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:

perboric acid (H3BO2(O2)), monosodium salt trihydrate [1]; perboric acid, sodium salt, tetrahydrate [2]; perboric acid (HBO(O2)), sodium salt, tetrahydrate; sodium peroxoborate, hexahydrate [3]

EC Numbers: 239-172-9 [1]; 234-390-0 [2]

CAS Numbers: 13517-20-9 [1]; 37244-98-7 [2]; 10486-00-7 [3]

Index Numbers: 005-018-00-2; 005-018-01-X

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1 PHYSICAL HAZARDS

Not assessed in this CLH-proposal.

2 HEALTH HAZARDS

Acute toxicity

2.1 Acute toxicity - oral route

2.1.1 Animal data

2.1.1.1 [Study 1] Acute oral toxicity study of PBS-4 in rats (OECD Guideline 401 (Acute Oral Toxicity)).

Study reference:

Study report (1987a). Acute oral toxicity of sodium perborate tetrahydrate in rats.

Detailed study summary and results:

Test type

Acute oral toxicity of sodium perborate tetrahydrate in rats according to
OECD Guideline 401 (Acute Oral Toxicity)

GLP: not specified

Test substance

- Sodium perborate tetrahydrate
- Degree of purity: unknown

Test animals

- Species/strain/sex: rat/ Wistar Bor:WISW (SPFTNO)/males and females
- No. of animals per sex per dose: 3/sex/dose group
- Source: Winkelmann GmbH & Co. KG, Borchen
- Age at study initiation: m: 49 days; f: 63 days
- Weight at study initiation: m: 137 - 148 g; f: 131 - 152 g
- Fasting period before study: overnight prior to dosing
- Housing: one animal per cage
- Diet: ad lib.
- Water: ad lib.
- Acclimation period: >= 5 days

Administration/exposure

- Mode of administration : oral (gavage)

- Doses/concentration levels: Doses: 2150, 2610 and 3160 mg/kg bw
- Vehicle: 1 % aqueous Tragant suspension
- Concentration in vehicle: 215, 261 or 316 mg/mL
- Amount of vehicle: 10 mL/kg bw

Details on study design

- Post exposure observation period: 14 days
- Frequency of observations: Clinical signs: 6 - 8 hrs post-dosing and thereafter once daily; Mortality: 1 - 2 times daily; Bodyweight: before dosing and days 7 and 14 post-dosing. Immediately in died animals.
- Necropsy of survivors and animals found dead performed: yes (gross)
- Statistics: According to Miller & Tainter (1944). Probit analysis (95 % CI) for both sexes.

Results

- LD50 males: : 2 670 mg/kg bw
- LD50 females: 2 360 mg/kg bw
- LD50 males/females: 2 567 mg/kg bw

- Mortality:
    - 2150 mg: no mortality
    - 2610 mg: 1/3 m and 3/3 f died within 48 hrs after dosing
    - 3160 mg: 3/3 m and 3/3 f died within 24 hrs after dosing
- Clinical signs: >= 2150 mg: decrease in locomotor activity, tremors, diarrhoea, rough fur, staggering gait, impaired general conditions
- Body weight: No significant changes
- Gross pathology: Tympany, aqueous to yellow liquid in stomach and intestine, reddening of glandular stomach.
- No adverse findings in surviving animals.

Executive summary of applicant

With Sodium Perborate Tetrahydrate an acute oral toxicity study was performed according to OECD Guideline 401. Three Wistar rats per sex and group were dosed once via gavage with 2150, 2610 and 3160 mg/kg bw. Animals were frequently monitored for clinical signs, body weight and mortality. All animals were examined for signs of macroscopic changes.

The acute oral toxicity for Sodium Perborate Tetrahydrate was calculated with 2670 mg/kg for males, 2360 mg/kg for females and 2567 mg/kg for combined sexes.

2.2 Acute toxicity - dermal route

2.2.1 Animal data

2.2.1.1 [Study 1] Acute dermal toxicity study in rats with PBS-1 (OECD TG 402, non-GLP).

Study reference:

Study Report (1987b). Acute dermal toxicity of sodium perborate monohydrate in rabbits.

Detailed study summary and results:

Test type

Acute dermal toxicity (OECD TG 402, non-GLP).

Test substance

- Sodium perborate monohydrate (PBS-1)
- Degree of purity: unknown

Test animals

- Species/strain/sex: rabbit/New Zealand White/males and females
- No. of animals per sex per dose: 5/sex

Administration/exposure

- Mode of administration: Occlusive dermal application
- Duration of test/exposure period: Single dermal dose, 24 h exposure
- Doses/concentration levels: 2000 mg/kg bw
- Post exposure observation period: 14 days

Results and reliability

- LD50 > 2000 mg/kg bw
- The acute dermal toxicity study (OECD TG 402) performed in rabbits with sodium perborate monohydrate established an LD_{50} > 2000 mg/kg bw. Clinical signs such as diarrhoea, few faeces, yellow nasal discharge and anogenital soiling were reported. One male rabbit died on day 13 post-treatment revealing abnormalities of the gastrointestinal tract, spleen, liver and lung. On day 1 post-treatment, 2/9 surviving rabbits showed skin irritation, which decreased in severity during the 14-day observation period, and distended intestines at the necropsy evaluation. No statistically significant effects on body weight were recorded.

2.3 Acute toxicity - inhalation route

2.3.1 Animal data

2.3.1.1 [Study 1] Acute inhalation toxicity study with PBS-4 in rats (non-guideline, GLP).

Study reference:

Study report (1987c). Acute inhalation toxicity of sodium perborate tetrahydrate in rats.

Detailed study summary and results:

Test type

Acute inhalation toxicity of sodium perborate tetrahydrate in rats, standard acute method, similar to OECD TG 403; GLP-compliant. Data concerning macroscopic or histopathological examinations not reported.

Test substance

- Sodium perborate tetrahydrate (PBS-4)
- CAS No. 10486-00-7
- Degree of purity: 98.6%
- Impurities (or a note that the impurities do not affect the classification)
- Batch number: 16.703 (Haskell Lab.)
- MMAD: 3.3 – 4.2 µm (dust)

Test animals

- Species/strain/sex: rats/ Crl:CD BR/males
- No. of animals per sex per dose: 6/dose group
- Age and weight at the study initiation: 8 weeks, 230 – 290 g

Administration/exposure

- Type of inhalation exposure: nose-only
- Duration of test/exposure period: 4 hours
- Doses/concentration levels: 0.16, 0.48, 1.10 and 2.90 mg/L
- Post exposure observation period: 14 days
- Statistical methods: LC 50 was calculated separately by Probit Analysis.

Characterization of Sodium Perborate Tetrahydrate Atmospheres and Associated Rat Mortality

Concentration (mg/m^3) Mean	S.D.	Range	% Particles < 10 um AD[a]	MMD(um)[b]	Mortality (# deaths/# exposed)
160	23	140 - 210	94	3.3	0/6
480	130	280 - 640	94	3.5	1/6
1100	240	720 - 1500	93	3.5	3/6
2900	1500	870 - 4900	86	4.2	5/6

[a] Percent by weight of particles with aerodynamic diameter (AD) less than 10 um.
[b] Mass median aerodynamic diameter.

Results

- LC50 value : 1.16 mg/L
- All deaths occurred within 24 hours.
- Clinical signs: During or immediately following exposure, rats from all groups exhibited gasping, red nasal discharge, and compound-covered faeces. Rats from the >= 480 mg/m3 groups also exhibited laboured breathing.

- Body weight: Rats typically had slight to severe (up to 18 % of initial body weight) weight losses within 24 hours of exposure.
- Pathology: no data.

Characterization of Sodium Perborate Tetrahydrate Atmospheres and Associated Rat Mortality

Concentration (mg/m^3) Mean	S.D.	Range	% Particles < 10 um AD[a]	MMD(um)[b]	Mortality (# deaths/# exposed)
160	23	140 - 210	94	3.3	0/6
480	130	280 - 640	94	3.5	1/6
1100	240	720 - 1500	93	3.5	3/6
2900	1500	870 - 4900	86	4.2	5/6

[a] Percent by weight of particles with aerodynamic diameter (AD) less than 10 um.
[b] Mass median aerodynamic diameter.

2.4 Skin corrosion/irritation

Not assessed in this CLH-proposal.

2.5 Serious eye damage/eye irritation

Not assessed in this CLH-proposal.

2.6 Respiratory sensitisation

Not assessed in this CLH-proposal.

2.7 Skin sensitisation

Not assessed in this CLH-proposal.

2.8 Germ cell mutagenicity

Not assessed in this CLH-proposal.

2.9 Carcinogenicity

Not assessed in this CLH-proposal.

2.10 Reproductive toxicity

2.10.1 Animal data

2.10.1.1 Adverse effects on sexual function and fertility

2.10.1.1.1 [Study 1] 28-day repeated dose toxicity study in rats administered PBS-4 (OECD TG 407, Limit test; GLP).

Study reference:

Study report (1989). Sodium perborate tetrahydrate 4-week oral toxicity study after repeated administration in rats. Unpublished report.

Detailed study summary and results:

Test type

Repeated dose 28-day oral toxicity study in rats, according to OECD TG 407, GLP-compliant.

Only one dose level – limit test.

Test substance

- Sodium perborate tetrahydrate (PBS-4)
- CAS No. 10486-00-7
- Degree of purity - >98%

Test animals

- Species/strain/sex – rat/WISW (SPFCpb)/males and females
- No. of animals per sex per dose – 5/sex
- Age and weight at the study initiation – 7 weeks; males: 139-173 g; females: 108-131 g

Administration/exposure

- route of administration – oral (gavage)
- duration and frequency of test/exposure period – once daily, 7 days/week for 4 weeks
- doses/concentration levels, rationale for dose level selection – 1000 mg/kg bw/day; dose rationale based on a dose finding study
- control group – 1% aqueous tylose suspension
- test substance concentration of the vehicle suspension – 215 mg/L

Description of test design:

- CAGE SIDE OBSERVATIONS: 1 - 2 times daily incl. observations for mortality
- DETAILED CLINICAL OBSERVATIONS: Daily check for clinical signs (behavioural changes, first occurrence, progress, intensity and duration of signs of toxicity). Prior to study start and at termination: testing of reflexes (pain, pinna and corneal reflexes) as well as examinations of eyes, teeth, or hearing.
- BODY WEIGHT: once weekly, starting with pre-study period.
- FOOD CONSUMPTION: once weekly
- FOOD EFFICIENCY: No data
- WATER CONSUMPTION: No data
- OPHTHALMOSCOPIC EXAMINATION: prior to study start and at termination in all animals

- HAEMATOLOGY: during week 4 in all animals

  Anaesthetic used for blood collection: CO2 anaesthesia. Parameters examined: RBC, Hct, Hb, WBC, MCH, MCHC, MCV, thrombocytes (platelets) and differential leucocyte count

- CLINICAL CHEMISTRY: during week 4 in all animals

  Parameters examined: Alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, BUN, Ca, Cl, CHE, CK, creatinine, gamma-glutamyltransferase, glucose, inorganic phosphorus, K, Na, total bilirubin, total cholesterol, total protein, triglycerides

- URINALYSIS: during week 4 in all animals

  Parameters examined: bilirubin, glucose, haemoglobin, ketones, leucocytes, nitrite, osmolality, pH-value, protein, urobilinogen and microscopic sediment examination in animals whose urine state showed pathological changes in leucocytes, protein, or haemoglobin.

- GROSS PATHOLOGY: all animals
- HISTOPATHOLOGY: adrenal glands, bone (sternum), bone marrow smears, brain, caecum, colon, duodenum, heart, ileum, jejunum, kidneys, liver, lungs, ovaries, rectum, skin, spleen, stomach, testes.
- STATISTICS: Dunnett or Steel-test.

Results and discussion

- Clinical symptoms: The only clinical symptom observed in almost all treated rats was salivation, in males also ofreddish colour. One male additionally performed stilted gait, sunken sides and piloerection. During the last 4 days of treatment piloerection was observed in two additional animals. Mortality did not occur during the study.

- Food consumption and body weight: The food consumption as well as the body weight gain were reduced in males after start of administration. The difference reached about 15% in week 4 for both parameters. Females vere not affected.

- Clinical pathology: Slight decreases were present in treated animals of both sexes in red blood cell count heamoglobin content and hematocrit. The value of mean corpuscular haemoglobin concentration was slightly increased, white blood cell count and absolute lymphocyte count were decreased only in males. The number of platelets was slightly elevated in both males and females . Reduction of absolute lymphocyte number with corresponding decrease in white blood cell count was also present in females but without statistical significance.

- Clinical chemistry: slight but statistically significantly decreased values were found for alkaline phosphatase, total protein and cholinesterase in both sexes. The serum levels of bilirubin, inorganic phosphorus and potassium were increased in males only.

- Urinanalysis: no alterations were present.

- Organ weights: the aboslute wights of hearts, brains, kidneys and testes were reduced in male sonly. The relative weight (to body weight) of these organs were not affected. Slight increases were noticed in the relative weight of adrenals in males and livers in females.

- Necropsy: reduction of spleen size in 2 treated males.

- Histopathology: test substace-related finsings in the spleen in males only, and in the stomach in males and females. Mild general reduction of splenic parenchyma in males without specific finsings. The gastric changes consist of minimal to slight acanthosis and hyperkeratosis in the forestomach, slight to moderate hyperplasia of fundic mucosa, especially of the gastric pits.

ORGAN/BODY WEIGHT RATIOS SUMMARY
Week 5 (Main-Group)
MALES

		GROUP 1 CONTROL	GROUP 2 1000
B.- Weight	MEAN	249	206 *
(GRAM)	ST.DEV.	7	29
	T STAT	---	-3.18
	MINIMUM	243	180
	MAXIMUM	260	251
	N	5	5
Brain	MEAN	0.70	0.77
(%)	ST.DEV.	0.02	0.08
	T STAT	---	2.26
	MINIMUM	0.68	0.67
	MAXIMUM	0.72	0.85
	N	5	5
Heart	MEAN	0.45	0.45
(%)	ST.DEV.	0.05	0.04
	T STAT	---	0.10
	MINIMUM	0.40	0.40
	MAXIMUM	0.51	0.50
	N	5	5
Liver	MEAN	4.42	4.17
(%)	ST.DEV.	0.69	0.25
	T STAT	---	-0.77
	MINIMUM	3.95	3.90
	MAXIMUM	5.61	4.50
	N	5	5
Kidney l.	MEAN	0.39	0.39
(%)	ST.DEV.	0.03	0.02
	T STAT	---	-0.27
	MINIMUM	0.36	0.37
	MAXIMUM	0.42	0.41
	N	5	5
Kidney r.	MEAN	0.37	0.39
(%)	ST.DEV.	0.03	0.03
	T STAT	---	1.36
	MINIMUM	0.35	0.36
	MAXIMUM	0.41	0.43
	N	5	5
Adrenal l.	MEAN	0.009	0.012 *
(%)	ST.DEV.	0.001	0.002
	T STAT	---	2.64
	MINIMUM	0.008	0.010
	MAXIMUM	0.011	0.014
	N	5	5
Adrenal r.	MEAN	0.008	0.011 **
(%)	ST.DEV.	0.001	0.001
	T STAT	---	3.82
	MINIMUM	0.006	0.009
	MAXIMUM	0.009	0.012
	N	5	5
Testis l.	MEAN	0.84	0.84
(%)	ST.DEV.	0.03	0.07
	T STAT	---	0.00
	MINIMUM	0.80	0.78
	MAXIMUM	0.87	0.94
	N	5	5
Testis r.	MEAN	0.85	0.85
(%)	ST.DEV.	0.04	0.08
	T STAT	---	0.07
	MINIMUM	0.80	0.76
	MAXIMUM	0.90	0.95
	N	5	5

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

ORGAN/BODY WEIGHT RATIOS SUMMARY
Week 5 (Main-Group)
FEMALES

		GROUP 1 CONTROL	GROUP 2 1000
B.- Weight	MEAN	157	159
(GRAM)	ST.DEV.	12	9
	T STAT	---	0.36
	MINIMUM	142	150
	MAXIMUM	171	172
	N	5	5
Brain	MEAN	1.02	0.99
(%)	ST.DEV.	0.10	0.09
	T STAT	---	-0.43
	MINIMUM	0.90	0.88
	MAXIMUM	1.13	1.12
	N	5	5
Heart	MEAN	0.50	0.49
(%)	ST.DEV.	0.06	0.02
	T STAT	---	-0.34
	MINIMUM	0.45	0.46
	MAXIMUM	0.59	0.51
	N	5	5
Liver	MEAN	4.44	4.87 *
(%)	ST.DEV.	0.27	0.26
	T STAT	---	2.56
	MINIMUM	4.18	4.51
	MAXIMUM	4.88	5.12
	N	5	5
Kidney l.	MEAN	0.38	0.39
(%)	ST.DEV.	0.03	0.04
	T STAT	---	0.41
	MINIMUM	0.34	0.36
	MAXIMUM	0.41	0.43
	N	5	5
Kidney r.	MEAN	0.39	0.39
(%)	ST.DEV.	0.04	0.03
	T STAT	---	0.20
	MINIMUM	0.35	0.36
	MAXIMUM	0.43	0.43
	N	5	5
Adrenal l.	MEAN	0.018	0.020
(%)	ST.DEV.	0.004	0.002
	T STAT	---	1.22
	MINIMUM	0.016	0.017
	MAXIMUM	0.024	0.022
	N	5	5
Adrenal r.	MEAN	0.018	0.017
(%)	ST.DEV.	0.004	0.004
	T STAT	---	-0.24
	MINIMUM	0.015	0.012
	MAXIMUM	0.024	0.022
	N	5	5
Ovar l.	MEAN	0.036	0.030
(%)	ST.DEV.	0.010	0.004
	T STAT	---	-1.33
	MINIMUM	0.029	0.023
	MAXIMUM	0.054	0.034
	N	5	5
Ovar r.	MEAN	0.036	0.033
(%)	ST.DEV.	0.004	0.004
	T STAT	---	-1.22
	MINIMUM	0.031	0.026
	MAXIMUM	0.041	0.037
	N	5	5

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

ORGAN/FINDING		DOSE GROUP:	01		02	
		SEX:	M	F	M	F
		NO.ANIMALS:	5	5	5	5
LIVER	NO. EXAM.:		5	5	5	5
- Mononucl.c.inf. foc.			1	4	2	5
- Small inflammat.foci			3	5		4
KIDNEYS	NO. EXAM.:		5	5	5	5
- Pelvic dilatation				1		
- Basophilic tubules			1		2	1
- Hyaline casts						1
- Mononucl.c.inf. foc.			1	1		2
- Nephritis interstit.			1		1	
HEART	NO. EXAM.:		5	5	5	5
- Mononucl.c.inf. foc.			1	1	2	2
- Arteritis			1			
- Fibrosis subendocard				1		
SPLEEN	NO. EXAM.:		5	5	5	5
- Increas.hematopoies.						1
- Reduction parenchyma					5	
LUNGS	NO. EXAM.:		5	5	5	5
- Heomorrhage acute			1		2	
- Histiocytosis focal			3	4	3	5
STOMACH	NO. EXAM.:		5	5	5	5
- Hemorrhage acute					1	
- Cyst(s) mucosal				1		
- Akanthos./Hyperkerat				1	4	4
- Hyperplasia fund.muc					5	4
- Gastritis subacute						1
BRAIN	NO. EXAM.:		5	5	5	5
- Hemorrhage acute			2	1	1	1

2.10.1.1.2 [Study 2] Two-year feeding study with boric acid in rats

Study reference:

Study report (1966d). Two-year dietary feeding study with boric acid in rats. REACH Registration dossier for boric acid, publicly available at https://www.echa.europa.eu/sv/web/guest/registration-dossier/-/registered-dossier/15472/1.

Study report (1967). Two-year dietary feeding study with boric acid in rats. REACH Registration dossier for boric acid, publicly available at https://www.echa.europa.eu/sv/web/guest/registration-dossier/-/registered-dossier/15472/1.

Guideline:

- No guideline followed for 90-day oral repeated dose toxicity studies.
  No guideline specified for the reproductive toxicity study, but conforms to the standard three-generation, 2 litters per generation multi-generation studies normally used at the time.

Reliability :

- Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species /strain: Rat, Sprague-Dawley (male/female)**,** Beagle dogs (male/female)

Test material:

- Boric acid or borax
- Purity: unknown

Materials and methods:

1. **90-day oral repeated dose toxicity study in rats and dogs (Study 1 and 2)**

Male and female rats er group were placed for a period of 90 days on dietary concentrations of borax or boric acid at 52.5, 175, 525, 1750 and 5250 ppm as boron equivalent added with thorough mixing to the basal diet on a w/w basis. In addition, five young male and five female beagle dogs per group were placed for a period of 90 days on dietary concentrations of borax or boric acid at 17.5, 175 and 1750 ppm as boron equivalent added to the laboratory diet. In both studies all animals were individually caged.

Route of administration: oral, feed

Exposure: 90 days

Doses / Concentrations:

- in rats: 0, 52.5, 175, 525, 1750 and 5250 ppm boron, equivalent to 0, 4.7, 15.7, 47.2, 157.5 and 472.5 mg B/kg

bw/day, respectively

- in dogs: 0, 17.5, 175, and 1750 ppm boron, equivalent to 0, 0.4, 4.3 and 43.7 mg B/kg bw/day, respectively

No. of animals: 10 rats/sex/dose group and 5 dogs/sex/dose group

Body weights: Body weights and food consumption were measured at weekly intervals.

Clinical observations: Hematologic studies included packed cell volume, hemoglobin, erythrocyte count, total and differential leukocyte counts on all dogs initially, at 2 and 4 wk and at termination. Biochemical studies including blood urea nitrogen, blood sugar, serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase were performed at the same time. Urine samples were analyzed for specific gravity, pH, protein, sugar, bilirubin, acetone and sediment at similar intervals. Survivors were sacrificed after 90 days on the diet.

Necropsy evaluation: the weights of brain, thyroid, liver, spleen, kidney, adrenals and testes were recorded.The tissues preserved in buffered formalin and studied histopathologically were brain. pituitary, thyroids, lung, heart, liver, spleen, kidneys, adrenals, pancreas, small and large intestines, urinary bladder, testes, ovary (for rat only), bone and bone marrow.

Statistics: Numerical deviation from the control observations were evaluated by conventional statistical tests using $P < 0.05$ as the fiducial limit (Snedecor,1956).

2. **Reproductive toxicity study (Study 3)**

Prior to initiation of the first breeding phase, the male and female rats were maintained in individual cages and fed their respective diets for 14 wk. After the 14 wk feeding period, 1 male and 2 females were placed in each breeding cage. At 24 hr after birth, the litters were reduced to a maximum of 8 progeny to be raised. The first filial generation (F1A) was carried through weaning and discarded. The parental generation (P1) was rebred to produce their second litter (F1B). At the time of weaning, 16 females and 8 males each from the control and test groups were selected at random and designated the second parental generation (P2) for continuation of the reproduction study. These animals were bred to produce the F2A and F2B litters as before. The F2B litter became the P3 generation and were bred to produce the F3A and F3B litters.

Route of administration: oral, feed

Exposure: from the beginning of the study (14 weeks pre-mating exposure) until sacrifice of parents P1, and from weaning until sacrifice of the F1- and F2-generations

Doses / Concentrations: 0, 117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day

No. of animals: 8 males/dose group and 16 females/dose group

Body weights: Body weight and food consumption were recorded weekly.

Necropsy evaluation: With the exception ofthe P1, P2 and P3, control and test groups, necropsies were performed on all rats.

Statistics: Numerical deviation from the control observations were evaluated by conventional statistical tests using $P < 0.05$ as the fiducial limit (Snedecor,1956).

3. **Two-year feeding study with boric acid in rats (Study 4)**

The control group of 70 male and 70 female weanling rats (Sprague-Dawley strain) received the basal diet. Test groups of 35 male and 35 female weanling rats each received a diet containing borax or boric acid at 117, 350 and 1170 ppm as boron equivalent, for a period of 2 yr. All animals were individually housed and provided with free access to the diet and drinking water.

Route of administration: oral, feed

Exposure: 2 years

Doses / Concentrations: 0, 117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day

No. of animals: 70/sex/dose group for controls and 35/sex/dose group for the treatnebt

Body weights: Data on body weight, food consumption and toxic signs were recorded regularly (interval not specified).

Clinical parameters: Biochemical studies and urine analyses (as described in study 1 above) were carried out on all dogs at similar intervals. Pooled urine samples from 5 rats each were analyzed at 6,18 and 24 mo. Samples of blood for hematologic studies were taken from representative rats in each group at seven intervals during the 2 yr feeding period.
Organ weights: Organ weights (organs involved were described in study 1 above) were recorded and organ weight/body weight rations were calculated.
Histopathology examination: Sections of tissues involved (as described in Stuyd 1 above) were examined for histopathologic alteration.
Necropsy evaluation: Five rats of both sexes from each group at 6 and 12 mo, and all survivors at 2 yr were sacrificed and necropsied.
Statistics: Numerical deviation from the control observations were evaluated by conventional statistical tests using $P < 0.05$ as the fiducial limit (Snedecor, 1956).

Results:

1. **90-day oral repeated dose toxicity study in rats and dogs (Study 1 and 2)**

- **Rats**

Clinical observations: the physical appearance of the rats receiving either borax or boric acid at levels at and below 525 ppm boron were generally comparable to those of the controls throughout the study. Rats fed 1750 and 5250 ppm of boron as borax or boric acid had a rapid respiration, inflamed eyes, swollen paws, and desquamated skin on the paws and tails. These animals appeared excited when handled. All males had a shrunken scrotum during the last weeks of the study.
Mortality: One rat each at 52.5 and 1750 ppm of boron (in borax) died during the study. At 5250 ppm of boron, both borax and boric acid killed all rats within 3 to 6 wk.
Feed consumption: Growth and food utilization efficiency were significantly reduced for males fed borax at 1750 ppm boron content and for both males and females at 5250 ppm boron. Boric acid at 525 ppm (or less) as boron equivalent did not affect the growth, food consumption and food efficiency. At 1750 ppm boron levels, boric acid reduced growth and food consumption in both males and females.
Organ weights: Borax at 52.5 ppm as boron equivalent caused an increase in the weight of brain, spleen, kidneys and ovaries in female rats, while boric acid at the same level caused an increase in liver weight; no changes of organ weights occurred in male rats. Increase in kidney weight was observed in males fed borax at 175 ppm boron content.
Rats which received either borax or boric acid at 525 ppm of boron showed organ weights comparable to those of the controls. Male rats fed boron compounds at 1750 ppm boron content had a significant decrease in body weight and the weights of liver, spleen, kidneys and testes; borax at this level also caused a reduction in brain weight, while boric acid lowered adrenal weight.

Table: Changes in body weights in male rats administered 1750 ppm boron

	Control	Borax	Boric acid
Body	477 + 31	215 + 90[b]	268 ± 44[b]
Brain	2.13 ± 0.17	1.86 ± 0.11[b]	1.97 ± 0.15
Thyroids	0.019 ± 0.004	0.015 ± 0.002	0.016 ± 0.004
Liver	16.85 ± 1.35	7.04 ± 1.42[b]	7.41 ± 1.61[b]
Spleen	0.78 ± 0.12	0.34 ± 0.09[b]	0.40 ± 0.11[b]
Kidneys	3.08 ± 0.34	1.92 ± 0.32[b]	1.89 ± 0.33[b]
Adrenals	0.046 ± 0.006	0.039 ± 0.008	0.037 ± 0.009[b]
Testes	3.50 ± 0.26	0.79 ± 0.17[b]	0.83 ± 0.11[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

Female rats which received the same dose levels of either borax or boric acid had decreases in body weight and weights of liver, spleen and ovaries; in addition, boric acid caused a fall in adrenal weight.

Table: Changes in body weights in female rats administered 1750 ppm boron

	Control	Borax	Boric acid
Body	247 ± 21	222 ± 28[b]	216 ± 28[b]
Brain	1.91 ± 0.09	1.91 ± 0.13	1.91 ± 0.17
Thyroids	0.015 ± 0.003	0.015 ± 0.003	0.018 ± 0.006
Liver	7.90 ± 1.20	6.40 ± 1.46[b]	6.38 ± 1.16[b]
Spleen	0.52 ± 0.12	0.39 ± 0.11[b]	0.38 ± 0.06[b]
Kidneys	1.88 ± 0.15	1.75 ± 0.28	1.73 ± 0.19
Adrenals	0.05 ± 0.01	0.040 ± 0.009[b]	0.047 ± 0.007
Ovaries	0.124 ± 0.02	0.071 ± 0.025[b]	0.090 ± 0.030[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

An increase in brain/body weight ratio occurred in female rats fed borax at 52.5 ppm, while boric acid at the same dose level was accompanied by a decrease in brain/body weight ratio in male rats. Borax caused an increase in kidney/body weight ratio at 525 ppm.Both boron compounds, when fed to rats at 1750 ppm, caused increases in brain, thyroids and adrenal/body weight ratios in the males.

Table: Changes in body weight: body weight ratios (%) for male rats administered 1750 ppm boron

	Control	Borax	Boric acid
Brain	0.447 ± 0.003	0.81 ± 0.20[c]	0.75 ± 0.11[c]
Thyroids	0.814 ± 0.002	0.007 ± 0.002[c]	0.006 ± 0.002[c]
Liver	3.54 ± 0.27	2.96 ± 0.23[b]	2.77 + 0.34[b]
Spleen	0.17 ± 0.02	0.14 ± 0.02	0.15 ± 0.04
Kidneys	0.65 ± 0.08	0.81 ± 0.08[c]	0.71 ± 0.09
Adrenals	0.010 ± 0.001	0.015 ± 0.002[c]	0.014 ± 0.003[c]
Testes	0.73 ± 0.06	0.34 ± 0.04[b]	0.32 ± 0.07[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.
[c] Significantly higher than controls, $p < 0.05$.

In addition to these findings, borax also increased kidney/body weight ratio. There was an increase in brain/body weight ratios in female rats receiving either borax or boric acid at 1750 ppm boron content.

Table: Changes in organ weight: body weight ratios (%) for female rats administered 1750 ppm boron

	Control	Borax	Boric acid
Brain	0.78 ± 0.07	0.90 ± 0.12[c]	0.90 ± 0.14[c]
Thyroids	0.006 ± 0.001	0.007 ± 0.001	0.008 ± 0.003[c]
Liver	3.19 ± 0.21	3.06 ± 0.26	2.95 ± 0.22[b]
Spleen	0.20 ± 0.03	0.18 ± 0.03	0.18 ± 0.04
Kidneys	0.75 ± 0.05	0.79 ± 0.06	0.81 ± 0.05
Adrenals	0.022 ± 0.002	0.019 ± 0.005	0.022 ± 0.002
Ovaries	0.05 ± 0.01	0.034 ± 0.010[b]	0.042 ± 0.013

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.
[c] Significantly higher than control, $p < 0.05$.

Furthermore, borax decreased ovaries/body weight ratio: boric acid increased thyroids and decreased liver/body weight ratios. There were no changes of organ/brain weight ratios in the rats fed either borax or boric acid at 52.5, 175 and 525 ppm as boron equivalent. Both borax and boric acid at 1750 ppm boron content caused decreases in liver, spleen, kidneys and testes/brain weight ratios in male rats.

Table: Organ: brain weight ratios (%) for male rats administered 1750 ppm boron

	Control	Borax	Boric acid
Thyroids	0.88 ± 0.22	0.81 ± 0.16	0.79 ± 0.21
Liver	795 ± 68	378 ± 72[b]	373 ± 58[b]
Spleen	37.0 ± 5.0	18.0 ± 4.0[b]	20.0 ± 5.0[b]
Kidneys	146 ± 20	103 ± 16[b]	95.2 ± 11.1[b]
Adrenals	2.2 ± 0.3	2.1 ± 0.4	1.9 ± 0.4
Testes	165 ± 14	42.4 ± 7.7[b]	42.4 ± 5.8[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

Female rats receiving boron compounds at the Furthermore, borax at 1750 ppm boron content also caused a decrease
in adrenals/brain weight ratio in female rats.
Necropsy examination: Necropsies performed on the animals that died (one each from 52.5 and 1750 ppm boron levels of borax and all rats at 5250 ppm boron level of borax and boric acid) showed congestion of liver and kidneys,
bright red lungs and in several animals a swollen appearance of the brain, small gonads and a thickened pancreas. Microscopic examination of the tissues revealed complete atrophy of testes in all males fed either borax or boric acid
at 1750 ppm as boron equivalent, partial atrophy in 4 males at 525 ppm of borax and in 1 at 525 ppm of boric acid. Spermatogenic arrest was found in 1 male at 525 ppm of borax. The adrenals of the majority of the males and several
females at 1750 ppm boron equivalent of borax revealed a slight to moderate increase in lipid content and the size of
the cells in the zona reticularis; the adrenals of 4 males at 1750 ppm boron content of boric acid had similar changes
but to a lesser degree.

- **Dogs**

Clinical observations: dogs, with one exception, fed both borax and boric acid at 17.5, 175 and 1750 ppm as boron equivalent were essentially normal in appearance, behavior,elimination, body weights and food consumption.
Mortality: One male dog at 1750 ppm level of boron as borax died of diarrhea on day 68 of the study and showed congested kidneys and severe congestion of the mucosa of small and large intestines.
Clinical parameters: Hematologic, biochemical and urine values were within normal limits except for 2 male and 3 female dogs in the high borax level group (1750 ppm boron content). These animals had decreased packed cell volume and hemoglobin values during the study.
Organ weights: The spleen/body weight ratio in male dogs at 17.5 ppm level of boron as borax was significantly lower than that of the controls. At 175 ppm boron content, as boric acid, a decrease in testes/body weight ratio was observed. Both borax and boric acid caused significant decreases in thyroids and testes/body weight ratios in dogs at 1750 ppm boron content.

Table: Mean body weights, organ weights and organ:body weight ratios (%) of male and female dogs administered 1750 ppm boron

	Control		Borax		Boric acid	
	Weight	Ratio	Weight	Ratio	Weight	Ratio
Male						
Body weight	8.5 ± 1.9		9.3 ± 0.8		8.6 ± 1.0	
Thyroids	0.77 ± 0.14	0.009 ± 0.001	0.59 ± 0.13	0.006 ± 0.001[b]	0.48 ± 0.18	0.006 ± 0.002[b]
Testes	1.72 ± 3.3	0.20 ± 0.03	9.6 ± 3.4	0.10 ± 0.03[b]	10.5 ± 1.5	0.12 ± 0.02[b]
Female						
Body weight	6.2 ± 2.0		7.7 ± 1.2		9.0 ± 2.3	
Brain	68.7 ± 7.1	1.1 ± 0.4	80.3 ± 3.1[c]	1.10 ± 0.15	72.3 ± 2.7	0.85 ± 0.23
Liver	190.0 ± 47.0	2.8 ± 0.5	257 ± 47	3.3 ± 0.5	345 ± 49	4.1 ± 1.2[c]

[a] All numbers are expressed as mean ± SD for four male and five female dogs. All lower levels are comparable to controls.
[b] Significantly lower than control at $p < 0.05$
[c] Significantly higher than control at $p < 0.05$.

Other organs including spleen, liver, kidneys and adrenals were found to be within normal limits. Neither borax nor boric acid at 17.5 and 175 ppm boron content levels produced any changes in organ weights and organ/body weight ratios in female dogs. Increases in brain/body weight ratio and liver/body weight ratio occurred in dogs fed 1750 ppm boron content levels of borax and boric acid, respectively.

Histopathology examination: No histologic alterations were seen in dogs fed 175 ppm (or less) of boron in boric acid. Both borax and boric acid at the 1750 ppm boron level produced severe testicular atrophy in all male dogs. Degeneration of the spermatogenic epithelium was generally complete. Red blood cell destruction, as indicated by the presence of hemosiderin in the reticular cells of the liver and spleen and the proximal tubules of the kidney, was somewhat greater in the animals that received borax than in those that received boric acid. The thyroid gland of the borax treated males presented a slightly greater proportion of solid epithelial nests and minute follicles than was found in the control animals, In the adrenal gland, the zona reticularis was consistently increased in width in borax fed dogs and only in boric acid treated female dogs. The high level of boric acid (1750 ppm boron content) also increased the width of the zona glomerulosa in the adrenals of the female dogs. The zona fasciculata was, in general, somewhat decreased in width. The thyroids of the two females were infiltrated by lymphoid tissue, and one was rather markedly atrophied.

2. Reproductive toxicity study (Study 3)

There were no adverse effects on the reproduction of rats receiving a diet containing either borax or boric acid at 117 and 350 ppm as boron equivalent. Litter size, weights of progeny and appearance were normal compared with those of the controls. The overall fertility indices for the two test compounds at levels of 177 and 350 ppm boron were significantly higher than those of the controls. Live birth indices were within normal limits in the test groups.

Table: Fertility indices for F1, F2 and F3 filial generations of rats (5.9 and 17.5 mg B/kg bw/day administered as boric acid or borax)

Index	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day
Fertility index [a]	**Borax**					
	P1-F1A			P1-F1B		
	62.5	68.8	75	60	62.5	75
	P2-F2A			P2-F2B		
	81.3	81.3	100	80	75	93.8
	P3-F3A			P3-F3B		
	68.8	87.5	100[b]	68.8	87.5	100[b]
	Boric acid					
	P1-F1A			P1-F1B		
	62.5	87.5	81.3	60	87.5	75
	P2-F2A			P2-F2B		
	81.3	93.8	93.8	80	93.8	93.8
	P3-F3A			P3-F3B		
	68.8	100[b]	87.5	68.8	93.8	93.8

[a] Fertility index: number of pregnancies/number of matings x 100.

[b] Significantly higher than controls.

Histopathological examination: No gross abnormalities were observed in the organs examined from either parents or weanlings. Evidence was also found of decreased ovulation in the majority of the ovaries examined from the same level females sacrificed following the reproduction study (data not shown).

Mating: The high level test groups fed both borax and boric acid at 1170 ppm as boron equivalent were found to be sterile. An attempt to obtain litters by mating the treated females with the males fed only the basal diet was not successful. Microscopic examination revealed the lack of viable sperm in the atrophied testes of all males at the 1170 ppm boron equivalent level of both borax and boric acid.

For all filial generations (i.e. F1, F2 and F3), for both low- and mid-dose groups, the litter size, weights of progeny and appearance were not statistically significantly different from controls (data not shown). No other information on maternal toxicity is reported.
At 58.5 mg/kg bw/day there were no offspring produced from P1 animals.

Table: Live birth indices for F1, F2 and F3 filial generations of rats (5.9 and 17.5 mg B/kg bw/day administered as boric acid or borax)

Index	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day
Borax						
Live birth index[a]	P1-F1A			P1-F1B		
	98.4	98.4	100	99.1	99.2	99.4
	P2-F2A			P2-F2B		
	97.8	99.4	96.9	98.6	92.4	98.8
	P3-F3A			P3-F3B		
	100	100	99.4	100	100	100
	Boric acid					
	P1-F1A			P1-F1B		
	98.4	96	97.2	99.1	99.4	100
	P2-F2A			P2-F2B		
	97.8	100	99.4	98.6	99.4	97.9
	P3-F3A			P3-F3B		
	100	99.5	97.9	100	99	98.8

[a] Live birth index = number of pups born alive/number of born pups x 100.

3. Two-year feeding study in rats (Study 4)

Clinical observations: The appearance and behavior of the rats fed both borax and boric acid at 117 and 350 ppm as boron equivalent in the diets were generally comparable with those of the controls. The following gross signs were observed among the rats at the highest level (1170 ppm boron content): coarse hair coats, scaly tails, a hunched position, swelling and desquamation of the pads of the paws, abnormally long toenails, shrunken appearance of the scrotum of the males, inflamed eyelids and bloody discharge of the eyes. Onset of these signs was at the beginning of the second month. They became more frequent and pronounced by the end of the first year, but remained relatively unchanged during the second year.
Feed consumption: Both borax and boric acid at 1170 ppm as boron equivalent lowered food consumption during the first 13 wk and suppressed growth in rats throughout the 2 yr study.
Clinical parameters: Low packed cell volume and hemoglobin values found at many intervals during the study are considered to be significant in male and female rats fed borax at 1170 ppm as boron equivalent, and in female rats which received the same level of boric acid. Biochemical values and urine analyses were found to be within normal limits in rats which received different levels of both boron compounds.
Organ weights: The testes weights and testes/body weight ratios were significantly lower, whereas the brain and thyroid/body weight ratios were significantly higher than those of the controls (data not shown).

Histopathology examination: There were no histologic alterations in the organs of rats fed either borax or boric acid at 117 and 350 ppm levels as boron equivalent for 2 yr. Atrophic testes were found in all males receiving 1170 ppm boron in both borax and boric acid at 6, 12 and 24 mo. Microscopic examination revealed atrophied seminiferous epithelium and decreased tubular size in the testes.

Table: Testes atrophy was observed at 24 months

Dose level (mg B/kg bw/day)	**0**	**5.9**	**17.5**	**58.5**
No. of animals	3/10	1/10	4/10	10/10

Conclusion
Rats exposed to the high dose of 336 mg/kg bw boric acid (corresponding to a level of 58.5 mg B/kg bw) were sterile. Microscopic examination of the atrophied testes of all males in this group showed no viable sperm. The authors also reported evidence of decreased ovulation in about half of the ovaries examined from the females exposed to 58.5 mg B/kg bw and only 1/16 matings produced a litter from these high dose females when mated with control male animals. There were no adverse effects on reproduction reported at exposures of 34 and 100 mg/kg bw boric acid (5.9 and 17.5 mg B/kg bw). The authors reported no adverse effects on fertility, lactation, litter size, progeny weight or appearance in rats exposed to either 5.9 or 17.5 mg B/kg bw. Also, no gross abnormalities were observed in the organs examined from either parents or weanlings from these dose groups.

2.10.1.2 Adverse effects on the development of the offspring

2.10.1.2.1 [Study 1] Prenatal developmental toxicity study with PBS-4 in rats (OECD TG 414, GLP).

Study reference:

Study report (1995). Sodium perborate tetrahydrate: teratogenesis study in rats by oral route. Unpublished report.

Detailed study summary and results:

Test type

Prenatal developmental toxicity study in rats, according to OECD TG 414, GLP-compliant, no deviations.

Test substance

- Sodium perborate tetrahydrate (PBS-4)
- CAS No. 10486-00-7
- Degree of purity – unknown
- Batch number – CONFIDENTIAL

Test animals

- Species/strain/sex – rat/Crl:CD (SD) BR/females
- No. of animals per sex per dose – 25 females/dose group
- Age and weight at the study initiation – approx. 11 weeks old, 200-225 g at receipt.

Administration/exposure

- route of administration – oral (gavage)
- duration and frequency of test/exposure period – daily from day 6 through day 15 of pregnancy (GD 6 – 15)
- doses/concentration levels, rationale for dose level selection - The dosages had been established in accordance with the Sponsor and on the basis of the results of the preliminary teratogenesis study (results of the preliminary study not available).

Group 1	l % Methylcellulose 400 cps aqueous solution as control article
Group 2	100 mg/kg/day of PBS-4
Group 3	300 mg/kg/day of PBS-4
Group 4	1000 mg/kg/day of PBS-4

Administration volume - 10 mL/kg/day, calculated for each animal on the basis of the last body weight recorded.

- test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:

  Results show that formulates at the concentrations of 10 and 200 mg/ml are stable up to 2 hours if stored at room temperature. All formulates were stored accordingly. Formulates proved also homogeneous if kept magnetically stirred. Concentration checks were performed according to the method provided by the Sponsor, twice during the experiment.

Date of sampling	**Concentration mg/ml**		
	Expected	**Observed**	%Δ
February, 1995	10	9.868	- 1.32
	30	30.396	+ 1.32
	100	100.000	0
March, l 995	10	9.668	- 3.32
	30	30.204	+ 0.68
	100	98.687	- 1.31

Values were rounded off at the last decimal. Percent differences were calculated before rounding off.

Description of test design:

- details on mating procedure

  At the start of the mating period, the cages of males were alternated in close proximity with the cages of females. Every evening (4 evenings/week) the 2 females of each cage were mated with one sexually mature male for 16 hours at a time. Every morning, a vaginal smear was taken with a metal loop from each female and examined at the microscope, to ascertain copulation. The day on which the presence of spermatozoa was found was considered day 0 of pregnancy for that female.

- parameters assessed for P and F1

CLINICAL SIGNS AND BEHAVIOR

The rats were observed daily for physical appearance, behavior and clinical signs. Any deviation from the norm was recorded. During the treatment period, the animals were observed for any possible reaction to the treatment.

MORTALITY

The female rats found dead would have been subjected to autopsy to detect the cause of death. Corpora lutea and implantations would have been counted, whenever possible. The organs with gross alterations would have been fixed in formalin for histologic examination, if necessary.

ABORTION

The female rats presenting signs of abortion (vaginal bleeding) would have been left alive and autopsied at day 20 of gestation. Implantations would have been counted, whenever possible.

BODYWEIGHT

The dams were weighed on days 0, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20 of gestation.

FOOD CONSUMPTION

The leftover amounts of the weighed food was recorded on days 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18 and 20 of pregnancy in order to calculate the mean food consumption in g/animal/day.

PARAMETERS INVESTIGATED

On day 20 of gestation, the dams were killed by cervical dislocation after preliminary CO_2 anesthesia. A gross pathology examination was performed with special emphasis to the gastro-intestinal tract, stomach, intestine (duodenum, jejunum, ileum, cecum, colon, rectum) and organs with gross alterations were fixed in formalin for histologic examination. The following parameters were recorded:

- gravid uterus weight
- number of corpora lutea
- number of implantations
- number of resorptions - early: only placenta visible; late : placenta and embryo visible
- number and sex of viable fetuses
- number and sex of dead fetuses (fetuses without spontaneous movements and breathing)
- individual fetus weight
- individual placental weight

A gross external examination was performed on all fetuses immediately.
The externally malformed fetuses were fixed in order not to lose the evidence of malformation.
Half of the fetuses per litter were cleared and examined for skeletal malformations, anomalies and variants.
The remaining half of the fetuses were preserved in Bouin's fluid for examination by the Wilson technique.

As far as possible, the distribution per litter for examination by clearing or by Wilson's technique was equal by sex.

The observations were classified as follows:

- malformations: rare and/or usually lethal (such as hydrocephaly, thoracocele, acephalia, amelia, phocomelia, celosomia etc.)
- anomalies: more frequent and not lethal (such as reduced cranial ossification, hemorrhages etc.)
- variants: common in the control populations and often definable only in terms of continuous variable gradients: i.e. poor ossification of sternebrae, pubis or other.

The uteri of apparently non-pregnant females were stained using the method of Salewski and examined for the presence of early resorption sites.

DATA EVALUATION AND STATISTICAL ANALYSIS

All raw data were recorded on appropriate forms bound in numbered registers, and the numerical data were stored and processed by a computer system. The fertility index was expressed as the percent ratio between the number of females having evident signs of pregnancy with respect to the number of females that had positive vaginal smears.The mean body weight of each group including the maternal weight excluding gravid uterus was calculated from the weight of the gravid females. Calculation was also made of maternal body weight excluding gravid uterus. Absolute body weight gain was calculated at the different scheduled times and on day 6 of gestation (1st day of treatment). The mean food consumption of each group was calculated at different days in order to have the mean daily food consumption. Litter weight, mean fetal weight and placental weight were calculated from individual fetal or placental weights. Fetal losses were subdivided into pre-and post-implantation and were counted per litter.

Group mean values were calculated from individual data in two ways:

- Mean A: calculated on all the surviving females having evident signs of pregnancy including those that presented 100% post-implantation losses.
- Mean B: calculated only on those females with viable fetuses at term.

The external, visceral and skeletal malformations, anomalies and variants found are presented in tables and described for each litter. The malformations found in the dead fetuses were presented only in the Appendices. The expression "normal values" refers to data culled from our laboratory experience. Data recorded from observations were expressed both as individual and as group mean values (M. ± S.D.). Frequencies were expressed both as absolute and as percent values.The following tests were used for the purpose of comparing treated and control groups: To compare frequencies, the heterogeneity test (CHI square 2xN) and Fisher's exact test were applied. The Trend test was also applied. The probability of Trend test was recorded alongside the groups under the word "TREND". All these tests were one-tailed.

Results and discussion

Maternal effects:

- Clinical signs and mortality – no clinical signs or behavioural changes and no mortality were observed at any dose level in the dams.
- Body weight and body weight gain in dams - dose-dependently and stat. sign. ($p<0.05$) reduced bodyweight and bodyweight gain at 300 and 1000 mg/kg bw/day.
- Significantly lower mean daily food consumption was observed, in comparison with the control group, in the 1000 mg/kg/day group on days 8, 9 and 10 of gestation, in the 300 mg/kg/day group on days 9, 14, 16 and 20 of gestation and in the 100 mg/kg/day group only on day 9 of gestation. A significantly lower mean daily food consumption was observed also during the interval days 0-6 of gestation in the 300 mg/kg/day group. This finding was considered incidental since the treatment starts on day 6 of gestation.
- Autopsy done on females did not show any pathological finding.

TABLE 6. - Observation on gravid females (p. 1)

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000	TREND (P)
Gravid at term	21	20	20	19	
With res. visible at Salewski	0/21 0.00%	0/20 0.00%	2/20 10.00%	1/19 5.26%	>0.05
With early resorptions	7/21 33.33%	7/20 35.00%	7/20 35.00%	* 13/19 68.42%	.0058
With late resorptions	1/21 4.76%	0/20 0.00%	0/20 0.00%	2/19 10.53%	>0.05
With resorptions	8/21 38.10%	7/20 35.00%	9/20 45.00%	* 14/19 73.68%	.0041
With only resorptions	0/21 0.00%	0/20 0.00%	2/20 10.00%	1/19 5.26%	>0.05
With dead fetuses	0/21 0.00%	0/20 0.00%	0/20 0.00%	1/19 5.26%	.0428
With only viable fetuses	13/21 61.90%	13/20 65.00%	11/20 55.00%	* 5/19 26.32%	.0041

TABLE 7. - Frequencies per group (p. 1)

Mean "A"

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000	TREND (P)
No. of females	21	20	20	19	
Corpora lutea	369	345	332	352	
Implantations	320/369 86.72%	301/345 87.25%	272/332 81.93%	*** 272/352 77.27%	.0001
Res. visible at Salewski	0/320 0.00%	0/301 0.00%	* 4/272 1.47%	3/272 1.10%	.0313
Early resorptions	8/320 2.50%	7/301 2.33%	12/272 4.41%	*** 24/272 8.82%	.0001
Late resorptions	1/320 .31%	0/301 0.00%	0/272 0.00%	2/272 .74%	>0.05
Total no. of resorptions	9/320 2.81%	7/301 2.33%	* 16/272 5.88%	*** 29/272 10.66%	.0001
Dead fetuses	0/320 0.00%	0/301 0.00%	0/272 0.00%	1/272 .37%	.0410
Live fetuses	311/320 97.19%	295/301 98.01%	* 256/272 94.12%	*** 242/272 88.97%	.0001
Live male fetuses	155/311 49.84%	156/295 52.88%	119/256 46.48%	122/242 50.41%	>0.05
Live female fetuses	156	139	137	120	

TABLE 8. - Values per litter (p. 1)
(Mean, S.D., n)

Mean "A"

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000	ANOVA
			**	***	N ***
Gravid uterus wt .....(g)	86.64 20.890 (21)	84.77 11.462 (20)	68.29 23.904 (20)	55.77 16.363 (19)	
					P
Corpora lutea .....(no.)	17.57 2.874 (21)	17.25 2.468 (20)	16.60 3.789 (20)	18.53 3.454 (19)	
					N
Res. visible at Salewski ..(no.)	.00 .000 (21)	.00 .000 (20)	.20 .696 (20)	.16 .688 (19)	
				*	P *
Early resorptions .....(no.)	.38 .590 (21)	.35 .489 (20)	.60 .883 (20)	1.26 1.240 (19)	
					N
Late resorptions .....(no.)	.05 .218 (21)	.00 .000 (20)	.00 .000 (20)	.11 .315 (19)	
				**	P **
Total no. of resorptions ..(no.)	.43 .598 (21)	.35 .489 (20)	.80 1.005 (20)	1.53 1.307 (19)	
					P
Live fetuses .....(no.)	14.81 3.124 (21)	14.75 2.197 (20)	12.80 5.258 (20)	12.74 4.293 (19)	

ANOVA - "P" = Parametric / "N" = Non Parametric / "-" = Not processed

Foetal effects:

- Body weight: Dose-dependent decreases in the live foetal weight ($p<0.05$ at 300 and 1000 mg/kg bw/day) and litter weight ($p<0.05$ at 1000 mg/kg bw/day; lower than the HCD foetal weight) were also seen.
- Malformations: Six externally malformed fetuses with ablepharia, acrania, exencephaly and/or with macroglossia, cleft palate, cleft lip and facial cleft were found in two litters of the 100 mg/kg/day group: four in one litter and two in the other one.
  No externally malformed fetus was found at the two highest dosages. The skeletal examination showed the following malformed fetuses:
  - one with scoliosis in litter no. 12 of the control group;
  - 2 with fused ribs at 1000 mg/kg/day: one in litter no. 78 and one in litter 84.

  A significantly higher frequency of skeletal anomalies was found at 1000 mg/kg/day. When the statistical analysis was done district by district significance levels were found again at 1000 and also at 300 mg/kg/day for various incomplete ossifications and wavy ribs. At 100 mg/kg/day the supraoccipital incomplete ossification was statistically significant. However this significant value (38.89%) was over the upper limit of the colony data (0%-35.90%).

  The number of fetuses with skeletal variants was similar in all the experimental groups. As for the skeletal variants examined district by district significance levels were reached at 1000 mg/kg/day. At 300 mg/kg/day significance levels were reached for the 5th and the 6th sternebrae unossified and for the 4th sternebra incomplete ossification. At 100 mg/kg/day the only statistical significance was related to the 5th sternebra unossified.

However the value (60.42%) was over the upper limit of the range of the historical data (15.91% - 51.16%).

The visceral examination showed the following malformed fetuses at the 1000 mg/kg/day group:

- one in litter no. 76 with monolateral microphthalmia; one with vascular ring in litter no. 78;
- one with bilateral hydronephrosis in litter no. 84;
- two in litter no. 85: one with displaced aortic arch and displaced Botallus duct (Ductus arterious) and one with monolateral hypoplasia of the kidney;
- two in litter no. 91: one with monolateral microphthalmia and one with bilateral anophthalmia; one with vascular ring in litter no. 94;
- one with displaced aortic arch and displaced Botallus duct (Ductus arterious) in litter no. 96;
- one with monoliteral microphthalmia in litter no. 97;
- one with double aortic arch in litter no. 100.

The plurimalformed fetuses found in 2 litters at 100 mg/kg/day were considered incidental, since these kinds of malformations were not present at the highest dosages and since they were present only in 2 litters.

	HCD on 2146 foetuses (1992-1993)	**Dose levels (mg/kg bw/day)**			
		0	**100**	**300**	**1000**
No. of pregnant females	-	21	20	20[&]	19[#]
No. of litters	-	21	20	18	18
Maternal body weight (g; group mean and gain)					
GD 6	-	288.7	283.6	277.6	282.2
GD 15	-	333.3	329.5	315.8*	314.3*
GD 20	-	410.9	400.3	369.4*	366.0*
GD 0-20, gain	-	153.8	144.5	119.2*	110.9*
GD 0-20 (gain excluding gravid uterine weight)	-	67.2	59.7	50.9*	55.1
Reproductive parameters					
Gravid uterine weight (g)	-	86.6	84.8	68.3*	55.8*
No. dams with early resorptions	-	7/21	7/20	7/20	13*/19
No. dams with late resorptions	-	1/21	0/20	0/20	2/19
No. of implantations/no. of corpora lutea	-	320/369	301/345	272/332	272*/352
No. of early resorptions	-	8	7	12	24*
Total no. of resorptions	-	9	7	16*	29*
Post-implantation loss (%)	-	2.91	2.39	13.54*	15.20*
Foetal parameters					
No. of live foetuses	-	311	295	256*	242*
No. of dead foetuses	-	0	0	0	1

		HCD on 2146 foetuses (1992-1993)	Dose levels (mg/kg bw/day)			
			0	**100**	**300**	**1000**
No. of live foetuses/litter		14.4 ± 3.3	14.80	14.75	14.22	13.44
No. of resorptions/litter		0.7 ± 1.1	0.43	0.35	0.80	1.53*
Live foetus weight (g)		3.7 ± 0.4	3.69	3.57	3.28*	2.4*
Live litter weight (g)		53.9	54.97	52.62	46.49	32.52*
Placenta weight (g)		0.5 ± 0.08	0.5	0.51	0.48	0.37*
Malformations, abnormalities and variations as reported by the study authors (Study Report, 1995b)						
No. of foetuses examined for skeletal/visceral		-	156/155	144/145	129/127	123/119
Malformations (%)	External	0 – 0.120	0	2*[a]	0	0
	Skeletal	0	0.64[b]	0	0	1.62[b]
	Overall visceral, including:	0.020	0	0	0	9.20*[c]
	Cardio-vascular effects	-	0	0	0	5.88*[c]
	Eye effects	-	0	0	0	3.36[c]
Abnormalities (%)	External	0.115	0	0	0	0
	Overall skeletal, including:					
	Wavy rib ([d])	-	1.30	0.70	13.20*	7.30*
	Supraoccipital incomplete ossification ([e])	0 – 35.90	26.92	38.89*	45.73*	76.42*
	Overall visceral, including:	0.415	0.64	1.37	1.57	7.56*[f]
	Enlarged lateral ventricles of the brain	-	0	0	0	1.68[f]
	Absence of renal papillae	-	0.64	0.70	0.78	6.72*[f]
Variations (%)	**Overall skeletal, including:**					
	Reduced rib XIII unilateral ([g])	-	0	0	1.55	4.07
	Reduced rib XIII bilateral ([g])	-	0.64	0	1.55	4.88
	Ribs XIII punctate unilateral ([h])	-	0.64	2.08	0	14.63*
	Ribs XIII punctate bilateral ([h])	-	1.28	1.39	2.33	6.50
	Ribs XII/XIII (i)	-	0.64	2.1	0.78	11.38*
	Ribs XIII/XIV ([j])	-	1.92	0.69	0	0
	Ribs XIV punctate unilateral ([k])	-	1.92	0.69	0	0
	Unossified 5th sternbrae	19.910 – 51.160	35	60.42*	70.50*	100*
	Unossified 6th sternbrae	-	25	34	54.20*	89.43*
	Incomplete	-	7	11.11	15.50*	48.78*

		HCD on 2146 foetuses (1992-1993)	Dose levels (mg/kg bw/day)			
			0	100	300	1000
	ossification of 4th sternbrae					
	Overall visceral, including:					
	Dilated or convoluted ureter	-	28.40	44.82*	42*	79*
	Dilated renal pelvis	-	4.51	17.24*	11*	38.60*

[&] Two dams with complete resorptions

[#] One dam with complete resorptions

* Statistically significant effect $p < 0.05$; statistical analysis by Chi-squared and Fischer's exact test or ANOVA parametric or nonparametric, where applicable, compared to controls

[a] 6 plurimalformed foetuses (of 295 total foetuses): ablepharia (5), acrania (6), exencephaly (6), exophthalmia (3), macroglossia (6), cleft palate (5), cleft lip (2), facial cleft(1)

[b] 1 foetus with scoliosis and bifurcated 8th rib (controls); 2 foetuses of 2 different litters with fused ribs (1000 mg/kg bw/day)

[c] 11 foetuses: microphthalmia or anophthalmia (4), vascular ring (2), bilateral hydronephrosis (1), displaced or double aortic arch (3), displaced botallus duct (2), hypoplasia of kidney (1); the 7 foetuses with cardio-vascular effects were from 5 different litters; the 4 foetuses with eye effects were from 3 different litters

[d] wavy rib: 2 (from 2 different litters), 1, 17 (from 5 different litters) and 9 (from 3 different litters) foetuses at **0, 100, 300 and 1000 mg/kg bw/day, respectively**

[e] Supraoccipital incomplete ossification in 251 foetuses: 42 (from 14 litters), 56 (from 20 litters), 59 (from 15 litters) and 94 (from 16 litters) at 0, 100, 300 and 1000 mg/kg bw/day, respectively

[f] 9 foetuses: dilated lateral cerebral ventricles (2 foetuses of the same litter), absence of renal papillae (8 foetuses of 4 different litters), hemorrhagic kidney (1)

[g] Reduced rib XIII unilateral in 0, 0, 2 and 5 foetuses at 0, 100, 300 and 1000 mg/kg bw/day, respectively
Reduced rib XIII bilateral in 1, 0, 2 and 6 foetuses at 0, 100, 300 and 1000 mg/kg bw/day, respectively

[h] Ribs XIII punctate unilateral in in 1, 3, 0 and 18 foetuses at 0, 100, 300 and 1000 mg/kg bw/day, respectively
Ribs XIII punctate bilateral in 2, 2, 3 and 8 at 0, 100, 300 and 1000 mg/kg bw/day, respectively

[i] Ribs XII/XIII in 1, 3 (from the same litter), 1 and 14 (from 10 different litters) foetuses at 0, 100, 300 and 1000 mg/kg bw/day, respectively

[j] Ribs XIII/XIV in 3, 1, 0 and 0 foetuses at 0, 100, 300 and 1000 mg/kg bw/day, respectively

[k] Ribs XIV punctate unilateral in 3, 1, 0 and 0 foetuses at 0, 100, 300 and 1000 mg/kg bw/day, respectively

TABLE 9. - Observation on fetuses (p. 1)

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000	TREND (P)
Viable fetuses	311	295	256	242	
Skeletally examined fetuses	156	144	129	123	
Wilson's examined fetuses	155	145	127	119	
With external malformations	0/311 0.00%	* 6/295 2.03%	0/256 0.00%	0/242 0.00%	>0.05
With skeletal malformations	1/156 .64%	0/144 0.00%	0/129 0.00%	2/123 1.63%	>0.05
With visceral malf. (Wilson's)	0/155 0.00%	0/145 0.00%	0/127 0.00%	*** 11/119 9.24%	.0001
With external anomalies	0/311 0.00%	0/295 0.00%	0/256 0.00%	0/242 0.00%	>0.05
With skeletal anomalies	92/156 58.97%	89/144 61.81%	84/129 65.12%	*** 117/123 95.12%	.0001
With visceral anom. (Wilson's)	1/155 .65%	2/145 1.38%	2/127 1.57%	*** 11/119 9.24%	.0001
With skeletal variants	153/156 98.08%	143/144 99.31%	129/129 100.00%	123/123 100.00%	>0.05
With visceral var. (Wilson's)	28/155 18.06%	*** 53/145 36.55%	** 43/127 33.86%	*** 66/119 55.46%	.0001

TABLE 10. - Observation on fetuses (p. 1)
(Mean, S.D., no. of litters)

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000	ANOVA
With external malf. .....(no.)	.00 .000 (21)	.30 .979 (20)	.00 .000 (18)	.00 .000 (18)	N
With external anomalies ...(no.)	.00 .000 (21)	.00 .000 (20)	.00 .000 (18)	.00 .000 (18)	-
With skeletal malf. .....(no.)	.05 .218 (21)	.00 .000 (20)	.00 .000 (18)	.11 .323 (18)	N
With skeletal anomalies ...(no.)	4.38 2.397 (21)	4.45 2.212 (20)	4.67 2.425 (18)	** 6.50 1.917 (18)	N *
With skeletal variants ....(no.)	7.29 1.554 (21)	7.15 1.496 (20)	7.17 1.425 (18)	6.83 1.505 (18)	N
With visc.malf.(Wilson's) .(no.)	.00 .000 (21)	.00 .000 (20)	.00 .000 (18)	*** .61 .698 (18)	N ***
With visc.anom.(Wilson's) .(no.)	.05 .218 (21)	.10 .308 (20)	.11 .323 (18)	* .61 1.092 (18)	N *
With visc.var. (Wilson's) .(no.)	1.33 1.494 (21)	* 2.65 1.785 (20)	2.39 1.975 (18)	** 3.67 2.544 (18)	N **

TABLE 14. - Skeletal examination (p. 1)
(no. of cases, %)

Malformation

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	156	144	129	123
General observation				
scoliosis	1 .64%	0	0	0
Ribs				
8th, bifurcated	1 .64%	0	0	0
fused	0	0	0	2 1.63%
Vertebrae				
8th thoracic centrum, hemivertebra	1 .64%	0	0	0

TABLE 14. - Skeletal examination (p. 7)
(no. of cases, %)

Variants

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	156	144	129	123
Head				
unossified hyoid bone	24 15.38%	12 8.33%	14 10.85%	36 29.27%
hyoid body, incomplete ossification	4 2.56%	2 1.39%	8 6.20%	20 16.26%
Ribs				
12/13	1 .64%	3 2.08%	1 .78%	14 11.38%
13/14	3 1.92%	1 .69%	0	0
13th, punctate, unilateral	1 .64%	3 2.08%	0	18 14.63%
13th, punctate, bilateral	2 1.28%	2 1.39%	3 2.33%	8 6.50%
14th, punctate, unilateral	3 1.92%	1 .69%	0	0
13th, reduced, unilateral	0	0	2 1.55%	5 4.07%

TABLE 14. - Skeletal examination (p. 8)
(no. of cases, %)

Variants

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	156	144	129	123
Ribs				
13th, reduced, bilateral	1 .64%	0	2 1.55%	6 4.88%
Sternum				
5th sternebra, butterfly	1 .64%	0	0	0
1st sternebra, asymmetric	0	0	0	4 3.25%
2nd sternebra, asymmetric	0	1 .69%	3 2.33%	9 7.32%
3rd sternebra, asymmetric	0	3 2.08%	3 2.33%	10 8.13%
4th sternebra, asymmetric	5 3.21%	10 6.94%	7 5.43%	2 1.63%
5th sternebra, asymmetric	2 1.28%	2 1.39%	2 1.55%	0
2nd sternebra, bipartite	0	0	0	3 2.44%

TABLE 14. - Skeletal examination (p. 9)
(no. of cases, %)

Variants

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	156	144	129	123
Sternum				
3rd sternebra, bipartite	1 .64%	0	0	0
4th sternebra, hemisternebra	1 .64%	0	0	0
5th sternebra, hemisternebra	15 9.62%	8 5.56%	9 6.98%	0
6th sternebra, hemisternebra	0	0	1 .78%	0
1st sternebra, unossified	0	0	1 .78%	14 11.38%
2nd sternebra, unossified	2 1.28%	0	1 .78%	15 12.20%
3rd sternebra, unossified	0	0	0	8 6.50%
4th sternebra, unossified	0	0	0	46 37.40%

TABLE 14. - Skeletal examination (p. 10)
(no. of cases, %)

Variants

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	156	144	129	123
Sternum				
5th sternebra, unossified	54 34.62%	87 60.42%	91 70.54%	123 100.00%
6th sternebra, unossified	40 25.64%	49 34.03%	70 54.26%	111 90.24%
1st sternebra, incomplete ossification	5 3.21%	2 1.39%	5 3.88%	59 47.97%
2nd sternebra, incomplete ossification	12 7.69%	14 9.72%	17 13.18%	61 49.59%
3rd sternebra, incomplete ossification	3 1.92%	1 .69%	4 3.10%	64 52.03%
4th sternebra, incomplete ossification	11 7.05%	16 11.11%	20 15.50%	60 48.78%
5th sternebra, incomplete ossification	79 50.64%	45 31.25%	27 20.93%	0
6th sternebra, incomplete ossification	63 40.38%	50 34.72%	43 33.33%	9 7.32%

TABLE 15. - Visceral examination (p. 1)
(no. of cases, %)

Malformation

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	155	145	127	119
General observation				
vascular ring	0	0	0	2 1.68%
anophthalmia, bilateral	0	0	0	1 .84%
microphthalmia, right	0	0	0	2 1.68%
microphthalmia, left	0	0	0	1 .84%
aortic arch, displaced, right	0	0	0	2 1.68%
botallus duct, displaced, right	0	0	0	2 1.68%
double aortic arch	0	0	0	1 .84%
Kidney				
hydronephrosis, bilateral	0	0	0	1 .84%

TABLE 15. - Visceral examination (p. 1)
(no. of cases, %)

Malformation

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined feluses	155	146	127	119
General observation				
vascular ring	0	0	0	2 1.68%
anophthalmia, bilateral	0	0	0	1 .84%
microphthalmia, right	0	0	0	2 1.68%
microphthalmia, left	0	0	0	1 .84%
aortic arch, displaced, right	0	0	0	2 1.68%
botallus duct, displaced, right	0	0	0	2 1.68%
double aortic arch	0	0	0	1 .84%
Kidney				
hydronephrosis, bilateral	0	0	0	1 .84%
Kidney				
hypoplasia, left	0	0	0	1 .84%

TABLE 15. - Visceral examination (p. 3)
(no. of cases, %)

Anomalies

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	155	145	127	119
Brain				
lateral cerebral ventricles, dilated	0	0	0	2 1.68%
General observation				
neck, subcutaneous hematoma	0	1 .69%	0	0
Kidney				
absence of renal papilla, right	1 .65%	1 .69%	1 .79%	1 .84%
absence of renal papilla, left	0	0	0	2 1.68%
absence of renal papilla, bilateral	0	0	0	5 4.20%
hemorrhagic, left	0	0	0	1 .84%

TABLE 15. - Visceral examination (p. 4)
(no. of cases, %)

Anomalies

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	155	145	127	119
Trunk				
subcutaneous, hemorrhage	0	0	1 .79%	0

TABLE 15. - Visceral examination (p. 5)
(no. of cases, %)

Variants Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	155	145	127	119
Kidney				
renal pelvis dilated, right	2 1.29%	7 4.83%	5 3.94%	13 10.92%
renal pelvis dilated, left	0	6 4.14%	3 2.36%	3 2.52%
renal pelvis dilated, bilateral	5 3.23%	12 8.28%	6 4.72%	30 25.21%
Ureter				
convoluted, right	2 1.29%	5 3.45%	2 1.57%	2 1.68%
convoluted, left	8 5.16%	12 8.28%	13 10.24%	2 1.68%
convoluted, bilateral	18 11.61%	34 23.45%	23 18.11%	53 44.54%
dilated, right	2 1.29%	2 1.38%	0	4 3.36%
dilated, left	2 1.29%	2 1.38%	5 3.94%	3 2.52%

Variants Dose (mg/kg/day)	Gr# 1 0	Gr# 2 100	Gr# 3 300	Gr# 4 1000
no. of examined fetuses	155	145	127	119
Ureter				
dilated, bilateral	12 7.74%	10 6.90%	10 7.87%	30 25.21%

2.10.2 Human data

2.10.2.1 Adverse effects on sexual function and fertility

2.10.2.1.1 [Study 1] Investigation of environmental boron exposure on Y:X sperm ratio

Study reference:

Yalçin, C. Ö., Üstündağ, A., & Duydu, Y. (2019). Is There an Association Between Extreme Levels of Boron Exposure and Decrease in Y: X Sperm Ratio in Men? Results of an Epidemiological Study. Turkish Journal of Pharmaceutical Sciences, 16(1), 96

2.10.2.1.2 [Study 2] Investigation of environmental boron exposure on Y:X sperm ratio and sex ratio of offspring

Study reference:

Duydu, Y., Başaran, N., Yalçın, C. Ö., Üstündağ, A., Aydın, S., Anlar, H. G., Bacanli, M., Aydos, K., Atabekoglu, C.S., Golka, K., Ickstadt, K., Scwerdtle, T., Werner, M., Meyer, S. and Bolt, H. M. (2019). Boron-exposed male workers in Turkey: no change in sperm Y: X chromosome ratio and in offspring's sex ratio. Archives of toxicology, 93(3), 743-751.

2.10.2.1.3 [Study 3] Investigation of environmental boron exposure on human reproduction

Study reference:

Bolt, H. M., Başaran, N., & Duydu, Y. (2020). Effects of boron compounds on human reproduction. Archives of toxicology, 94(3), 717-724.

2.10.2.2 Adverse effects on the development of the offspring

2.10.2.2.1 [Study 1] Pre- and postnatal environmental boron exposure and infant growth (prospective study, mother-child cohort)

Study reference:

Hjelm, C., Harari, F., & Vahter, M. (2019). Pre-and postnatal environmental boron exposure and infant growth: Results from a mother-child cohort in northern Argentina. Environmental research, 171, 60-68.

2.11 Specific target organ toxicity – single exposure

Not assessed in this CLH-proposal.

2.12 Specific target organ toxicity – repeated exposure

Not assessed in this CLH-proposal.

2.13 Aspiration hazard

Not assessed in this CLH-proposal.

3 ENVIRONMENTAL HAZARDS

Not assessed in this CLH-proposal.