4	94.9	67.3	
	SD	46.01	48.99	42.91	72.68	
	n	22	24	20	21 *	DN
21-24	MEAN	107.2	101.5	87.4	102.7	
	SD	48.26	43.14	35.44	77.72	
	n	22	24	19	17	NS
24-27	MEAN	87.9	104.9	93.3	100.9	
	SD	45.62	38.11	26.95	51.44	
	n	21	23	16	14	NS
27-28	MEAN	102.4	97.2	104.0	103.6	
	SD	63.97	47.73	43.29	42.59	
	n	20	23	16	14	NS

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Gross pathological findings:

Darker or brighter bulges, larger brownish areas, dark discoloration and dark points in the lungs as well as black points in the stomach wall, markedly filled gall bladder with bloody content, markedly full stomach (in ten does at 300 mg/kg bw/day) filled with food or with dry content, empty intestines and distended gall bladder with bloody content both in two does were attributed to the treatment at the 300 mg/kg bw/day dose group. Stomach filled to distention was also recorded for one doe in the 30 mg/kg bw/day group, however just one with the slighter observation "fuller stomach than usual" in the 100 mg/kg bw/day group hence based on the lack of dose response these changes were not attributed to the treatment in these groups. The same female at 30 mg/kg bw/day had bloody fluid in the abdomen as a single observation and in the low dose hence not attributed to the treatment. Pinhead-sized or point-like haemorrhages or/and brownish discoloration, reddish mottled lungs, pale liver, pinched spleen were seen in the groups unrelated to the treatment. Gas filled intestines in one doe at the high dose could be in association with the treatment/reduced

food consumption. The observations “vaginal orifice tainted with blood or anal region tainted with faeces” was in association with abortion in the 100 and 300 mg/kg bw/day group or weak faeces in one female in the 300 mg/kg bw/day group.

Pre- and post-implantation loss:

Increase of early embryonic death and post-implantation loss (statistically significant if the number and percent of resorptions evaluated and not statistically significant if the mean number and SD calculated, probably due to the high standard deviation) as well as slight decrease (without a statistical significance) of mean number of viable fetuses was indicated. The number of does with total post-implantation loss was four in the 300 and none in the other groups and judged to be due an effect of the test item.

No treatment related adverse effect was indicated in the pre- implantation loss, the mean number of implantations, late embryonic death, dead fetuses and the sex distribution in the dose groups.

Moreover the total intrauterine mortality (sum, %) and pre-implantation loss was statistically significantly lower ($p<0.01$) at 100 mg/kg bw/day, which is not considered to be of biological relevance.

Summary of intrauterine mortality, viable fetuses, sex distribution

(mean, SD)

GROUPS (mg/kg bw/day):		Control	30	100	300	
NUMBER OF DAMS:		23	25	20	25	
Corpora Lutea	Mean:	12.3	11.7	12.4	10.9	
	SD:	2.55	2.84	2.70	1.98	NS
Preimplantation Loss %	Mean:	16.8	14.9	8.8	11.6	
	SD:	24.08	23.08	12.33	8.69	NS
Implantation	Mean:	10.3	10.0	11.5	9.6	
	SD:	3.44	3.35	3.25	1.93	NS
Early Embryonic Death %	Mean:	4.8	6.6	6.6	30.7	
	SD:	9.06	12.74	10.66	45.68	NS
Late Embryonic Death %	Mean:	0.3	0.3	1.0	0.5	
	SD:	1.23	1.70	2.80	1.91	NS
Dead Fetuses %	Mean:	2.3	1.9	0.5	2.3	
	SD:	5.02	4.58	1.92	4.68	NS
Postimplantation Loss %	Mean:	7.4	8.8	8.1	33.4	
	SD:	10.20	14.00	10.15	44.34	NS
Total Intrauterine Mortality %	Mean:	24.0	22.6	15.8	40.0	
	SD:	21.34	24.35	15.45	40.44	NS
Viable fetuses	Mean:	8.3	9.0	10.3	6.5	
	SD:	3.92	3.38	2.86	4.42	NS
Male fetuses %	Mean:	48.5	53.6	52.8	51.6	
	SD:	15.31	15.64	19.63	19.38	NS
Female fetuses %	Mean:	51.5	46.4	47.2	48.4	
	SD:	15.31	15.64	19.63	19.38	NS

REMARKS : NS – Not Significant
* – $p < 0.05$
** – $p < 0.01$
U – Mann-Whitney U - test Versus Control
DN – Duncan's multiple range test

(sum, %)

GROUPS (mg/kg bw/day):		Control	30	100	300
NUMBER OF DOES:		23	25	20	25
Corpora Lutea	Sum:	284	292	248	273
Preimplantation Loss	Sum:	48	43	19 **	32
(Data compared to no. of corpora lutea)	%:	17	15	8	12
Implantation	Sum:	236	249	229	241
Early Embryonic Death	Sum:	13	16	12	36 **
(Data compared to no. of implantations)	%:	6	6	5	15
Late Embryonic Death	Sum:	1	1	2	1
(Data compared to no. of implantations)	%:	0	0	1	0
Dead Fetuses	Sum:	6	5	1	4
(Data compared to no. of implantations)	%:	3	2	0	2
Postimplantation Loss	Sum:	20	22	15	41 **
(Data compared to no. of implantations)	%:	8	9	7	17
Total Intrauterine Mortality	Sum:	68	65	29 **	60
(Data compared to no. of corpora lutea)	%:	24	22	12	22
Viable fetuses	Sum:	191	216	164	91
Male fetuses	Sum:	94	116	86	46
(Data compared to no. of viable fetuses)	%:	49	54	52	51
Female fetuses	Sum:	97	100	78	45
(Data compared to no. of viable fetuses)	%:	51	46	48	49

REMARKS:
* = $p < 0.05$; CH^2
** = $p < 0.01$; CH^2

Total litter losses by resorption: no effects observed

Early or late resorptions: no effects observed

Dead fetuses: no effects observed

Changes in pregnancy duration: not examined

Changes in number of pregnant: not examined

Other effects: no effects observed

Placental weight: There was no significant difference in the mean placental and relative placental weight.

Results (fetuses)

Fetal body weight changes:

Significantly lower fetal weight ($p<0.01$ if the sexes evaluated together, $p<0.05$ for females and lower but statistically not significant for males) and crown-rump length were observed in the 300 mg/kg bw/day dose group.

One litter was completely affected (i.e. 10/10 fetuses). The maternal animal (# 031740146) of this litter revealed clinical signs such as reduced food consumption, body weight loss and no or reduced feces during gestation.

Summary of fetal and placental weight

LITTER MEANS OF FETAL AND PLACENTAL WEIGHT

		DOSE GROUPS (mg/kg bw/day)											
		Control			30 mg/kg bw/day			100 mg/kg bw/day			300 mg/kg bw/day		
		M+F	M	F	M+F	M	F	M+F	M	F	M+F	M	F
Fetal weight (g)	MEAN	35.2	35.7	34.2	33.8	34.0	33.4	33.9	34.3	33.9	29.71	30.54	29.31
	SD	4.59	5.86	4.26	4.18	4.25	4.37	5.66	6.42	5.36	5.22	5.47	5.13
	n	20	20	20	23	23	23	16	16	16	10	10	10
											**		*
											DN	NS	DN
Placental weight (g)	MEAN	6.5	6.6	6.3	6.1	6.2	6.0	6.2	6.2	6.3	5.9	6.0	5.8
	SD	0.72	1.06	0.62	1.01	1.01	1.15	0.99	1.16	1.18	1.25	1.35	1.21
	n	20	20	20	23	23	23	16	16	16	10	10	10
											NS	NS	NS
Relative placental weight (g/g)	MEAN	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	SD	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
	n	20	20	20	23	23	23	16	16	16	10	10	10
											NS	NS	NS
Crown-rump length (cm)	MEAN	91.5	92.2	90.7	89.8	90.0	89.5	89.3	89.7	89.4	84.8	85.4	84.5
	SD	5.45	7.39	5.07	3.38	3.51	3.70	4.75	6.06	4.03	6.51	6.70	6.41
	n	20	20	20	23	23	23	16	16	16	10	10	10
											**	**	**
											DN	U	DN

Remarks: NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

Reduction in number of live offspring: no effects observed

Changes in sex ratio: no effects observed

Changes in litter size and weights: no effects observed

Changes in postnatal survival: not examined

External malformations:

The number of evaluated litters/fetuses was 20/191, 23/216, 16/164 and 10/91 in the control, 30, 100 and 300 mg/kg bw/day groups, respectively. There was no significant difference in the litter incidence (11 (55%), 8 (35%), 7 (44%) and 6 (60%)) in the control, 30, 100 and 300 mg/kg bw/day groups respectively regarding the all over fetal malformation.

Malformation

The number of the affected litters was one both in the control and the 100 mg/kg bw/day group, respectively. A fetus was found with multiply malformed head (skull bones and facial bones absent ca. from the line of the oral orifice and upper line of ears, ca. 1/3 part of brain present, brain not covered by meninges and skull bones, tongue present, anophthalmia bilateral, maxilla absent) in the 100 mg/kg bw/day dose group. The placenta of this fetus was fused with the next late resorption which could cause a disturbance in the placenta functioning and evolving of this malformation. Considering this and the fact that it was a single case in the test item treated groups this malformation was not considered to be a consequence of the treatment. In addition, cleft palate was found in one control fetus.

Variations

The incidence of abnormalities increased significantly ($p<0.01$) in the 300 mg/kg bw/day group due to growth retardation (body weight below 22.43 g for males and 21.79 g for females or crown-rump length below 77.48 mm for males and 76.71 mm for females) which were evaluated as external variations.

Placentas

The placenta of the malformed fetus was fused with the next implantation (late resorption) and paler lobe of a placenta was found in the 100 mg/kg bw/day dose group as well as two placentas were fused with each other in the 30 mg/kg bw/day dose group. These placenta changes were not attributed to the treatment considering the low incidence and different type.

Results of exteral, visceral and skeletal examinations

(percentile litter means and SD)

		DOSE GROUPS (mg/kg bw/day) Control	30	100	300	
EXTERNAL EXAMINATION						
Litters examined	N	20	23	16	10	
Fetuses examined	N	191	216	164	91	
Fetuses with abnormalities	Mean	3.3	3.2	5.6	14.0	
	SD	7.76	5.86	9.90	31.53	NS
Variation	Mean	2.5	3.2	5.1	14.0	
	SD	7.12	5.86	9.97	31.53	NS
Malformation	Mean	0.8	0.0	0.5	0.0	
	SD	3.73	0.00	1.92	0.00	NS
VISCERAL EXAMINATION						
Litters examined	N	20	23	16	10	
Fetuses examined	N	191	216	164	91	
Fetuses with abnormalities	Mean	4.4	5.7	5.2	5.3	
	SD	5.81	8.19	6.76	5.72	NS
Variation	Mean	3.3	5.3	5.2	4.4	
	SD	5.53	8.24	6.76	5.78	NS
Malformation	Mean	1.1	0.4	0.0	0.9	
	SD	3.25	1.90	0.00	2.87	NS
SKELETAL EXAMINATION						
Litters examined	N	20	23	16	10	
Fetuses examined	N	191	216	164	91	
Fetuses with abnormalities	Mean	27.1	21.4	23.3	41.6	
	SD	18.97	19.24	23.53	22.79	NS
Variation	Mean	20.3	15.6	17.3	30.2	
	SD	19.96	13.25	19.13	21.13	NS
Malformation	Mean	6.8	5.8	6.0	11.4	
	SD	9.92	10.31	7.69	13.49	NS

Remarks: NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

(sum, %)

			DOSE GROUPS (mg/kg bw/day) Control	30	100	300	
EXTERNAL EXAMINATION							
Litters examined		N	20	23	16	10	
Fetuses examined		N	191	216	164	91	
Fetuses with abnormalities		N	6	8	11	14	**
		%	3	4	7	15	
	Litters	N	4	6	6	3	
		%	20	26	38	30	
Variation		N	5	8	10	14	**
		%	3	4	6	15	
	Litters	N	3	6	5	3	
		%	15	26	31	30	
Malformation		N	1	0	1	0	
		%	1	0	1	0	
	Litters	N	1	0	1	0	
		%	5	0	6	0	
VISCERAL EXAMINATION							
Litters examined		N	20	23	16	10	
Fetuses examined		N	191	216	164	91	
Fetuses with abnormalities		N	8	11	8	5	
		%	4	5	5	5	
	Litters	N	8	10	7	5	
		%	40	43	44	50	
Variation		N	6	10	8	4	
		%	3	5	5	4	
	Litters	N	6	9	7	4	
		%	30	39	44	40	
Malformation		N	2	1	0	1	
		%	1	0	0	1	
	Litters	N	2	1	0	1	
		%	10	4	0	10	
SKELETAL EXAMINATION							
Litters examined		N	20	23	16	10	
Fetuses examined		N	191	216	164	91	
Fetuses with abnormalities		N	52	44	42	38	*
		%	27	20	26	42	
	Litters	N	18	19	12	9	
		%	90	83	75	90	
Variation		N	40	33	31	28	
		%	21	15	19	31	
	Litters	N	14	18	10	9	
		%	70	78	63	90	
Malformation		N	12	11	11	10	
		%	6	5	7	11	
	Litters	N	9	7	7	5	
		%	45	30	44	50	

Remarks:
* = $p < 0.05$; CH^2
** = $p < 0.01$; CH^2

Summary of external abnormalities

TYPES OF EXTERNAL ABNORMALITIES

(Sum., %)

			DOSE GROUPS (mg/kg bw/day) Control	30	100	300
Number of Does		N	20	23	16	10
Number of Fetuses examined		N	191	216	164	91
Number of Fetuses with abnormalities		N	6	8	11	14 **
		%	3	4	7	15
	Litters	N	4	6	6	3
		%	20	26	38	30
Variation		N	5	8	10	14 **
		%	3	4	6	15
	Litters	N	3	6	5	3
		%	15	26	31	30
Malformation		N	1	0	1	0
		%	1	0	1	0
	Litters	N	0	0	1	0
		%	0	0	6	0
Fetal variations						
- Retarded in body weight		N	4	6	8	13 **
		%	2	3	5	14
	Litters	N	2	5	5	2
		%	10	22	31	20
- Retarded in crown rump length		N	6	7	10	13 **
		%	3	3	6	14
	Litters	N	4	5	5	3
		%	20	22	31	30
Fetal malformations						
- Cleft palate		N	1	0	0	0
		%	1	0	0	0
	Litters	N	1	0	0	0
		%	5	0	0	0
- Multiple malformed head		N	0	0	1	0
		%	0	0	1	0
	Litters	N	0	0	1	0
		%	0	0	6	0
Placenta abnormalities						
- Fused with a resorption		N	0	0	1	0
		%	0	0	1	0
	Litters	N	0	0	1	0
		%	0	0	6	0
- Fused		N	0	1	0	0
		%	0	0	0	0
	Litters	N	0	1	0	0
		%	0	4	0	0
- One lobe paler		N	0	0	1	0
		%	0	0	1	0
	Litters	N	0	0	1	0
		%	0	0	6	0

REMARKS:
* = $p < 0.05$; CH^2
** = $p < 0.01$; CH^2

Skeletal malformations:

The incidence of fetuses with skeletal abnormalities (variations and malformations) increased in the 300 mg/kg bw/day dose group ($p<0.05$) but with a similar litter incidence. The litter incidence of malformations was similar in the groups (9 (45%), 7 (30%), 7 (44%), 5 (50%), while the incidence of variations was higher (not statistically significant) at 300 mg/kg bw/day.

Malformation

Absent skull bones and short mandible was found in one fetus at 100 mg/kgbw/day and were not judged to be related to the treatment (discussed at external examination). Sternebral (split xiphoid or other sternal cartilage, slightly wider sternum), rib (fused), and vertebral (absent cervical arch, asymmetric thoracic

centrum including cartilage, dumb-bell shaped cartilage of thoracic centrum, fused lumbar arches, multiple malformed vertebrae) malformations occurred without a dose response or a statistical significance. If the incidence of fused sternum is summarized (3, 6, 9, 5 in the control, 30, 100 and 300 mg/kg bw/day groups respectively), a statistically significant increase was seen in the 100 mg/kg bw/day ($p<0.05$) group and not at 300 mg/kg bw/day. Considering that this malformation was observed also in the control group (3 (15%) litter and 4 (2%) fetal incidence), and that this malformation occurs also in control fetuses according to the Background pregnancy and fetal data, the appearance was not attributed to the treatment. Multiply malformed ribs and vertebrae were found in one fetus in the 30 and in three in the 300 mg/kg bw/day, latter statistically significantly increased. Fused ribs however were seen only in the control (four fetuses) and 30 mg/kg bw/day (one fetus) groups. Multiply malformed vertebrae may occur in fetuses of does treated with inactive substances according to the Background pregnancy and fetal data. The increased incidence of fused sternum and multiply malformed ribs and vertebrae were suspected to be secondary to the maternal toxicity in the 300 mg/kg bw/day dose group. One fetus (the same as with malformation at external examination) had besides cleft palate slightly shorter mandible, misshapen skull and facial bones, fused sternebra and bent scapula unilateral.

Variations

Enlarged anterior or/and posterior fontanel, irregular shape of anteriorfontanel, slightly shorter maxilla, incomplete ossification of sternum, misaligned, bipartite, dumb-bell shaped sternebra, misshapen ossification of one sternebra, slightly pinched sternal cartilage, supernumerary ossificationpoint in sternum, fusing tendency of sternebra, lack of sternal connection of 7th rib, bent and or interrupted 13th rib, dumb-bell shaped, bipartite or/and asymmetric ossification thoracic centrum, bipartite, dumb-bell shaped or and asymmetric coccygeal, less than 14 ossified coccygeal, unossified pubic, talus, pollex, less than 3.5 ossified metacarpal, less than 7/7 ossified proximal and middle phalanges were recorded as skeletal variations.

Dose related statistical significance ($p>0.01$) was observed in the 300 mg/kg bw/day group due to increased incidence of delayed ossification of proximal and middle phalanges which was in association with the lower body weight and crown-rump length.

Summary of types of skeletal abnormalities

(sum, %)

DOSE GROUPS (mg/kg bw/day)			Control	30	100	300
Number of Litters		N	20	23	16	10
Number of Fetuses examined		N	191	216	164	91
Number of Fetuses with abnormalities		N	52	44	42	38 *
		%	27	20	26	42
	Litters	N	18	19	12	9
		%	90	83	75	90
Variation		N	40	33	31	28
		%	21	15	19	31
	Litters	N	14	18	10	9
		%	70	78	63	90
Malformation		N	12	11	11	10
		%	6	5	7	11
	Litters	N	9	7	7	5
		%	45	30	44	50
Fetal variations						
Skull						
- anterior fontanelle larger or slightly larger		N	5	3	7	1
		%	3	1	4	1
	Litters	N	3	3	2	1
		%	15	13	13	10
- anterior and posterior fontanelle enlarged (slight)		N	0	1	0	0
		%	0	0	0	0
	Litters	N	0	1	0	0
		%	0	4	0	0
- anterior fontanelle irregular shape		N	2	0	2	0
		%	1	0	1	0
	Litters	N	1	0	1	0
		%	5	0	6	0
- maxilla shorter (slight)		N	0	1	0	0
		%	0	0	0	0
	Litters	N	0	1	0	0
		%	0	4	0	0

(sum, %)

DOSE GROUPS (mg/kg bw/day)			Control	30	100	300
Number of Litters		N	20	23	16	10
Number of Fetuses examined		N	191	216	164	91
Sternebra						
- less than 5 ossified		N	1	0	0	1
		%	1	0	0	1
	Litters	N	1	0	0	1
		%	5	0	0	10
- bipartite		N	2	3	2	3
		%	1	1	1	3
	Litters	N	2	3	1	2
		%	10	13	6	20
- misaligned		N	1	1	1	1
		%	1	0	1	1
	Litters	N	1	1	1	1
		%	5	4	6	10
- dumb-bell shaped		N	1	1	1	0
		%	1	0	1	0
	Litters	N	1	1	1	0
		%	5	4	6	0
- one sternebrae misshapen ossification		N	0	1	1	1
		%	0	0	1	1
	Litters	N	0	1	1	1
		%	0	4	6	10
- cartilage pinched (slight)		N	1	0	0	0
		%	1	0	0	0
	Litters	N	1	0	0	0
		%	5	0	0	0
- supernumerary ossification point		N	1	0	0	2
		%	1	0	0	2
	Litters	N	1	0	0	1
		%	5	0	0	10
- fusing tendency		N	0	1	2	0
		%	0	0	1	0
	Litters	N	0	1	2	0
		%	0	4	13	0
Ribs						
- 7th not connected to sternum		N	0	1	0	1
		%	0	0	0	1
	Litters	N	0	1	0	1
		%	0	4	0	10
- interrupted 13th		N	4	7	2	1
		%	2	3	1	1
	Litters	N	3	5	2	1
		%	15	22	13	10
- 13th interrupted and bent		N	0	0	0	1
		%	0	0	0	1
	Litters	N	0	0	0	1
		%	0	0	0	10

(sum, %)

DOSE GROUPS (mg/kg bw/day)		Control	30	100	300
Number of Litters	N	20	23	16	10
Number of Fetuses examined	N	191	216	164	91
Vertebrae					
- thoracic centrum bipartite or/and asymmetric	N	4	1	0	1
	%	2	0	0	1
Litters	N	3	1	0	1
	%	15	4	0	10
- thoracic centrum asymmetric ossification	N	0	2	0	0
	%	0	1	0	0
Litters	N	0	2	0	0
	%	0	9	0	0
- thoracic centrum dumb-bell shaped or asymmetric dumb-bell shaped ossification	N	8	5	10	8
	%	4	2	6	9
Litters	N	5	5	5	5
	%	25	22	31	50
- thoracic centrum dumb-bell shaped cartilage (slight)	N	0	0	1	0
	%	0	0	1	0
Litters	N	0	0	1	0
	%	0	0	6	0
- coccygeal bipartite and dumb-bell shaped or and asymmetric ossification	N	0	2	0	0
	%	0	1	0	0
Litters	N	0	2	0	0
	%	0	9	0	0
- coccygeal less than 14 ossified	N	0	1	0	0
	%	0	0	0	0
Litters	N	0	1	0	0
	%	0	4	0	0
Pelvic girdle					
- pubic not ossified	N	3	2	0	2
	%	2	1	0	2
Litters	N	3	2	0	2
	%	15	9	0	20
Forelimb/hindlimb					
- talus not ossified	N	2	1	3	0
	%	1	0	2	0
Litters	N	1	1	2	0
	%	5	4	13	0
- metacarpal less than 3.5 ossified	N	0	0	1	0
	%	0	0	1	0
Litters	N	0	0	1	0
	%	0	0	6	0
- pollex not ossified	N	7	11	11	8
	%	4	5	7	9
Litters	N	6	7	5	4
	%	30	30	31	40
- proximal and middle phalanges less than 7/7 ossified	N	13	15	19	16 **
	%	7	7	12	18
Litters	N	5	10	6	6
	%	25	43	38	60

(sum, %)

DOSE GROUPS (mg/kg bw/day)			Control	30	100	300
Number of Litters		N	20	23	16	10
Number of Fetuses examined		N	191	216	164	91
Fetal malformations						
Skull						
- skull bones absent, short mandible		N	0	0	1	0
		%	0	0	1	0
	Litters	N	0	0	1	0
		%	0	0	6	0
Sternebra						
- fused		N	3	4	7	3
		%	2	2	4	3
	Litters	N	2	3	4	3
		%	10	13	25	30
- misaligned and fused		N	0	1	2	2
		%	0	0	1	2
	Litters	N	0	1	2	2 *
		%	0	4	13	20
- misaligned, bipartite and fused		N	0	2	0	0
		%	0	1	0	0
	Litters	N	0	2	0	0
		%	0	9	0	0
- summarized all fused		N	3	6	9 *	5
		%	2	3	5	5
	Litters	N	2	5	5	4
		%	10	22	31	40
- split in cartilage (slight)		N	1	0	0	0
		%	1	0	0	0
	Litters	N	1	0	0	0
		%	5	0	0	0
- xiphoid split or xiphoid split, slight		N	3	6	3	3
		%	2	3	2	3
	Litters	N	3	4	2	3
		%	15	17	13	30
- whole sternum wider (slight)		N	0	1	0	0
		%	0	0	0	0
	Litters	N	0	1	0	0
		%	0	4	0	0
Ribs						
- fused		N	4	1	0	0
		%	2	0	0	0
	Litters	N	4	1	0	0
		%	20	4	0	0
Ribs and vertebrae						
- multiple malformed		N	0	1	0	3 *
		%	0	0	0	3
	Litters	N	0	1	0	2 *
		%	0	4	0	20

(sum, %)

DOSE GROUPS (mg/kg bw/day)		Control	30	100	300
Number of Litters	N	20	23	16	10
Number of Fetuses examined	N	191	216	164	91
Fetal malformations					
Vertebrae					
- cervical arch absent unilateral	N	1	1	0	0
	%	1	0	0	0
Litters	N	1	1	0	0
	%	5	4	0	0
- thoracic centrum asymmetric including cartilage	N	0	1	0	1
	%	0	0	0	1
Litters	N	0	1	0	1
	%	0	4	0	10
- thoracic centrum cartilage dumb-bell shaped	N	0	1	0	0
(and dumb-bell shaped or bipartite ossification)	%	0	0	0	0
Litters	N	0	1	0	0
	%	0	4	0	0
- lumbar arches fused	N	0	1	0	0
(and dumb-bell shaped or bipartite ossification)	%	0	0	0	0
Litters	N	0	1	0	0
	%	0	4	0	0
- multiple malformed vertebrae	N	0	0	1	0
	%	0	0	1	0
Litters	N	0	0	1	0
	%	0	0	6	0
General					
- multiply malformed skeleton (malformed skull,	N	1	0	0	0
cleft palate, bent scapula)	%	1	0	0	0
Litters	N	1	0	0	0
	%	5	0	0	0

Remarks:
* – $p < 0.05$; CH^2
** – $p < 0.01$; CH^2

Visceral malformations:

There were no test item related adverse effects on the visceral development of fetuses.

Malformations

There was no significant difference in the number of malformed fetuses (2, 1 and 1 in the control, 30 and 300 mg/kg bw/day dose group respectively). Partial deficiency in the thalamus tissue was found in one fetus in the 300 mg/kg bw/day group. One fetus was found with an absence of one kidney at 30 mg/kg bw/day. Considering the low incidence, these malformations were not attributed to an effect due to the test item. Two fetuses had malformation of the great arteries/heart in the control group (enlarged right ventricle, thickened arteria pulmonalis and thin aortic arch originating directly next to arteria pulmonalis from the right ventricle).

Variations

Slightly or moderately enlarged space between cerebral hemisphere and thalamus or skull, slightly or moderately dilated IIIrd brain ventricle, convoluted- or hydroureter, malpositioned testis were found with single cases or low incidences and without a dose response, hence not attributed to the treatment.

Table 91: summary of types of visceral abnormalities

TYPES OF VISCERAL ABNORMALITIES

(Sum., %)

			DOSE GROUPS (mg/kg bw/day)			
			Control	30	100	300
Number of Litters		N	20	23	16	10
Number of Fetuses examined		N	191	216	164	91
Number of Fetuses with abnormalities		N	8	11	8	5
		%	4	5	5	5
	Litters	N	8	10	7	5
		%	40	43	44	50
Variation (Fetuses)		N	6	10	8	4
		%	3	5	5	4
	Litters	N	6	9	7	4
		%	30	39	44	40
Malformation (Fetuses)		N	2	1	0	1
		%	1	0	0	1
	Litters	N	2	1	0	1
		%	10	4	0	10
Fetal variations						
Brain						
- enlarged space between cerebral hemisphere and thalamus, slight or moderate		N	0	2	0	0
		%	0	1	0	0
	Litters	N	0	2	0	0
		%	0	9	0	0
- enlarged space between cerebral hemisphere and skull (vertex)		N	1	0	1	0
		%	1	0	1	0
	Litters	N	1	0	1	0
		%	5	0	6	0
- dilated IIIrd ventricle, slight or moderate		N	2	2	1	1
		%	1	1	1	1
	Litters	N	2	2	1	1
		%	10	9	6	10
Ureters						
- convoluted ureter		N	3	6	5	3
		%	2	3	3	3
	Litters	N	3	6	5	3
		%	15	26	31	30
- hydroureter		N	0	0	1	0
		%	0	0	1	0
	Litters	N	0	0	1	0
		%	0	0	6	0
Gonads						
- malpositioned testis		N	0	1	0	0
		%	0	0	0	0
	Litters	N	0	1	0	0
		%	0	4	0	0

(Sum., %)

		DOSE GROUPS (mg/kg bw/day)			
		Control	30	100	300
Number of Litters	N	20	23	16	10
Number of Fetuses examined	N	191	216	164	91
Fetal malformations					
Brain					
- partial deficiency in thalamus tissue	N	0	0	0	1
	%	0	0	0	1
Litters	N	0	0	0	1
	%	0	0	0	10
Great arteries or/and heart					
- enlarged right ventricle, arteria pulmonalis thickene	N	1	0	0	0
	%	1	0	0	0
Litters	N	1	0	0	0
	%	5	0	0	0
- thin aortic arch, originates directly next to arteria pulmonalis (from right ventricle)	N	1	0	0	0
	%	1	0	0	0
Litters	N	1	0	0	0
	%	5	0	0	0
Kidneys					
- absent unilateral	N	0	1	0	0
	%	0	0	0	0
Litters	N	0	1	0	0
	%	0	4	0	0

REMARKS:

* – $p < 0.05$; CH^2

** – $p < 0.01$; CH^2