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:

Calcium bromide

EC Number: 232-164-6

CAS Number: 7789-41-5

Index Number: 035-RST-VW-Y

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Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

CONTENTS

1 PHYSICAL HAZARDS

Not assessed in the CLH report.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 [Study 1] Pharmacokinetics of oral and intravenous bromide in human volunteers (no guideline)

Study reference:

A6.2/09, Doc. No. 592-008

Vaiseman N., Koren G. and Pencharz P. (1986). Pharmacokinetics of Oral and Intravenous Bromide in Normal Volunteers. Clinical Toxicology, 24(5), 403-413.

2.1.2 [Study 2] Kinetics and distribution of sodium bromide in rat (no guideline study)

Study reference:

A6.2/17, Doc. No. 592-075

Pavelka S., Babický A., Vobecký M., Lener J. and Švandová E. (2000a). Bromide Kinetics and Distribution in the Rat I. Biological Trace Elements Research, Vol. 76, 57-66.

2.1.3 [Study 3] Distribution of sodium bromide in rat (no guideline study)

Study reference:

Pavelka S., Babický A. Vobecký M. and Lener J. (2000b). Bromide Kinetics and Distribution in the Rat II. Biological Trace Element Research, Vol. 76, 67-74.

2.1.4 [Study 4] Distribution of sodium bromide in rat (no guideline study)

Study reference:

A6.4.1/03, Doc. No. 592-007

Van Logten M. J., Wolthuis M., Rauws A. G., Kroes R. (1973). Short-term Toxicity Study on Sodium Bromide in Rats. Toxicology 1, 321-327.

2.1.5 [Study 5] Distribution of sodium bromide in rat (no guideline study)

Study reference:

A6.4.1/04, Doc. No. 592-005

Van Logten, M. J., et al (1974). Semichronic Toxicity Study of Sodium Bromide in Rats. Toxicology 2, 257-267.

2.1.6 [Study 6] Influence of Dietary Chloride on Bromide Excretion in the Rat (no guideline study)

Study reference:

Rauws A.G. and van Logten M.J. (1975). The Influence of Dietary Chloride on Bromide Excretion in the Rat. 1975:Toxicology, 3, 29-32.

2.1.7 [Study 7] Intravenous and oral administration of sodium bromide to healthy Beagle dogs (no guideline study)

Study reference:

A6.2/01, Doc. No. 592-029

Trepanier L.A. and Babish J.G. (1995). Pharmacokinetic Properties of Bromide in Dogs after the Intravenous and Oral Administration of Single Doses. Research in Veterinary Science, 58, 248-251.

2.1.8 [Study 8] Biological half-life of bromide ions in humans (no guideline study)

Study reference:

A6.2/10, Doc. No. 592-010

Söremark R. (1960). The biological half-life of bromide ions in human blood. Acta physiol. Scand., 50, 119-123

2.1.9 [Study 9] Distribution of ammonium bromide in mouse (no guideline study)

Study reference:

A6.2/16. Doc. No. 592-084

Söremark R. and Ullberg A. (1960). Distribution of bromide in mice. an autoradiographic study with 82Br. International Journal of Applied Radiation and Isotopes, Vol 8, 192-197

2.1.10 [Study 10] Bromide pharmacokinetics in vivo in dog (no guideline study)

Study reference:

A6.2/03, Doc. No. 592-032

March, P.A. Podell, M., Sams, R.A. (2002). Pharmacokinetics and toxicity of bromide following high-dose oral potassium bromide administration in healthy beagles. J. Vet. Pharmacol. Therap. 25, 425-432

2.1.11 [Study 11] Dermal penetration (in vivo and in vitro) of bromide in human, pig and rabbit (no guideline study)

Study reference:

A6.2/05, Doc. No. 592-045

Tregear, R. T. (1966). The Permeability of Mammalian Skin to Ions. The Journal of Investigative Dermatology, Vol. 40, No. 1, 16-23.

2.1.12 [Study 12] Distribution study of bromide in guinea pig (no guideline study)

Study reference:

Shani J., Barak S., Ram M., Levi D., Pfeifer Y., Schlesinger T., Avrach W. W., Robberecht H. and Van Grieken R. (1982). Serum Bromide Levels in Psoriasis. Pharmacology, 25:297-307.

2.1.13 [Study 13] Dermal penetration of bromide in human and Guinea pig (males/females)

Study reference:

A6.2/06, Doc. No. 592-044

Shani, J. et al (1985). Skin Penetration of minerals in Psoriatics and Guinea-Pigs Bathing in Hypertonic Salt Solutions. Pharmacological Research Communications, Vol. 17, No. 6.

3 HEALTH HAZARDS

3.1 Acute toxicity

Not assessed in the CLH report.

3.2 Skin corrosion/irritation

Not assessed in the CLH report.

3.3 Serious eye damage/eye irritation

Not assessed in the CLH report.

3.4 Respiratory sensitisation

Not assessed in the CLH report.

3.5 Germ cell mutagenicity

Not assessed in this CLH report

3.6 Carcinogenicity

Not assessed in this CLH report

3.7 Reproductive toxicity

3.7.1 Animal data

ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

3.7.1.1 [Study 1] Two-generation reproductive toxicity study of sodium bromide in rat

Reference Study report, 2016a

Guideline Similar to OECD 416; 2-generation reproduction study. Deviations: Male and female P generation rats were paired twice (excluding high dose group), owing to reduced pregnancy rate in intermediate dose group. The offspring from the first pairing formed the F1a generation, dosed from Day 21 postpartum and selected for post-weaning assessments (including reproductive assessments and production of the F2a litters).

The offspring from the second pairing of the P generation rats formed the F1b generation, terminated at day 40 postpartum.

Reliability Klimisch 1

Species / strain Rat, Crl:CD(SD) rats

The body weight range for the treated male rats was 145 g to 189 g on the day after arrival and was 167 g to 212 g at assignment to study. The male rats were approximately 45 days of age upon arrival at the Testing Facility.

The body weight range for the treated female rats was 103 g to 138 g on the day after arrival and was 124 g to 146 g at assignment to study. The female rats were approximately 37 days of age upon arrival at the Testing Facility.

Test material Sodium bromide

CAS Number: 7647-15-6

Purity: 99.5%

Study design The design of this study was based on the study objectives, the overall product development strategy for the test substance, EPA guideline OPPTS870-3800,1 and OECD 416 Guideline.

Male and female P generation rats in Groups 1 through 3 were paired twice, owing to reduced pregnancy rate in Group 3. The first litter formed the F1a generation, dosed from Day 21 postpartum and selected for post-weaning assessments (including reproductive assessments and production of the F2a litters).

The (F1b) litter from the second cohabitation of the P generation rats was terminated at day 40 postpartum.

Groups 4 (Male) and 5 (Female; 350 or 500 mg/kg/day, respectively) were terminated at the end of the P generation owing to poor condition in parental animals and low viability in the F1a pups.

ADMINISTRATION / EXPOSURE

Route of administration: Oral gavage

Analytical verification of doses or concentrations: not specified

Doses / Concentrations: 0, 50, 150, 350/500 (M/F) mg/kg bw/day

No. of animals per sex per dose:

24/sex/group in P generation mating to produce F1a.

23/sex in control group, 23/24 (M/F) in low dose group, 23/22 (M/F) in intermediate dose group in in P generation mating to produce F1b.

23/sex in control group, 22/sex in low dose group, 15/sex in intermediate dose group in F1a generation mating to produce F2.

P generation

P generation males were given the test or control substance formulations once daily by oral gavage beginning 10 weeks before the first cohabitation period, during cohabitation(s) and, gestation, littering and post-partum periods until all F1a and for Groups 1 to 3, F1b generation pups were weaned and continuing through to the day before euthanasia (183-186 dosing days).

P generation females were given the test or control substance formulations once daily beginning 10 weeks before the first cohabitation, during the cohabitation, gestation, littering and postpartum periods until all F1a and (for Groups 1 to 3) F1b generation pups were weaned and continuing through to the day before euthanasia (>181 days). Any dam in the process of parturition was not given the test or control substance formulations until the following work day.

F1 generation

The F1a and F1b generation pups were not directly given the test or control substance formulations prior to weaning, but may have been exposed to the test or control substance formulations during maternal gestation (in utero exposure) or via maternal milk or excreta during the lactation period.

One weaned pup per sex from each available litter in Groups 1 to 3 were selected for the F1a generation (Subset A, rearing and mating) were administered the test or control substance formulations once daily beginning on Day 21 postpartum, for at least 10 weeks before cohabitation, during the cohabitation, gestation, littering and post-partum periods until all F2 generation pups were weaned PND 21), and continuing through to the day before euthanasia (PND 22).

The selected F1b generation weaned pups were administered the test or control substance formulations (0, 50, or 175 mg/kg bw/day) once daily beginning on Day 21 postpartum and continuing through to the day before euthanasia (PND 40).

F2 Generation

The F2 generation pups were not directly given the test or control substance formulations, but may have been exposed to the test or control substance formulations during maternal gestation (in utero exposure) or via maternal milk or excreta during the lactation period. Euthanasia was PND 22.

OBSERVATIONS AND EXAMINATIONS

Parental animals:

The following parameters and end points were evaluated in this study for the P generation: viability, clinical signs, maternal behavior, body weights, body weight changes, food consumption, estrous evaluation, reproductive capacity, gross necropsy findings, ovarian and uterine examinations, male reproductive assessments, organ weights, and histopathological examinations.

Male Reproductive Assessments:

- Sperm Motility
- Sperm Concentration
- Sperm Morphology
- Spermatid Counts

The following natural delivery/reproductive parameters were reported:

- Duration of Gestation: The duration of gestation was calculated from DG 0 to the day the first pup was observed.
- Mating index Percentage of pairings that resulted in matings.
- Fertility Index: Percentage of matings that resulted in pregnancies
- Gestation Index: Percentage of pregnancies that resulted in birth of live litters
- Number of offspring per litter: Live and dead pups
- Number of implantation sites.
- General condition of dam and litter during the postpartum period.
- Live birth index: Percentage of pups born alive
- Viability Indices: Percentage of pups alive day 0 postpartum that survived to 4 days postpartum
- Lactation Index: Percentage of pups alive day 4 that survived 21 days postpartum

Litter observations:

Parameters examined: fertility, viability, body weight

Gross examination of dead pups: organ weights were determined for adrenals, thyroid, pituitary, testes, prostate, ovaries and uterus

Histopathology / organ weigths: organ weights were determined for adrenals, thyroid, pituitary, testes, prostate, ovaries and uterus.

Findings **CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS - FIRST COHABITATION**

PERIOD)

Administration of 350 mg/kg/day to males and 500 mg/kg/day of sodium bromide to females produced severe toxicity, characterized by increased mortality (4 males and 9 females died or were terminated early). A total of 0, 1, 1 and 4 (16.7%) males and 2, 1, 2 (8.3%), and 9 (9 out of 24 animals means 37.5%) female rats in the 0, 50, 175 and 350/500 mg/kg/day dose groups, respectively did not survive to scheduled euthanasia.

Adverse clinical observations in high dose group included dehydration, ungroomed coat, chromodacryorrhea, hunched posture, ptosis, urine-stained abdominal fur, decreased motor activity, chromorhinorrhea, ataxia, piloerection, low carriage, thin body condition, and bradypnea, with effects generally more severe in males. In the 175 mg/kg/day dose group, similar clinical signs occurred but they were less marked and at a lower incidence, especially in females.

Males

Vehicle Control

Clinical observations in control animals during the treatment period were limited to sparse hair coat, scabs on the neck or mouth, and soft and liquid feces in up to 6 of the 24 male rats in this group.

50 mg/kg/day

Clinical observations in the 50 mg/kg/day dose group were considered not treatment-related because two were secondary to an injury which occurred in one animal and the other observations demonstrated no clear pattern of effect and/or the sign did not persist.

Transient observations included soft and liquid feces in five males, and one or two males with chromodacryorrhea, urine stained abdominal fur, piloerection, a scab, a mass under the skin, and rales. In low dose group one male was found dead study day 8.

175 mg/kg/day

The incidence of adverse observations in males in this dose group was higher than controls.

Mild dehydration, ungroomed coat and chromodacryorrhea, occurred in five to six males in this group and hunched posture, urine stained abdominal fur, chromorhinorrhea and ptosis occurred in two or three males in this group, but these signs did not occur in any control group males and occurred in significantly increased numbers ($p \leq 0.01$) of males in the 350 mg/kg/day dose group. Swollen limbs and a subcutaneous mass-missing/broken incisors occurred one to two males in this group and soft, liquid feces occurred in three, but these finding were less frequent or absent at the higher dose level. In intermediate dose group one male (study day 114) was euthanized prior to study termination due to severe clinical observations.

350 mg/kg/day

Adverse effects of treatment at 350 mg/kg/day included increased ($p \leq 0.01$) incidence of dehydration, ungroomed coat, chromodacryorrhea, hunched posture, ptosis, urine-stained abdominal fur, decreased motor activity, chromorhinorrhea, ataxia, piloerection, low carriage, thin body condition, and bradypnea.

Scabs, subcutaneous masses, sparse hair coat, rales, cold to touch, purple hindpaw, missing/broken incisor, convulsions, flaking skin and scant feces limited use of the hindlimbs were observed but were considered unrelated to the test substance because only 1 or 2 males were affected, and there was no pattern to the onset and duration of any sign. (4 males died at 350 mg/kg/day).

In high dose group three males (study days 78, 79, 168) were euthanized prior to study termination due to severe clinical observations and one male was found dead study day 81.

	0 mg/kg bw/d	**50 mg/kg bw/d**	**175 mg/kg bw/d**	**350 mg/kg bw/d**
Number of animals	24	24	24	24
Mortality	0	1	1	4
Clinical observation during pre-mating		chromodacryorrhea 2 urine-stained abdominal fur 1 piloerection 1	dehydration: total 6 mild 6 ungroomed coat 6 chromodacryorrhea 5 hunched posture 2	dehydration: total 24** mild 24** moderate 11** ungroomed coat 21** chromodacryorrhea 9**

			ptosis 2 urine-stained abdominal fur 3 chromorhinorrhea 2	hunched posture 19** abdominal fur 15** decreased motor activity 12** chromorhinorrhea 10** ataxia 8** piloerection 7** low carriage 5** thin body condition 4** bradypnea 3** ptosis 19**
Mean food consumption [g/rat/day] during pre-mating study days 1-8	24.0 ± 2.1	24.6 ± 1.8	24.9 ± 1.7	25.5 ± 1.9
Food consumption [g/rat/day] during premating study days 29-36	26.6 ± 2.3	27.0 ± 3.0	25.1 ± 2.2	23.4 ± 4.4**
Food consumption [g/rat/day]] during pre-mating study days 64 - 71b (b. Last value recorded before the first cohabitation period)	28.1 ± 3.0	28.6 ± 3.1	24.8 ± 2.1**	22.8 ± 2.4**
Food consumption [g/rat/day] study days 120-127d (d. Last value recorded before the second cohabitation period)	28.8 ± 2.9	28.1 ± 3.6 [23]a	24.7 ± 2.4** [23]a	22.2 ± 2.4** [21]a
Food consumption [g/rat/day] study days 1-183	27.6 ± 2.6	27.8 ± 2.6 [23]a	24.6 ± 1.8** [23]a	23.0 ± 1.7** [20]a
Body weight [g] pre-mating day 1	219.2 ± 12.9	219.1 ± 12.8	219.3 ± 12.8	219.4 ± 12.1
Body weight [g] pre-mating day 36	424.2 ± 31.7	436.3 ± 36.1	425.2 ± 35.0	417.2 ± 31.6
Body weight [g] pre-mating day 71 (last value recorded before mating)	522.5 ± 45.7	537.9 ± 53.0	503.0 ± 47.2	472.3 ± 40.4**
Body weight [g] mating day 16 (DS 86)	542.6 ± 50.5	557.0 ± 54.5	508.9 ± 47.8*	452.6 ± 34.7**
Body weight [g] study day 92	553.1 ± 52.1	567.1 ± 55.4	516.0 ± 47.0*	460.2 ± 36.3**
Body weight [g] study day 127 (last value recorded before second mating)	602.0 ± 61.2	622.7 ± 62.2	551.0 ± 50.5** (91.5% of ctrl)	490.0 ± 37.8**
Body weight [g] mating day 14 (DS 141)	621.4 ± 64.9	632.5 ± 62.6	550.6 ± 49.4**	498.1 ± 35.2**
Body weight [g] study day 148	628.8 ± 65.2	640.9 ± 63.2	558.1 ± 50.5**	500.6 ± 37.3**
Body weight [g] study day 169	653.6 ± 71.5	667.9 ± 70.1	570.6 ± 50.9**	490.0 ± 41.4**

Body weight [g] study day 183	666.0 ± 74.1	687.8 ± 75.3	578.3 ± 51.1**	502.1 ± 39.0**

Table: General toxicity in males – P-generation

[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead or euthanized due to adverse clinical observations.
b. Excludes values for rats that were in cohabitation during this interval.
c. Excludes values that could not be calculated, as well as those associated with spillage.
d. Last value recorded before the second cohabitation period.

** Significantly different from the control group value (p≤0.01).

Females

Vehicle Control

Minimal and sporadic common clinical observations occurred in this group. No adverse signs occurred during the pre-mating period, and a single female had chromodacryorrhea during the gestation period. Single females had ungroomed coat, chromodacryorrhea, soft and liquid feces and/or sparse hair coat on the limbs during the lactation period. In control group one female was found dead lactation day 6, and one additional female (extension; not included in clinical observations) was found dead gestation day 21.

50 mg/kg/day

Clinical observations were minimal and sporadic and demonstrated no clear pattern of effect and/or did not persist. None were considered related to treatment with sodium bromide.

No females had any adverse clinical observations during the premating period. A mass on the snout and scab on the mouth were noted in one female during gestation. Two females (2939, 2948) had ungroomed coat, two (2944, 2936) had soft and liquid feces and two (2930, 2948) had sparse hair coat on the limbs during lactation. In low dose group one female was found dead gestation day 22.

175 mg/kg/day

Clinical observations were minimal and sporadic. Most signs that occurred were in the two females that were euthanized due to adverse clinical observations. The signs did not persist and demonstrated no clear pattern of effect. None were considered related to treatment with sodium bromide.

A single female had a mass on a swollen snout during the premating period. During lactation chromorhinorrhea, soft and liquid feces and piloerection occurred in single females.

500 mg/kg/day

In high dose group nine females (study day 58 (pre-mating) and 77 (mating), lactation days 0, 6, 26, 27, 28, 53, 68) were euthanized prior to study termination due to severe clinical observations.

The number of females (2 to 9) with chromodacryorrhea, mild dehydration and ungroomed coat during the premating period was significantly increased (p≤ 0.01) compared to control group females. Single cases of females with ataxia, rales. During gestation, the number of females with mild dehydration, urine-stained abdominal fur, thin body condition, hunched posture, chromodacryorrhea, ungroomed coat, ptosis and/or ataxia were all significantly increased (p≤ 0.01) compared to control group females. A single female had rales during gestation.

During the lactation period, the number of females with dehydration, ungroomed coat, hunched posture, decreased motor activity, ataxia, and pale body were all significantly increased (p≤ 0.01) compared to control group females. The number affected ranged from 1 to 5 and most of the signs that occurred only in a single female occurred in a rat that was euthanized early.

Table: General toxicity in females – P-generation, during first pairing

	0 mg/kg bw/d	**50 mg/kg bw/d**	**175 mg/kg bw/d**	**500 mg/kg bw/d**
Mortality	2	1	2	9
Clinical observation during pre-mating	(N=24)	(N=24)	(N=24)	(N=24) Chromodacryorrhea (6**) Dehydration, mild (6**) Ungroomed coat (3**)
Clinical observation during gestation	(N=23, excluding animals that did not have a confirmed	(N=23)	(N=22)	(N=10k) Dehydration, mild (9**) Urine-stained abdominal fur (4**)

	mating date)			Thin body condition (3**) Hunched posture (3**) Chromorhinorrhea (3**) Chromodacryorrhea (2**) Ungroomed coat (2**) Ptosis (2**) Ataxia (2**)
Clinical observation during lactation	(N=23)	(N=22)	(N=16)	(N=6k) Dehydration, mild and moderate (5**) Ungroomed coat (4**) Hunched posture (4**) Decreased motor activity (1**) Ataxia (1**) Whole body: pale (1**)
Food consumption [g/rat/day] during pre-mating (d 1-71)	16.8 ± 1.6	17.2 ± 1.4	17.3 ± 2.3	17.1 ± 1.2 [23]b
Food consumption [g/rat/day] during gestation (d 0-7)	20.6 ± 2.8	22.0 ± 2.1	20.6 ± 3.0	20.2 ± 2.4 [15]d
Food consumption [g/rat/day] during gestation (d 7-10)	21.9 ± 2.4	23.7 ± 5.4	22.2 ± 3.3	21.9 ± 3.3
Food consumption [g/rat/day] during gestation (d 10-14)	22.3 ± 1.6	22.9 ± 2.1	22.4 ± 3.2	20.2 ± 1.9
Food consumption [g/rat/day] during gestation (d 14-20)	24.1 ± 2.6	24.6 ± 2.3	22.8 ± 2.6	20.7 ± 1.7**
Food consumption [g/rat/day] during gestation (d 0-20)	22.2 ± 2.2	23.2 ± 1.9	22.0 ± 2.5	20.6 ± 2.1
Food consumption [g/rat/day] during lactation (d 1-14)	54.0 ± 11.2 [21]c,e	52.6 ± 9.0 [20]e	46.7 ± 8.7* [15]c	19.4 ± 3.7** [5]c
Body weight [g] pre-mating day 1	150.0 ± 6.9	151.2 ± 5.7	149.5 ± 6.7	149.8 ± 6.4
Body weight [g] pre-mating day 36	237.6 ± 19.5	243.5 ± 22.2	244.3 ± 29.2	248.8 ± 19.0
Body weight [g] pre-mating day 71 (last value recorded before mating)	269.0 ± 25.7	276.8 ± 24.0	272.1 ± 32.8	263.3 ± 23.8
Body weight [g] GD 0	268.0 ± 25.4	276.1 ± 25.4	265.9 ± 31.9	251.3 ± 22.0
Body weight [g] GD 20	401.4 ± 37.6	411.0 ± 32.4	390.7 ± 44.4	358.2 ± 39.5*
Body weight [g] at lactation day 0,	310.9 ± 27.1	319.7 ± 26.3	305.6 ± 40.4	290.7 ± 20.0
Body weight [g] at lactation day 7	333.2 ± 29.0	341.3 ± 29.3	325.1 ± 33.2	297.0 ± 24.1*
Body weight [g] at lactation day 21	337.8 ± 26.4	344.6 ± 28.4	336.1 ± 35.6	292.8 ± 28.5**

* Significantly different from the control group value ($p \leq 0.05$).

** Significantly different from the control group value ($p \leq 0.01$).

[ ] = NUMBER OF VALUES AVERAGED

c. Excludes values for rats that were found dead or euthanized due to adverse clinical observations.

d. Because it is presumed that the pups begin to consume maternal food after Day 14 of lactation, maternal food consumption values were not tabulated after Day 14 of lactation.

e. Excludes values that were associated with spillage.

K. excludes rats that did not have a confirmed mating with a treated male rat and therefore, were paired with untreated male rats

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)

Reduced body weight gain was observed in high dose males from week 3 onwards and body weight at the end of the dosing period was 75% of control values: food intake was also lower from week 4 onwards. In high dose females, reduced body weight gain and food intake was observed only during late gestation and lactation.

At 175 mg/kg/day effects on body weight were only observed in males and were more moderate, with a terminal mean body weight of 86.8% of the control value, and significantly reduced food intake from week 6 onwards. Female food intake was reduced only in early lactation.

Administration of 50 mg/kg/day sodium bromide had no adverse effect on body weight, body weight gain or food intake in males or females of the P generation and any other findings were regarded as not adverse.

Body weight

Males

Body weight and body weight gain were not affected by sodium bromide at 50 mg/kg/day.

Mean body weight gain during the premating dosing period (DS 1 to 71) was 105% of the control group value. At the end of the dosing period (DS 183) mean body weight was 103.0% of the control group value. Transient body weight gains were limited to the first and last weeks of dosing, and weight loss occurred only during the mating period (week 19), as often observed due to increased activity during cohabitation).

At 175 mg/kg/day, mean body weight gain was significantly reduced ($p \leq 0.01$) over the premating dosing period (93.5% - DS 1 to 71) and at end of the dosing period (86.8% - DS 183).

Body weight gains were significantly increased ($p \leq 0.05$) in the first week of treatment and significant reductions in gain or weight losses ($p \leq 0.05$ to $p \leq 0.01$) were observed for the majority of intervals from week 6 onwards.

At 350 mg/kg/day mean body weight over the premating (DS1 to 71) and entire dosing period DS1 to 183) were significantly reduced ($p \leq 0.05$ to $p \leq 0.01$). Body weight gain was increased or significantly increased ($p \leq 0.05$ to $p \leq 0.01$) in weeks 1, 14, 16, and 26 and significantly reduced gain or weight loss ($p \leq 0.05$ to $p \leq 0.01$) was observed for the majority of intervals from week 3 onwards.

Females

Body weight and body weight gain of females during the premating period (DS 1 to 71) were not affected by doses of sodium bromide as high as 500 mg/kg/day. Body weights at the end of the premating period (DS 71) were 102.9%, 101.1% and 97.9% of the control group value for the 50, 175 and 500 mg/kg/day dose groups, respectively. Transient increases ($p \leq 0.05$ to $p \leq 0.01$) in body weight and /or body weight gain in weeks 1 and 4 in the 50 or 500 mg/kg/day dose groups were not considered related to sodium bromide.

During the gestation and lactation periods, body weight and body weight gain were not affected by doses of sodium bromide as high as 175 mg/kg/day. Body weights at the end of the gestation (DG 20) and lactation periods (DL21) were 102.3%, and 102% of the control group value for the 50 mg/kg/day dose groups, and 97.7% and 99.5% for the 175 mg/kg/day dose group.

At 500 mg/kg/day, the reduced body weight on DG 20 (89.2% of the control value, $p \leq 0.05$) represented reduced gains for DGs 0 to 20 and 14 to 20 ($p \leq 0.01$). Significantly lower body weights were observed from DL 7 onwards (DL7, $p \leq 0.05$, DL14 and 21, $p \leq 0.01$) and body weight gain over the entire lactation period (DL 0-21, 0.2g) was markedly lower than controls (26.2g, $p \leq 0.05$).

Food consumption

Males

Food consumption mean values of males were not affected by 50 mg/kg/day sodium bromide during the premating, gestation, and lactation period or until termination.

Mean food consumption values for males in the 175 mg/kg/day sodium bromide dose group were reduced ($p \leq 0.05$ to $p \leq 0.01$) for each weekly interval from week 6 (DSs 36 to 43) onwards and over the entire dosing period (DSs 1 to 183, [$p \leq 0.01$]).

Mean food consumption values in males treated at 350 mg/kg/day were reduced ($p \leq 0.01$) over the entire dosing period (DS 1 to 183) and for each interval from week 4 onwards ($p \leq 0.05$ to $p \leq 0.01$).

Females

Mean food consumption values of females were not affected by treatment with 50 mg/kg/day sodium

bromide

At 175 mg/kg/day, there was no effect of treatment in the pre-mating and gestation periods, but reductions on DL 4-7(p≤ 0.01) and DL 9 to 14 (p≤ 0.05) resulted in an overall reduced food intake for the lactation period assessed (DL 0-14, p≤ 0.05).

At 500 mg/kg/day, transient changes in the premating period (increased week 1, p≤ 0.01, decreased week 10, p≤ 0.05) were attributed to treatment, Decreased food intake was observed towards the end of gestation (DGs 14-20, p≤ 0.01) and for the lactation period assessed (DL 0-14, p≤ 0.01).

FERTILITY, PARTURITION AND SEXUAL FUNCTION (PARENTAL ANIMALS - FIRST COHABITATION PERIOD)

Owing to low numbers of confirmed matings in the high dose group, alternative pairings were included, and the cohabitation period extended, as follows:

Groups 1 to 3

Females that did not mate in the first 10 days of cohabitation were assigned alternate males (from the same dose group) and remained in cohabitation for up to 7 additional days.

Males that did not mate in the first 10 days were re-paired with untreated female rats (selected from retained spare females) for 7 days.

Groups 4 and 5

After 14 days of cohabitation with Group 5 females, Group 4 males not recorded as mated were re-paired with untreated female rats for up to 10 days.

Group 5 females that did not mate with the assigned group 4 male in the first 14 days of cohabitation were re-paired with untreated males and remained in cohabitation for up to 10 additional days.

All untreated female rats were used only to confirm mating/pregnancy and were terminated on DG21 or, if mating was not confirmed, within 14 days of the end of cohabitation**.**

Male Rats

Control

Of 24 males paired, 23 mated with treated (control) females and all of these females were pregnant. Two males (2207, 2218) were not recorded as mated with control females (although the female allocated to 2207 was subsequently found to be pregnant) and were re-paired with untreated females, both of which mated and became pregnant. The total number of control males mated was therefore 24, and all impregnated at least one female.

The mating index was 95.8% with treated females and 100% with treated & untreated females.

The fertility index was 100% with both. Two males (2201, 2202) mated and were re-assigned to 2 different control females (2907, 2918), only one of which (2918) mated and delivered.

50 mg/kg/day

One male (2248) died prior to mating and was excluded from calculations.

All 23 males paired with treated females, were confirmed mated, of which 2 (2226,2235) did not impregnate the female. One male which was not recorded as mated (2245) was re-paired with an untreated female, and both allocated females were subsequently found to be pregnant.

Two males (2225, 2227) mated early and were re-assigned to different treated females, which had not been recorded as mated with the first allocated male. Both of these females were subsequently pregnant, although delivery dates suggest one (2945) had likely mated with the first male allocated (2245).

In total, all males mated and but 2 (2226, 2235) did not impregnate a female. The mating index was 100% and the fertility index was 91.3% for males for both treated females and the combined treated/untreated females.

175 mg/kg/day

Of 24 males paired with treated females, 22 were confirmed mated. The 2 males not recorded as mated were then re-paired with untreated females: one (2265) mated and impregnated the female but the other (2258) did not mate.

Two males (2249,2250) mated and were re-assigned to 2 different treated females (2957, 2964), which had not been recorded as mated with the first allocated male (2258, 2265). Neither of these females mated

In total, only one male (2258) did not mate a female (treated/untreated). Sixteen males impregnated treated females and one impregnated an untreated female.

The mating index was 95.8% with all females, and 91.7% for males with treated females. The fertility index was 73.9% with all females and 72.7% with treated females, significantly lower than controls (p≤ 0.05), but only slightly below the historical control range (75-100%).

350 mg/kg/day

Three males died prior to cohabitation (2275, 2280, 2288), one male (2296) was not paired as there was no available female and the female allocated to one male (2285) died during cohabitation.

Of the remaining 19 males paired with treated females, 8 were confirmed mated, and there were 6 pregnant females. Two females were mated by the same male so the total number of males siring a pregnancy was 5.

12 males were re-paired with untreated females, of which 10 females were confirmed mated (83.3%) and 7 achieved a pregnancy (70%). Of these 12, one male (2274) had previously mated a treated female (2974) and achieved a pregnancy but all others were not recorded as mated.

Two males (2278, 2293) did not mate either allocated (treated/untreated) female. Of the 17 mated males, 11 impregnated at least one female.

One of the mated males (2284) was paired with 2 treated females (2985,2975- both of which mated and delivered) and one (2274) was paired with 3 treated females (2/3 mated, 1 delivered) and one untreated female (mated and pregnant).

The mating index was 89.5% with all females, and 42.1% with treated females. The fertility index was 64.7% with all females and 62.5% with treated females, both significantly lower than control values (p≤ 0.05 to p≤ 0.01).

There was also a difference in the timing of matings in the high dose animals: Significantly fewer matings (6/19) occurred in the first 5 days of cohabitation, when compared to controls (20/22).

Table. Male mating and fertility index – first cohabitation

Group **mg/kg/day**	**1** **(Control)** **0**	**2** **(Low)** **50**	**3** **(Intermediate)** **175**	**4** **(High)** **350**	**HCD**
Total (with treated females and untreated females)					Min- Max %
Mating Index %a N	100 24/24	100 23/23	95.8 23/24	89.5 17/19	75-100
Fertility Index %b N	100 24/24	91.3 21/23	73.9** 17/23	64.7** 11/17	75-100
With treated females					
Mating Index %a N	95.8 23/24	100 23/23	91.7 22/24	42.1 8/19	75-100
Fertility Index %b N	100 23/23	91.3 21/23	72.7* 16/22	62.5* 5/8	75-100
With untreated females					
Mating Index %a N	100 2/2	100 1/1	50 1/2	83.3 10/12	75-100
Fertility Index %b N	100 2/2	100 1/1	100 1/1	70 7/10	75-100

* Significantly different from the Group 1 value (p≤ 0.05).
** Significantly different from the Group 1 value (p≤ 0.01).
a Percent of pairings that resulted in matings (includes only one mating for each male that mated more than 1 female).
b Percent of matings that resulted in pregnancy (includes only one pregnancy for each male that impregnated more than 1 female).

Female rats

An adverse effect on mating index was observed in females treated at 500 mg/kg/day when paired with treated males, 45.5% (10/22) of pairings resulted in matings. However, only 2 females failed to mate with either a treated or an untreated male. There was no effect on mating index at 50 or 175 mg/kg/day.

The overall fertility index (treated/untreated females) was significantly lower than controls in females at 500 mg/kg/day ($p \leq 0.01$). When paired with treated males, fertility was markedly reduced at 500 mg/kg/day and marginally lower than the historical control range at 175 mg/kg/day.

There was no effect of treatment on fertility at 50 mg/kg/day.

Control

All 24 females which were paired with control males were mated and delivered, although the day of mating was not confirmed for one (2907) which was subsequently re-paired with a proven male. The mating and fertility indices were both 100%.

50 mg/kg/day

Of the 24 females paired with treated males, all mated (including 2945 for which no day of mating was recorded and was subsequently re-paired with a proven male) and 22 were pregnant.

The mating index was 100% and the fertility index, at 91.7%, was not significantly different from controls and was within the historical control range.

175 mg/kg/day

Of the 24 females paired, 22 were confirmed mated with treated males and 16 were pregnant.

The two females (2957, 2964) not recorded as mated were re-paired with an alternative, proven treated male but did not mate: the male (2258) initially allocated to 2957 appeared infertile as it did not mate an untreated female and in the second cohabitation mated the allocated treated female but it was not pregnant.

The mating index was 91.7% and the fertility index, at 72.7%, was significantly lower than control values, but only marginally below the historical control range.

500 mg/kg/day

Two females were excluded from calculations as one died before pairing and the other died during cohabitation.

Of 22 females paired with treated males, 10 were confirmed mated, including 2981 which was re-paired with proven male 2274. Female 2989 was also re-paired with 2274 but did not mate.

Six females were pregnant.

The 12 females which had not mated were re-paired with untreated males. Of these 2 were not confirmed as mated (2988, 2992) and only one (2982) was mated not pregnant.

In total, 20 of 22 females mated with treated/untreated males, and 15 were pregnant. Five females (2981, 2982, 2984, 2990, 2993) mated but were not pregnant, one of which (2982) was paired with an untreated male. Two females (2988, 2992) did not mate or deliver with either treated or untreated males.

The mating index was 45.5% for females mated with treated males and 90.9% including untreated males. The overall fertility index (treated/untreated males) was 75%, and with treated males was 60%, significantly lower than control females ($p \leq 0.05$ to $p \leq 0.01$).

Table. Female mating and fertility index – first cohabitation

Group **mg/kg/day**	**1** **(Control)** **0**	**2** **(Low)** **50**	**3** **(Intermediate)** **175**	**5** **(High)** **500**	**HCD**
Total (with treated males and untreated males)					Min- Max %

Mating Index %a N	NA	NA	NA	90.9 20/22	75-100
Fertility Index %b N	NA	NA	NA	75** 15/20	76-100
With treated males					
Mating Index %a N	100 24/24	100 24/24	100 22/24	45.5 10/22	75-100
Fertility Index %b N	100 24/24	91.7 22/24	72.7* 16/22	60** 6/10	76-100
With untreated males					
Mating Index %a N	NA	NA	NA	83.3 10/12	75-100
Fertility Index %b N	NA	NA	NA	90 9/10	76-100

NA = Not applicable.
* Significantly different from the Group 1 value ($p \leq 0.05$).
** Significantly different from the Group 1 value ($p \leq 0.01$).
a Percent of pairings that resulted in matings.
b Percent of matings that resulted in pregnancy.

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS - SECOND COHABITATION PERIOD)

Of the 23, 24 and 22 females in the 0, 50 and 175 mg/kg/day dose groups continued on study for a second mating, gestation and lactation, none showed adverse clinical observations related to sodium bromide.

The observations reported were not considered related to the test substance because most occurred in only one or two rats per group, the number of rats affected was not dose-dependent and/or the number of rats affected was greatest in the control group. Excluding signs that occurred only in the rats that were found dead, observations included: sparse hair coat, mild dehydration, swollen mouth or ears, a mass on the flank, scab on the lip, urine stained abdominal fur, ungroomed coat, chromodacryorrhea, soft or liquid feces and/or red perivaginal substance.

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS - SECOND COHABITATION PERIOD)

During the gestation and lactation periods values for body weight, body weight gain or food consumption were comparable between the groups for all intervals.

FERTILITY, PARTURITION AND SEXUAL FUNCTION (PARENTAL ANIMALS - SECOND COHABITATION PERIOD)

Owing to lower than expected pregnancy rates in the intermediate dose group from the first cohabitation, Groups 1 to 3 (0, 50 and 175 mg/kg/day) were retained for a second cohabitation period to produce F1b litters. Within each dose group, males and females were assigned different partners from the first pairing.

Male rats

In the second cohabitation period, there was no effect of treatment on male mating performance or fertility at 50 mg/kg/day. At 175 mg/kg/day there was a significant reduction in fertility index ($p \leq 0.01$) although the value was only slightly below the lower limit of the historical control range. The

mating index was lower than that of control males but within the historical control range. There was no adverse effect on the timing of mating, however, as all males mated within the first 5 days of cohabitation.

Control

One male (2210) was not paired as there was no female available. Of the 23 paired males, all mated and impregnated a female. One male (2201) was re-paired with an apparently non-mated female (2914) which was subsequently found to be mated and pregnant by the first allocated male (2218).

The mating and fertility indices were both 100%.

50 mg/kg/day

One male (2248) died prior to cohabitation. Of the 23 males paired, 22 were confirmed as mated and all mated females were pregnant. The female (2937) not mated with the first allocated male (2226) was re-paired with a proven male (2225) but did not mate. One male (2227) was

allocated 2 females (2928, 2948) both of which mated and were pregnant.

The mating index was therefore 95.6% and the fertility index was 100%.

175 mg/kg/day

One male (2252) died prior to cohabitation and one was not paired as no female was available.

Of the 22 males paired, 19 mated and 14 females were pregnant. Three mated males (2251, 2253, 2254) were re-paired with females (2955, 2967, 2972) which had not mated with the first allocated male, and these females did not mate at the second pairing.

The mating index was 86.4% and the fertility index was 73.7%, significantly lower than controls ($p \leq 0.01$).

Table Male Reproduction Index - Second Cohabitation Period

Group **mg/kg/day**	**1 (Control)** **0**	**2 (Low)** **50**	**3 (Intermediate)** **175**	**HCD**
Mating Index % N	100 23/23	95.6 22/23	86.4 19/22	75-100
Fertility Index % N	100 23/23	100 22/22	73.7** 14/19	75-100

** Significantly different from the Group 1 value ($p \leq 0.01$).

Female Rats

At the second cohabitation period, mating and fertility was not affected by 50 mg/kg/day sodium bromide, both indices were comparable to controls.

At 175mg/kg/day, the mating index was slightly lower than the control value and there was significant reduction in the fertility index ($p \leq 0.01$), although this was only slightly below the historical control range.

There was no effect of treatment on the timing of mating: all matings were within the first 5 days of the cohabitation period.

Table Female Reproduction Index - Second Cohabitation Period

Group **mg/kg/day**	**1 (Control)** **0**	**2 (Low)** **50**	**3 (Intermediate)** **175**	**HCD**
Mating Index %	100	95.8	86.4	75-100

N	23/23	23/24	19/22	
Fertility Index % N	100 23/23	100 23/23	73.7** 14/19	76-100

** Significantly different from the Group 1 value (p≤ 0.01).

OVERALL MATING AND FERTILITY PERFORMANCE (FIRST AND SECOND COHABITATION PERIODS)

In total, over both cohabitation periods, all males in Groups 1, 2 and 3 mated at least one female.

All control males impregnated at least one female and there was only one male in the 50 mg/kg/day group which did not achieve a pregnancy. At 175 mg/kg/day, although reduced pregnancy rates were observed at both cohabitation periods, unusually, the affected animals differed and in total only 2 males did not impregnate a female, giving an overall male fertility index of 91.7% and 77.3% for males and treated females (within the historical control range). This was likely a deficit in these males, as neither of the treated females allocated to one male became pregnant at the alternative pairing and the allocated untreated female was not mated, but the females allocated to the other male both became pregnant with alternative males.

At histopathology, minimal/mild spermatid retention was noted in the testes of both these males and both males had >10% abnormal sperm at seminology evaluation: one further male also had minimal lymphocytic infiltration of the epididymis: however, all these findings were observed in several other males in this group which achieved a pregnancy, and their results for other semen parameters were within the range of the remainder of the group. Spermatid retention (Sertoli cell and/or tubular, minimal/mild) was observed in 9 other males in this group but all impregnated at least one female, as did the remainder of the group, suggesting any effect on fertility may have been temporary and/or recoverable.

All females in Groups 1, 2 and 3 mated during either the first or second cohabitation periods. Five females in the 175 mg/kg/day group did not get pregnant from either pairing (with treated males only), giving an overall female fertility index of 77.3% (17/22), below the concurrent control value but within the historical control range. Only two of these females showed marked depletion of corpora lutea at histopathology and no corpora lutea at ovarian follicle examination.

One of these females had also shown extended estrus (9 days) and a further female, showed extended periods of diestrus (which may indicate pseudopregnancy). Another female which had marked depletion of corpora lutea at histopathology and no corpora lutea at follicle counting, was pregnant at the first pairing (but not at the second).

ORGAN WEIGHTS (PARENTAL ANIMALS)

Reproductive organs - weight

Males

Administration of 50 mg/kg/day of sodium bromide did not produce any effect on the terminal body weight, absolute weight of the reproductive organs, or the ratio of the organ weight to the terminal body or brain weight.

Terminal body weights were significantly reduced (p≤ 0.01) in the 175 and 350 mg/kg/day dose group compared to the control group value.

At 175 and 350 mg/kg/day, the reductions (p≤ 0.05 to p≤ 0.01) in absolute weight occurring in all reproductive organs evaluated reflected the reduced terminal body weights and were not considered adverse as the ratios of the reproductive organ weight to terminal body weight were all either comparable or significantly increased (p≤ 0.05 to p≤ 0.01) compared to the control group value.

The ratios of the reproductive organ weights to the brain weight were significantly reduced (p≤ 0.05 to p≤ 0.01) compared to control values but were not adverse as they reflected the reduced brain and body weights in these groups.

Table. Male reproductive organ weights – P generation

	Dose Level (mg/kg/day)	**HCDa**

	0	**50**	**175**	**350**	**Mean Min-Max**
No. Rats per Group	24	23	23	20	
Terminal Body Weight (grams)					
	672.5	691.7	580.4**	505.5**	
Epididymis Left					
Absolute value	0.8300 ± 0.0849	0.8269 ± 0.0521	0.7680± 0.0695**	0.6733 ± 0.0673**	0.7189 0.2600-0.8500
% of body weight	0.124 ± 0.013	0.120 ± 0.014	0.132± 0.014	0.132 ± 0.016	
% of brain weight	36.5 ± 3.8	35.9 ± 2.2	35.1 ± 3.1	31.2 ± 3.3**	
Cauda Epididymis Left					
Absolute value	0.3244 ± 0.0387	0.3203 ± 0.0244	0.2959 ± 0.421*	0.2434 ± 0.0395**	0.3108 0.1300-0.3920
% of body weight	0.049 ± 0.008	0.046 ± 0.007	0.050 ± 0.008	0.048 ± 0.010	
% of brain weight	14.3± 1.6	13.9 ± 1.0	13.5 ± 2.1	11.3 ± 1.9**	
Testis Left					
Absolute value	1.9532 ±0.1156	1.9217 ± 0.1244	1.7889 ± 0.1453**	1.6819 ± 0.1273 **	1.8124 1.3300-2.0565
% of body weight	0.290 ± 0.032	0.280 ± 0.031	0.308 ± 0.033	0.334 ± 0.040**	
% of brain weight	86.0 ± 4.8	83.4 ± 4.5	81.7 ± 6.6*	78.0 ± 7.0**	
Seminal Vesicles with Fluid					
Absolute value	2.1465 ± 0.2762	2.0164 ± 0.3269	1.8609 ± 0.2907**	1.6548 ± 0.3469**	1.8946 0.8100-2.6440
% of body weight	0.324± 0.063	0.295 ± 0.064	0.323 ± 0.065	0.328 ± 0.062	
% of brain weight	94.6 ± 13.0	87.4 ± 13.1	85.1 ± 14.0*	76.7 ± 16.2**	
Seminal Vesicles without Fluid					
Absolute value	0.9497 ± 0.1481	0.9197 ± 0.1577	0.8311 ± 0.1387*	0.7962 ± 0.1503***	0.8976 0.4300-1.2765
% of body weight	0.143 ± 0.027	0.133 ± 0.027	0.144 ± 0.030	0.156 ± 0.024	
% of brain weight	41.7 ± 6.2	39.9 ± 6.4	38.0 ± 6.7	36.9± 7.1	
Epididymis Right					
Absolute value	0.8394 ±	0.8210 ±	0.7904 ±	0.6643 ±	0.7311

	0.0711	0.0434	0.0622* 94% of control	0.0570** 79% of control	0.2700-0.8647
% of body weight	0.124 ± 0.014	0.119 ± 0.011	0.136 ± 0.015**	0.132 ± 0.014	
% of brain weight	37.0 ± 3.5	35.6 ± 2.1	36.1 ± 2.9	30.8± 2.9**	
Testis Right					
Absolute value	1.9295 ± 0.1239	1.9002 ± 0.1131	1.7929 ± 0.1441**	1.6804 ± 0.1272**	1.8201 1.3500-2.0706
% of body weight	0.289 ± 0.033	0.276 ± 0.031	0.311 ± 0.033*	0.333 ± 0.039**	
% of brain weight	84.9 ± 5.0	82.4 ± 4.0	81.9 ± 6.6	77.9 ± 6.7**	
Prostate					
Absolute value	1.5269 ± 0.2327	1.5254 ± 0.2487	1.4524 ± 0.2560	1.2519 ± 0.2249**	1.2294 0.5100-1.4972
% of body weight	0.227 ± 0.040	0.223 ± 0.046	0.251 ± 0.050	0.247 ± 0.040	
% of brain weight	67.3 ± 10.8	66.2 ± 10.9	66.4 ± 11.9	58.1 ± 10.8*	
Pituitary					
Absolute value	0.016 ± 0.003	0.015 ± 0.003	0.014± 0.003**	0.013± 0.003**	0.14 0.08-0.25
% of body weight	2.441 ± 0.506	2.234 ± 0.447	2.358 ± 0.563	2.517 ± 0.554	
% of brain weight	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1*	0.6 ± 0.1**	

Females

Administration of 50 or 175 mg/kg/day of sodium bromide did not produce any effect on the terminal body weight, or absolute or relative weight of the reproductive organs to the terminal body/brain weight. Administration of 500 mg/kg/day of sodium bromide reduced the absolute ovary weight to 63% of control value, and the ratio of the ovary weight to the terminal body weight and brain weight ($p \leq 0.01$). There was also an increase in pituitary weight, absolute ($p \leq 0.05$) and relative to body and brain weight ($p \leq 0.01$).

Two females with the highest absolute pituitary weight (2955, 2967, outside the concurrent control range) were also found to have marked depletion of corpora lutea at histopathology and no corpora lutea at follicle examination. There were, however, 3 other terminal kill females which had no corpora lutea which did not have elevated pituitary weight and the mean absolute pituitary weight was within the historical control range.

Table. Female reproductive organ weights – P generation

	Dose Level (mg/kg/day)				**HCDa**
	0	**50**	**175**	**500**	**Mean Min-Max**

No. Rats per Group	22	23	22	15	
Terminal Body Weight (grams)					
	323.5 ± 28.3	326.0 ± 23.1	331.0 ± 40.4	310.3 ± 24.8	
Ovary					
Absolute value	0.140 ± 0.019	0.154 ± 0.051	0.128 ± 0.032	0.088 ± 0.027**	0.135 0.098-0.206
% of body weight	0.042 ± 0.004	0.046 ± 0.017	0.040 ± 0.011	0.028 ± 0.008**	
% of brain weight	6.7 ± 0.9	7.4 ± 2.6	6.4 ± 1.7	4.5 ± 1.3**	
Uterus with cervix					
Absolute value	0.77 ± 0.17	0.76 ± 0.27	0.74 ± 0.19	0.77 ± 0.20	0.83 0.46-5.70
% of body weight	0.238 ± 0.051	0.231 ± 0.071	0.222 ± 0.051	0.248 ± 0.065	
% of brain weight	36.8 ± 7.9	36.5 ± 12.6	36.8 ± 9.0	39.0 ± 10.2	
Pituitary					
Absolute value	0.020 ± 0.006	0.020 ± 0.004	0.022 ± 0.007	0.026 ± 0.007*	0.018 0.013-0.035
% of body weight	6.273 ± 2.045	6.068 ± 1.474	6.547 ± 1.704	8.379 ± 2.340**	
% of brain weight	1.0 ± 0.3	0.9 ± 0.2	1.1 ± 0.4	1.3± 0.3**	

GROSS PATHOLOGY (PARENTAL ANIMALS)

No gross lesions related to treatment with sodium bromide occurred in males or females in any dose group. Four animals (one male and two females at 175 mg/kg/day and one male at 350 mg/kg/day) were noted to have a mass on their forelimbs; these correlated microscopically with abscesses. These, and the other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rat, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of sodium bromide.

HISTOPATHOLOGY (PARENTAL ANIMALS)

Reproductive organs - histopathology

Males

Test substance-related microscopic findings were noted in the reproductive tract of animals that had received 175 or 350 mg/kg/day sodium bromide, with a dose-related trend in incidence and severity. In males, a subtle increase in spermatid head retention was identified, particularly in stage XI tubules; less frequently in stage IX, X, or XII tubules. An increase in sperm retained at the surface of the tubular lumen was also noted. Increased debris (nucleated cells and amorphous eosinophilic material, which often surrounded sperm with curled tails) in the epididymis, also showed a test substance-related trend in males All males (20) at 350 mg/kg/day were affected with severity ranging from mild to moderate; at 175 mg/kg/day, 11/23 males were affected, with the majority showing only minimal changes and only 4 showing epididymal debris.

Females

In females test article-related depletion of corpora lutea was present in the ovaries of 3 females administered 175 mg/kg/day sodium bromide and 10 females treated at 500mg/kg/day surviving to terminal kill. A further high dose female, killed in week 23, also showed depletion of corpora lutea.

Other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rat, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of sodium bromide.

Ovarian Follicle Evaluation

No significant differences were noted in the number of primordial follicles in either left, right or both ovaries for animals treated with 50, 175 or 500 mg/kg/day sodium bromide compared to the Control group. Corpora lutea were present for all control females at the terminal kill, and only one animal in each of 50 mg/kg/day (2937) and 175 mg/kg/day (2956) dose groups had no corpora lutea present. Two further females from the 175 mg/kg/day group (2955, 2967, mated but not pregnant at the second cohabitation and killed on DG 25) had no corpora lutea present and another female (2972 killed DG 25) had corpora lutea present but they were largely regressing, although there was no effect on the overall number of follicles in these females.

There were, however, 3 other not mated/not pregnant females which did have corpora lutea present. At 500mg/kg/day, 5/8 terminal kill females evaluated had no corpora lutea present, and there were 5 further females terminated early which also had no corpora lutea.

As 3/20 females treated at 500 mg/kg/day in the recent 90 day study (Charles River laboratories Study No 20038591) had no corpora lutea present, and as a reduction in the number of corpora lutea per female was observed at the high dose level of 19200 ppm/kg diet (in excess of 1000 mg/kg body weight/day) in a published study (van Logten et al (197412), this finding may be related to administration of sodium bromide.

NATURAL DELIVERY AND LITTER OBSERVATIONS (P GENERATION TO PRODUCE F1A/B)

Natural Delivery First Pairing in the P-generation, and Litter Observations of F1a Litters through Weaning

Natural delivery and litter observations were not affected by the 50 mg/kg/day dose group of sodium bromide. There were no effects at 175 mg/kg/day, other than the slightly lower pregnancy rate described above (73.7%).

At 500 mg/kg/day, the number of dams with stillborn pups and number of dams with all pups dying before day 4 postpartum were significantly increased ($p \leq 0.01$). Delivered litter size, surviving pups per litter and live litter size were also reduced and the number of liveborn pups (determined at necropsy, see Section 3.13.2.1.) was reduced ($p \leq 0.01$). All pups died on or before postnatal day 5. There was evidence of poor maternal care as an increased ($p \leq 0.01$) number of litters had pups with mild to moderate dehydration, that were cold to touch, not nursing, had no milk band present and thin body condition.

Natural Delivery Second Pairing in the P-generation, and Litter Observations F1b Litters through Weaning

The number of pregnant dams in the 175 mg/kg/day dose group was significantly reduced (14/22; $p \leq 0.01$) compared with the control group (23/23).

No other parameter evaluated at natural delivery or during the preweaning period was affected by sodium bromide at 50 or 175 mg/kg/day. These parameters included duration of gestation, the number of dams with stillborn pups/no liveborn pups or total litter loss, the number of delivered, liveborn or stillborn pups, surviving pups per litter, sex ratio or pup weights. There was no effect on the gestation, viability or lactation index.

Significant increases ($p \leq 0.01$) in the number of dams with stillborn pups, and the number of liveborn pups in the 50 mg/kg/day dosage group were not considered related to the test substance because there was no adverse effect on litter size and increases were not apparent at the higher dose level.

First cohabitation (P generation to produce F1a)				
	Control	**50 mg/kg bw/d**	**175 mg/kg bw/d**	**500 mg/kg bw/d**
Number of females in cohabitation [a] N	24	24	24	22
Number of mated females[b]	24/24 (100.0)	24/24 (100.0)	22/24 (91.7)	10/22 (45.5)

N/N (%)				
Delivery observations				
Number of dams that delivered a litter N (%)	24 (100.0)	22 (100.0)	16 (100.0)	6 (100.0)
Duration of gestation mean±SD	22.8 ± 0.4	22.6 ± 0.5	22.9 ± 0.4	22.8 ± 0.4
Number of dams with stillborn pups N (%)	2 (8.3)	4 (18.2)	0 (0.0)	3 (50.0)**
Number of dams with no liveborn pups N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gestation index [d] N/N (%)	24/24 (100.0)	22/22 (100.0)	16/16 (100.0)	6/6 (100.0)
Dams with all pups dying Days 0-4 post partum N (%)	0 (0.0)	0 (0.0)	0 (0.0)	5 (83.3)**
Dams with all pups dying Days 5-21 post partum N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Second cohabitation (P generation to produce F1b)				
	Control	**50 mg/kg bw/d**	**175 mg/kg bw/d**	**-**
Number of females in cohabitation N	23	24	22	-
Number of mated females N (%)	23 (100.0)	23 (95.8)	19 (86.4)	-
Delivery observations				
Number of dams that delivered a litter N (%)	22 (100.0) (excludes values for rats that were found dead during gestation)	22 (100.0) (excludes values for rats that were found dead during gestation)	14 (100.0)	-
Duration of gestation mean±SD	22.7 ± 0.6	22.7 ± 0.5	22.8 ± 0.4	-
Number of dams with stillborn pups N (%)	0 (0.0) [e]	4 (18.2)**	0 (0.0)	-
Number of dams with no liveborn pups N (%)	0 (0.0) [e]	0 (0.0)	0 (0.0)	-
Gestation index N/N (%)	21/21 (100) [e]	22/22 (100.0)	14/14 (100.0)	-
Dams with all pups dying Days 0-4 post partum N (%)	0 (0.0) [e]	0 (0.0)	0 (0.0)	-
Dams with all pups dying Days 5-21 post partum	0 (0.0)	0 (0.0)	0 (0.0)	-

N (%)				

a. Excludes values for rats that were euthanized due to adverse clinical observations.

b. Restricted to rats with a confirmed mating date and rats that did not mate.

c. Number of pregnancies/number of rats that mated.

d.. Number of rats with live offspring/number of pregnant rats

e. Excludes rat 2915, which was observed delivering, but no pups were present at processing.

** Significantly different from the control group value (p≤0.01).

CLINICAL AND NECROPSY OBSERVATIONS (F1 OFFSPRING)

Clinical and Necropsy Observations - F1a Pups from Birth to Weaning

Maternal doses as high as 175 mg/kg/day did not affect physical development of the pups as evaluated by pinna unfolding, hair growth, tooth eruption and eye opening. No pups in the 500 mg/kg/day dose group survived to allow evaluation of these end points.

Pups which died and were necropsied preweaning in the 0, 50 and 175 mg/kg/day groups generally appeared normal with the exception of one control group pup with a small thymus and two pups in the 50 mg/kg/day maternal dose group, one of which had slight dilation of the renal pelvis and one with dilated brain ventricles. No milk in the stomach, dehydration, cold to touch, not nursing and thin body condition were noted in an increased number of litters and pups (p≤ 0.01) in the 500 mg/kg/day group. The absolute or relative weight of the brain, spleen and thymus did not differ among the groups.

Clinical and Necropsy Observations - F1b Pups through Weaning

Two pups from two different litters in the 175 mg/kg/day dose group had a bent tail, although one was only apparent on day 21 post-partum. This incidence was increased (p≤ 0.01) compared to the control group, but as neither pup was reported as abnormal at necropsy and as one control pup had a different tail abnormality (thread-like tail in conjunction with malformed hindlimbs) was considered unlikely to have been an effect of treatment. No other clinical or necropsy observations in the 50 or 175 mg/kg/day dose group occurred in more than a single litter. These observations included ungroomed coat, a scab, mild or moderate dehydration, pale body, purple body, corneal opacity, umbilical hernia, black tip of tail, and/or cold to touch.

Necropsy observations in the 50mg/kg/day group were limited to one stillborn pup with a domed head, and one pup on day 21 post-partum with a firm mass in the abdomen and slight dilation of the renal pelvis.

	Control	**50 mg/kg bw/d**	**175 mg/kg bw/d**	**500 mg/kg bw/d**
F1a				
Number of pregnant females	24	22	16	6
Delivered litters with one or more liveborn pups N	24	16	16	6[a]
Pups delivered (total) N Mean ± SD	333 13.9 ± 3.2	304 13.8 ± 2.3	205 12.8 ± 3.2	63 10.5 ± 5.8
Liveborn Mean ± SD N (%)	13.7 ± 3.4 329 (98.8)	13.5 ± 2.3 298 (98.0)	12.8 ± 3.2 205 (100.0)	9.3 ± 5.8 56(88.9)**
Stillborn Mean ± SD N(5)	0.1 ± 0.3 2 (0.6)	0.3 ± 0.7 6 (2.0)	0.0 ± 0.0 0 (0.0)	0.8 ± 1.2* 5 (7.9)**
Unknown vital status [b]	2	0	0	2
Pups found dead or presumed				

cannibalized N/N (%)				
Day 0	2/329(0.6)	0/298(0.0)	0/205(0.0)	2/ 56(3.6)**
Days 1-4	9/327(2.8)	4/298(1.3)	6/189(3.2)c	40/40(100.0)**[d]
Days 5-7	1/302(0.3)[e]	0/294(0.0)	0/183(0.0)	x
Days 8-14	0/301(0.0)[e]	0/294(0.0)	0/183(0.0)[c]	x
Days 15-21	1/301(0.3)[e]	0/294(0.0)	0/183(0.0)[c]	x
Viability index [f] % (N/N)	96.6 (318/329)	98.6 (294/298)	96.8 (183/189)[c]	0.0** (0/42)[d]
Lactation index [g] % (N/N)	99.3e (300/302)[e]	100.0 (294/294)	100.0[c] (183/183)[c]	--- ----
Surviving pups/litter Mean ± SD				
0	13.7 ± 3.4	13.5 ± 2.3	12.8 ± 3.2	9.3 ± 5.8
4	13.2 ± 3.2	13.4 ± 2.2	12.2 ± 3.0 [15][d]	0.0 ± 0.0** [5][d]
7	13.1 ± 3.2 [23][d]	13.4 ± 2.2	12.2 ± 3.0 [15][d]	0.0 ± 0.0** [5][d]
14	13.1 ± 3.2 [23][d]	13.4 ± 2.2	12.2 ± 3.0 [15][d]	0.0 ± 0.0** [5][d]
21	13.0 ± 3.2 [23][d]	13.4 ± 2.2	12.2 ± 3.0 [15][d]	0.0 ± 0.0** [5][d]
Percent male pups per number of pups sexed Day 0 Mean ± SD	52.5 ± 12.2	49.4 ± 12.5	41.3 ± 15.9*	60.4 ± 26.5
Live litter size at weighing Day 0 Mean ± SD	13.6 ± 3.3	13.5 ± 2.3	12.8 ± 3.2	9.0 ± 5.3
Pup weight/litter (g) Mean ± SD	6.6 ± 0.6	6.6 ± 0.5	6.4 ± 0.8	6.5 ± 1.1
F1b				
Number of pregnant females	21	22	14	
Delivered litters with one or more liveborn pups N	21	22	14	
Pups delivered (total) N Mean ± SD	288 13.7 ± 4.2	326 14.8 ± 2.0	195 13.9 ± 2.8	
Liveborn Mean ± SD N (%)	13.7 ± 4.2 288 (100.0)	14.4 ± 2.1 318 (97.5)**	13.9 ± 2.8 195 (100.0)	
Stillborn Mean ± SD N(%)	0.0 ± 0.0 0 (0.0)	0.4 ± 1.0 8 (2.4)**	0.0 ± 0.0 0 (0.0)	
Pups found dead or presumed cannibalized N/N (%)				
Day 0	1/288 (0.3)	0/318 (0.0)	1/195 (0.5)	
Days 1-4	1/287 (0.3)	2/318 (0.6)	0/194 (0.0)	
Days 5-7	0/286 (0.0)	0/316 (0.0)	1/194 (0.5)	
Days 8-14	0/286 (0.0)	2/316 (0.6)	2/193 (1.0)	
Days 15-21	0/286 (0.0)	0/314 (0.0)	0/191 (0.0)	

Viability index % (N/N)	99.3 286/288	99.4 316/318	99.5 194/195	
Lactation index % (N/N)	100.0 (286/286)	99.4 (314/316)	98.4 (191/194)	
Percent male pups per number of pups sexed Day 0 Mean ± SD	53.4 ± 9.6 52.8 ± 10.8 48.4 ± 12.3			
Live litter size at weighing Day 0 Mean ± SD	13.7 ± 4.2 14.4 ± 2.1 13.8 ± 2.8			
Pup weight/litter (g) Mean ± SD	6.8 ± 0.7 6.7 ± 0.7 6.6 ± 0.6			

a. Excludes female rats that did not have a confirmed mating with a treated male rat and therefore, were paired with untreated male rats.

b. Maternal cannibalization precluded identification of vital status at birth.

c. Excludes 16 pups from litter 2968, which were euthanized due to death of dam on Day 1 postpartum.

d. Excludes 14 pups from litter 2975, which were euthanized due to death of dam on Day 0 postpartum.

e. Excludes 16 pups from litter 2904, which were euthanized due to death of dam on Day 6 postpartum.

f. Number of live pups on Day 4 postpartum/number of liveborn pups on Day 0 postpartum.

g. Number of live pups on Day 21 (weaning) postpartum/number of live pups on Day 4 postpartum.

* Significantly different from the control group value ($p \leq 0.05$).

** Significantly different from the control group value ($p \leq 0.01$).

Physical Development – F1b pups Preweaning

There was no effect of treatment at 50 or 175 mg/kg/day on physical development. There were no intergroup differences in the average post-partum day and the progression (percentage of pups per litter/day) of pinna unfolding, hair growth, and eye opening. Tooth eruption in the 175 mg/kg/day dose group was significantly delayed ($p \leq 0.05$ to $p \leq 0.01$) on PNDs 11, 13 and 14 and for the average day 50% of the litters reached criterion. The delay was not considered adverse as it reflected the slightly lower pup body weight and the average day to criterion was less than a day different from the control group.

BODY WEIGHT (F1 OFFSPRING AT WEANING)

Body Weights - F1a Pups at Weaning

Terminal body weights on PND 21 for male pups in the 50 and 175 mg/kg/day dose groups were 96.0% and 90.9% of the control group value, respectively.

Terminal body weight on PND 21 for female pups in the 50 and 175 mg/kg/day dose groups was 95.2% and 94.1% of the control value, respectively.

Sexual Maturation - F1a Postweaning

Doses of sodium bromide had no effect on the timing of male or female sexual maturation at 50 or 175 mg/kg/day.

Clinical Observations, Body Weight, Body Weight Gain, Food Consumption and Necropsy Observations and Sexual Maturation (Vaginal Patency) - F1b Postweaning until PND 40

Post weaning, 21, 22 and 14 pups per sex in the 0, 50 and 175 mg/kg/day dose groups, respectively, were evaluated. No statistically significant or toxicologically important differences occurred in these pups in terms of clinical observations, body weight, body weight gain, food consumption, necropsy observations or the day vaginal patency was attained. Male puberty was not assessed owing to the timing of the kill.

ORGAN WEIGHTS (F1 OFFSPRING

Organ Weights F1a Pups at Weaning

At 175 mg/kg/day, a significant reduction ($p \leq 0.01$) in absolute brain weight compared to the control value was considered secondary to the lower terminal body weight, as there was no effect on relative brain weight.

There was no effect on the absolute or relative weight of the other organs weighed, i.e. spleen and thymus.

Organ Weights F1b Pups at Weaning

Maternal administration of sodium bromide at 50 or 175 mg/kg/day did not affect any male or female pup organ weights on postnatal day 22. The only difference was a lower ($p \leq 0.01$) male brain weight in the 175 mg/kg/day dose group, which was considered to reflect the slightly lower male pup body weight in this group compared to the control group. No other statistically

significant differences occurred.

A total of 23, 22 and 15 pups per sex per litter from Groups 1, 2 and 3 were selected from the F1a litters to form the F1 generation.

CLINICAL SIGNS AND MORTALITY (F1 GENERATION)

Mortality

No deaths related to sodium bromide occurred; All males in the 50 and 175 mg/kg/day dose groups survived to scheduled euthanasia. One control female (8115, mated but not pregnant) was killed owing to a fractured forelimb, and one female (8125) at 50mg/kg/day was killed on the day of delivery (owing to adverse clinical signs) and showed mild liver necrosis.

Clinical Observations

Males

Vehicle Control

Clinical observations in control animals during the treatment period were limited to excess salivation (slight), mild dehydration and soft and liquid feces, in 2, 3 and 1 males respectively.

50 and 175 mg/kg/day

Clinical observations in the 50 and 175 mg/kg/day dose groups were not considered treatment related because none occurred more than 3 males and none demonstrated a clear pattern of effect and/or the sign did not persist.

These observations were limited to transient observations in one or 2 animals of sparse hair coat on the neck, slight excess salivation, bent tail, portion of the tail missing, vocalization to touch, black tip of the tail, aggression on handling, mild dehydration, a scab on the back or neck, soft or liquid feces, abrasion on the back and/or chromorhinorrhea.

Females

Vehicle Control

Minimal and sporadic common clinical observations occurred in this group. During the premating period, three females had mild dehydration, one female had a scab, one had sparse hair coat on the limbs, two had missing/broken incisors and one had an abrasion on the forelimbs. During the gestation and lactation periods, similar sporadic signs and additional signs occurred primarily in one female that was euthanized due to a fractured limb (female 8115).

50 mg/kg/day

Minimal and sporadic common clinical observations occurred in this group. During the premating period, two females had mild dehydration, one female had a scab, two had sparse hair coat on the limbs and one had a swollen snout. During the gestation and lactation periods, similar sporadic signs occurred. Additional signs occurred, mostly in a female that was euthanized due to apparent complications with delivery (8125). This female had sparse hair coat (DP 82 to 96), and on the day of littering had decreased motor activity, pale extremities and ears, hunched posture, bradypnea, ungroomed coat, a red substance on the fur in the genital area and was cold to touch. This dam had delivered two still born pups, one pup that was born alive and died and 13 live pups, but delivery was

incomplete when it was euthanized as necropsy revealed 18 corpora lutea, 17 implantation sites, and 2 dead fetuses *in utero*. These changes were not considered related to sodium bromide because there was no effect at the higher dose level and the incidences were within the historical control ranges for this Testing Facility.

175 mg/kg/day

Minimal and sporadic common clinical observations occurred in this group, including single rats with mild dehydration, a scab, a mass, urine-stained abdominal fur and/or piloerection.

BODY WEIGHT AND FOOD CONSUMPTION (F1 GENERATION)

Body weight

Males

Body weight and body weight gain were not significantly affected by sodium bromide at 50 mg/kg/day. Mean body weights at the end of the premating dosing period (PND 91) and the end of the dosing period (PND 147) were 98.6% and 101.5 % of the control group value, respectively. Significantly increased body weight gain ($p \leq 0.05$) on PND 106 to 113, was not considered related to sodium bromide as it did not persist.

At 175 mg/kg/day mean body weights were significantly reduced ($p \leq 0.01$) from PND 71 to PND 148, with mean body weight at the end of the premating dosing period (PND 91) and at the end of dosing period (PND 147) at 89.9 % and 87.7% of the control group values, respectively.

Body weight gain was significantly reduced ($p \leq 0.01$) over the entire dosing interval (PND 22 to 148) and for specific intervals between PNDs 57 to 78 and 92 to 99 ($p \leq 0.05$ to $p \leq 0.01$).

Females

Body weight and body weight gain of females during the pre-mating dosing period (PND 21 to 91) were not affected by sodium bromide at 50 or 175 mg/kg/day. Body weights at the end of the premating period (PND 91) were 103 and 97.0% of the control group value for the 50 and 175 mg/kg/day dose groups, respectively.

A transient increase ($p \leq 0.05$) in body weight gain in the 50 mg/kg/day dose group in week 8 of dosing (DSs 49 to 55) was not attributed to sodium bromide.

Body weight and body weight gain of females during the gestation (DGs 0 to 20) or lactation periods (DLs 0 to 21) were not affected by doses of sodium bromide 50 or 175 mg/kg/day.

Body weights on DG 20 were 103.2% and 94.9% of the control group value for the 50 and 175 mg/kg/day dose groups, respectively. Reduced body weights ($p \leq 0.05$) on DG 7 and 10 and reduced ($p \leq 0.05$) body weight gain for DGs 0 to 7 in the 175 mg/kg/day dose group were not considered related to sodium bromide because they did not persist.

Body weights on DL 21 were 99.0% and 95.8 % of the control group value for the 50 and 175 mg/kg/day dose groups, respectively. Reduced body weights ($p \leq 0.01$) which occurred on DLs 0, 4, 7 and 14 in the 175 mg/kg/day dose group were not considered related to sodium bromide because they did not persist and weight gains were not affected.

Food Consumption

Male Rats

Mean food consumption values of males were not affected by the 50 mg/kg/day dose of sodium bromide.

Mean food consumption values for males in the 175 mg/kg/day sodium bromide dose group were significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) for each weekly interval from PNDs 49 to 147 resulting in a reduction ($p \leq 0.01$) in food consumption over the entire dosing period (PNDs 22 to 147).

Female Rats

Mean food consumption values during the premating period (PNDs 21 to 91) for females were not affected by sodium bromide at 50 or 175 mg/kg/day. No statistically significant differences occurred.

During the gestation and lactation periods, food consumption values were not affected by sodiumbromide at a dose of 50 mg/kg/day. In the 175 mg/kg/day dose group mean food consumption values were reduced in late gestation and early lactation with significant differences ($p \leq 0.05$ to $p \leq 0.01$) reported for DGs 14 to 20 and 0 to 20, and DLs 4 to 7, 7 to 9, and 0 to 14.

FERTILITY, PARTURITION AND SEXUAL FUNCTION (F1 GENERATION)

Male Rats

Mating and fertility was not affected by the 50 or 175 mg/kg/day dose of sodium bromide. All animals mated and the mean number of days to mating (days in cohabitation), and mating index were comparable among the groups. All males at 50 mg/kg/day sired a pregnancy and only one at 175 mg/kg/day did not (fertility index 93.3% compared to 95.6% in controls).

Female Rats

The average number of estrous stages in the 14 day assessment period was significantly reduced ($p \leq 0.05$) in the 175 mg/kg/day dose group (2.7) compared to the control group value (3.3) but there was no effect on mating (100%) or pregnancy rate (fertility index 93.3%).

There was no effect of treatment at 50 mg/kg/day as all animals mated and were pregnant.

ORGAN WEIGHTS (F1 GENERATION)

Organ Weights

Males

The terminal body weight and absolute weight of the brain, liver, kidneys, spleen, adrenals, thymus and thyroid/parathyroid were all unaffected by 50 mg/kg/day sodium bromide. A significant reduction in the ratio of the kidney weight to the terminal body weight in this group was not considered adverse as there was no effect on the absolute weight of the kidney, no histological change and no effect at the higher dose.

Absolute liver, kidney, spleen and thymus weights were significantly decreased ($p \leq 0.05$ to $p \leq 0.01$) in males treated at 175 mg/kg/day and the ratio of liver and kidney weights to the brain weight was significantly reduced ($p \leq 0.05$ to $p \leq 0.01$). These reductions reflected the lower terminal body weight (87.4% of the control group value, $p \leq 0.01$) and brain weight ($p \leq 0.01$) that occurred in this group, and were not considered adverse as there were no histological changes in these organs.

Females

The terminal body weight and absolute weight of the brain, liver, kidneys, spleen, adrenals, thymus and thyroid/parathyroid were all unaffected by the 175 mg/kg/day dose of sodium bromide. A significant reduction ($p \leq 0.01$) in the brain weight was not considered adverse as there was no effect on the histopathology of the brain. There was no adverse effect of treatment at 50 mg/kg/day.

Reproductive Organ Weights

Any statistically significant differences observed were attributed to changes in body weight or inherent variation (values within the range of historical control data). No clear effect of treatment with sodium bromide was concluded.

Males

Administration of 50 mg/kg/day of sodium bromide did not produce any effect on the absolute weight of the reproductive organs or weight relative to the terminal body/brain weight.

At 175 mg/kg/day, the absolute weight of the left cauda epididymis (88%), left testis (91%), left testis minus tunica albuginea, seminal vesicles with fluid (85%), and prostate (82%) were all significantly reduced ($p \leq 0.01$) compared to the control group and there was a slight increase (7%) in the weight of the left epididymis to body weight, but these changes reflected the reduced terminal body weight in the group and were therefore not considered adverse.

Females

There were no adverse effects on ovary, uterus or pituitary weights, absolute or relative (to brain or to body weight), in the 50 and 175 mg/kg/day dose groups.

GROSS PATHOLOGY (F1 GENERATION)

No gross lesions related to sodium bromide treatment occurred in males or females at 50 or 175 mg/kg/day.

HISTOPATHOLOGY (F1 GENERATION)

Spermatid head retention in the Sertoli cells and on the surface of the tubular lumen (minimal/mild),

was seen in 3 males of the F1 generation at 175 mg/kg/day but was considered a more marginal effect than that observed on the P generation, as one control male was also affected.

No adverse effect on corpora lutea was observed at histopathology.

Animals from the F1 generation also showed inflammatory cell infiltration in the lung at doses of 50 and 175 mg/kg/day, which was not dosage-dependent in females, although not present in control animals. Inflammatory cell infiltration in the lung was observed more frequently in males in the treated groups than controls (2, 6 and 7 in groups 1 to 3, respectively) but in females the incidence was higher in controls (14, 10 and 9 in groups 1 to 3, respectively).

Other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rat, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of sodium bromide

Sperm Evaluation

Administration of sodium bromide at a dose of 50 mg/kg/day did not affect any sperm parameter evaluated.

At 175 mg/kg/day, the number of motile sperm ($p \leq 0.01$) and the total count of sperm ($p \leq 0.05$) in the vas deferens were significantly reduced compared to the control value. However, as mean values for number motile and total count for all groups were within the historical control range for this Testing Facility, a relationship to treatment was considered questionable.

There was no significant effect at 175 mg/kg/day on percent motile or static sperm count from the vas deferens: cauda epididymal sperm count/density and testicular spermatid count were allcomparable to or higher than the concurrent control group and all values were within the historical control range.

Ovarian Follicle Evaluation

No significant differences were noted in the number of primordial follicles in either left, right or both ovaries for animals treated with 50 or 175 mg/kg/day sodium bromide compared to the Control group.

There appeared to be an overall increase of atretic follicles in the control and 50 mg/kg/day dose group animals and proportionally, all follicular types were better represented than was observed in the P-Generation animals in the same dose groups. This was most likely due to age differences (F1-generation animals were 131 days old at termination, compared to 183 days old for P-Generation animals) and not the test article. However, as the 175 mg/kg/day dose group females in the F1-Generation appeared to have fewer atretic follicles and follicular types were not as well represented, the possibility of an effect of sodium bromide treatment cannot be discounted.

NATURAL DELIVERY AND LITTER OBSERVATIONS, F2A LITTERS AND PUPS TO WEANING

A total of 22/23 (95.6%), 22/22 (100.0%) and 14/15 (93.3%) of the F1a females were pregnant and delivered a litter in the 0, 50 and 175 mg/kg/day dose groups, respectively.

No parameter evaluated at natural delivery or during the lactation period was affected by administration of sodium bromide at 50 or 175 mg/kg/day. These parameters included duration of gestation, number of dams with stillborn pups, mean number of implantation sites per dam, litter size, live litter size, mean number of stillborn pups, surviving pups/litter, percent male pups, number of pups alive and pup weights from PND 0 to 21. There was no total litter loss and no intergroup differences in viability or lactation index.

Although the number of pups dying between PND 8 and 14 in the 175 mg/kg/day dose group was statistically significant it was not considered biologically significant/adverse as only two pups died and this is within the historical control range of this Testing Facility.

There was no adverse effect of treatment on gestation, gestation index, litter size at birth of the F2 pups or pup viability, growth and physical development.

Clinical and Necropsy Observations, F2a litters and Pups to Weaning.

Sporadic in-life and necropsy observations occurred in all the groups. None of these observations were considered related to maternal administration of sodium bromide because most occurred in the control group, none occurred in more than two litters in any group and/or the number of litters affected was

not-dose dependent.

Clinical observations included cold to touch, purple colored limb, ungroomed coat, tip of tail or tail missing, pale body, scabbing, lacerations, abrasions, not nesting, not nursing, no milk band present, abdominal distension, mild dehydration, dry or necrotic eye and/or a black area on the abdomen. Necropsy observations occurred only in two 175 mg/kg/day dose group pups and included, slight dilation of the renal pelvis in one pup and an absent kidney in the other pup.

These findings occur spontaneously in this strain of rat and were considered unrelated to treatment at this dose level.

Anogenital Distance, Reflex and Physical Development F2a litters and Pups to Weaning.

Maternal administration of sodium bromide at 50 or 175 mg/kg/day did not affect the timing of pinna unfolding, hair growth, tooth eruption and eye opening in the male or female pups.

Anogenital distance measurements on PND 4 were comparable among the groups.

Conclusions **Summary**

350/500 mg/kg/day

At 350 mg/kg/day effects on sperm motility, morphology and sperm count were identified but there were no adverse effects on testicular spermatid counts or reproductive organ weights. No corpora lutea were found in the ovaries of 10 females in the 500 mg/kg/day dose group, but overall follicle counts were not affected and 6 of these females had at least one pregnancy.

There were no other treatment-related effects on female reproductive organ weights or histopathology. There were, however, slightly fewer estrous stages in the 14 day assessment period, owing to some females with extended periods of diestrus.

Only two males treated at 350 mg/kg/day did not mate with either a treated or untreated female (mating index 89.5%) but only 11/19 (64.7%) mated females became pregnant. There were no findings in either of the non-mated males which differed from mated males. All males in this group showed minimal/mild tubular spermatid retention and/or minimal- moderate Sertoli cell spermatid retention and 19/20 showed associated cellular debris in the epididymis. As these findings were also observed in males which died or were killed in week 12 they were likely present during the mating period. There was no apparent correlation, however, between the severity of the findings and pregnancy outcome of the pairings with treated or untreated females.

In females treated at 500 mg/kg/day, 20 of 22 females mated with treated or untreated males, and 15 were pregnant. Five females mated but were not pregnant, one of which was paired with an untreated male. Only 3 of these females had no corpora lutea present at follicle count. Two females did not mate or become pregnant with either treated or untreated males, one of which was also recorded as having no corpora lutea. The mating index was 45.5% for females mated with treated males and 90.9% (within the historical control range) including untreated males.

The overall female fertility index (with treated/untreated males) was 75% and with treated males was 60%, significantly lower than the control index ($p \leq 0.01$) for the treated/untreated males. Six pregnant females were recorded as having no corpora lutea, 4 of which mated only with an untreated male. There was also evidence of a delay in mating as only 6/20 matings occurred in the first 5 days of pairing, compared to 20/24 in control females.

There was no adverse effect on the duration of gestation or gestation index but no litters survived after day 5 post-partum. There were reductions in litter size at birth, the number of liveborn pups and pup survival. There was evidence of poor maternal care as pups were thin, cold to touch, not nursing, had no milk band present and had mild to moderate dehydration. Owing to reduced group size (due to unscheduled deaths/terminations), declining clinical condition, poor reproductive performance and a marked effect on pup viability, animals treated at 350/500 mg/kg/day were not re-paired for a second cohabitation and the high dose group was terminated at the end of the P generation.

175 mg/kg/day

Of 24 males paired with treated females in the first cohabitation period, 22 were confirmed mated and 16 of the mated females were pregnant. Of 2 males re-paired, one mated with the untreated females, so the mating index was 95.8% with all females and 91.7% with treated females, and the fertility index for treated and untreated females was 73.9%. In the second cohabitation period, 22 males were paired,

19 mated and 14 females were pregnant. The mating index was 86.4% and the fertility index was 73.7%, significantly lower than concurrent controls ($p \leq 0.01$) but only slightly lower than the historical control range.

In females, there was no adverse effect on estrous cycles. In the first cohabitation period, 22/24 females paired with treated males mated and 16 were pregnant. Two females did not mate and 6 females mated but did not deliver. In the second cohabitation period, 19/22 paired females mated and 14 were pregnant. Two of the 5 non-pregnant females had not been pregnant at the first cohabitation period, and one had not mated. Two of the females which did not mate had no corpora lutea at follicle count and histopathology, as did one female not pregnant at the second cohabitation.

At 175 mg/kg/day, other than the lower number of females which delivered, there were no adverse effects on duration of gestation, gestation index, number of pups born, pup viability, sex ratio, anogenital distance, growth or physical development (as assessed by pinna unfolding, hair growth, tooth eruption and eye opening) in either the F1a or F1b litters.

50 mg/kg/day

All males mated, but 2 did not impregnate a female at the first cohabitation. At the second cohabitation, 22 of 23 paired males mated and all mated males impregnated a female.

Of the 24 females paired with treated males at the first cohabitation period, all were confirmed mated and 22 were pregnant. In the second cohabitation period, only one female did not mate and all mated females were pregnant. There were no adverse effects on gestation or littering parameters or on pup survival, growth or physical development.

Control group

The mating index for males in the first cohabitation period was 95.8% and the fertility index was 100%. The mating and fertility indices for males in the second cohabitation period were 100%, and for females were 100% in both cohabitation periods. In comparison, values from historical control ranges for the species and strain at the laboratory were 75-100% for both mating and fertility for males, and 75-100% and 76%-100% for mating and fertility in females, respectively.

Conclusion

In conclusion, significant adverse effects were observed on clinical condition, body weight gain and food intake in the P generation, in males treated at 350 mg/kg/day and, to a lesser extent, in females treated at 500 mg/kg/day. Adverse effects on reproductive capacity were observed at these dose levels, with reduced male and female fertility, adverse effects on sperm count and morphology. All males also showed retained spermatid heads of minimal to moderate severity, and 10 females had depleted corpora lutea (although there was no effect on ovarian follicle counts, and no direct correlation with infertility). Litter size at birth was lower and pup viability, impaired by poor maternal care, was so poor as to preclude selection of a second generation. Similar but less marked effects on clinical condition, body weight and food intake were observed in males and females treated at 175 mg/kg/day. Mating performance was unaffected by treatment, and although fertility was reduced in both cohabitation periods, overall male fertility was within the historical control range, which may suggest transient and/or recoverable effects. Fewer males (11/23) showed retention of spermatids, the majority were described as minimal and there was no direct correlation with other effects. Five females were not pregnant at either pairing, but mating and fertility indices were within the historical control range and only 2 of these females had no corpora lutea. There was no adverse effect on gestation, littering, litter size or pup survival growth and development of the F1a or F1b litters. Effects in the F1 generation were limited to minimal/mild spermatid head retention in 3 males (compared to one control male) or irregularity of estrous cycle/differences in follicle counts, none of which adversely affected mating or fertility. There were no adverse effects on the F2 litters. There were no indicators of toxicity or adverse effects on reproductive parameters in either generation evaluated at 50 mg/kg/day of sodium bromide.

The NOAEL for parental toxicity, reproductive performance and pre- and postnatal development was therefore established by the study authors as 50 mg/kg/day.

3.7.1.2 [Study 2] Three-generation reproductive toxicity study, sodium bromide

Reference	A6.8.2/02, Doc. No. 592-002 Van Leeuwen, F. X. R. et al. (1983) Toxicity of Sodium Bromide in Rats – Effects on endocrine system and Reproduction. Fil. Che. Toxic; Vol 21, No. 4, 383-399
Guideline	Multi-generation reproductive toxicity, equivalent or similar to OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) according to Registrant(s). Deviations: No food consumption, pup body weights and litter size determination.
Reliability	Klimisch 2: reliable with restrictions. Not GLP-compliant. Remarks from Registrant(s): GLP was not obligatory at the time of study conduct and study was performed according to good experimental practice.
Species / strain	Rat, strain not specified
Test material	Sodium bromide Purity: not examined
Study design	A three-generation reproduction study in rats was performed using sodium bromide concentrations within a range of 75-19200 mg/kg diet, examining the effects on reproductive system. In three successive generations, at least two litters per female rat were raised. In the first generation, a third litter was raised for the investigation of transplacental transport of bromide. Furthermore, an additional litter was bred with parent animals of the highest dosage group which were changed to control diet in order to investigate reversibility of effects. **Doses / Concentrations:** 0, 75, 300, 1200, 4800 and 19200 mg NaBr/kg diet (nominal in diet, (corresponding to 0, 5.6, 22.5, 90, 360 and 1400 mg/kg bw/day with 1ppm = 0.075 mg/kg bw/day) No. of animals per sex per dose: 7-19 animals/sex/group **Parental animals:** Observations and examinations: CAGE SIDE OBSERVATIONS: No data DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: animals were observed for adverse clinical signs during the treatment period BODY WEIGHT: Yes - Time schedule for examinations: at start and termination of the study FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): not examined Estrous cyclicity (parental animals): No data Sperm parameters (parental animals): No data **Litter observations:** PARAMETERS EXAMINED: fertility, viability, body weight GROSS EXAMINATION OF DEAD PUPS: organ weights were determined for adrenals, thyroid, pituitary, testes, prostate, ovaries and uterus PARAMETERS EXAMINED: fertility, viability, body weight Postmortem examinations (parental animals): GROSS NECROPSY no data Postmortem examinations (offspring): HISTOPATHOLOGY / ORGAN WEIGTHS organ weights were determined for adrenals, thyroid, pituitary, testes, prostate, ovaries and uterus.

Statistics: No data

Reproductive indices: Fertility Index = no. of pregnancies x 100/no. of matings
Offspring viability indices: Viability Index = no. of pups alive at Day 5 x 100/no. of pups born alive.
Lactation Index = no. of pups alive at Day 21 x 100/no. of pups alive at Day 5

Findings

Results: P0 (first parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)

F0 animals: A dose-dependent decrease in thyroxine (T4) concentration was observed in the serum. Sodium bromide concentrations in the range of 125-2000 mg/kg diet in combination with ´chloride-free` diet in addition revealed decreased thyroxine concentrations in serum in animals treated at 500 and 2000 mg/kg. Uptake of radiolabelled iodide was measured within this experiment and showed significatly increased uptake after 500, but only slight increase after 125 and 2000 mg NaBr/kg diet.

F1 parents: No effects were described in the investigation.

REPRODUCTIVE FUNCTION: (PARENTAL ANIMALS)

Animals treated at 19200 mg/kg diet were not fertile, and fertility of the next lower dose level (4800 mg/kg diet) was reduced. Because of the diminished fertility in these dosage groups, second and third generations were bred only from the groups dosed with sodium bromide concentrations up to 1200 mg/kg diet. In these groups no effects related to treatment were found in the breeding results.

To investigate whether infertility occurred in males or in females, untreated males and females were mated with females and males of the highest dosage group. Of the treated females with untreated males only 20% became pregnant, and none of the untreated females mated with treated males became pregnant.

Reversibility of the observed effects were studied in animals fed a diet containing 19200 mg NaBr/kg for 7 month followed by a control diet for 3 months before mating. In contrast to the infertility of these animals observed before, fertility index was 62%, viability index 61% and lactation index 90%.

OTHER FINDINGS (PARENTAL ANIMALS)

The lactation index was comparable among all groups investigated.

Results: F1 generation

VIABILITY (OFFSPRING)

Pup viability at the 4800 ppm dose levels was significantly reduced but survival was shown to be greater in the second when compared to the first litter. All the young of the first litter alive on post-natal day 5 died before day 21 while all young alive on post-natal day 5 were still alive on day 21 in the second litter. No effects on breeding were observed at dose levels of 1200 ppm and below

BODY/ORGAN WEIGHTS (OFFSPRING)

Body- and organ-weight determination did not reveal a clear pattern of dose-related effects in neither of the three generations. Only the adrenals of females of the F0 generation showed a dose-dependent decrease in relative weight which could not be observed in later generations.

Litter observations:

Breeding results with fertility index, viability index and lactation index are given in table 6.8.2/02-1. No data for the dose groups on litter size and sex ratio are given.

Pup mean bodyweights on Day 21 did not differ between control and treatment groups (see table 6.8.1/02-1).

Viability of the young was greater in the second litter than in the first. Furthermore, during the lactation of the first litter all of the young alive at Day 5 died before Day 21. In the second litter, all animals alive at Day 5 were still alive at Day 21.

Macroscopic examination of all pups born during the entire experimental period provided no evidence

of anomalies.

Table 6.8.2/02-1:Breeding results in reproduction study on sodium bromide - fertility index, vability index, lactation index and mean bodyweight

	0 ppm	75 ppm	300 ppm	1200 ppm	4800 ppm	19200 ppm
Fertility index*						
F0	70	70	72	65	25	0
F1	62	54	44	53	-	-
F2	52	67	30	45	-	-
Viability index**						
F0	90	98	96	92	32/61¥	-
F1	92	88	80	97	-	-
F2	96	98	93	98	-	-
Lactation index***						
F0	95	96	95	94	0/100¥ -	-
F1	93	85	72	80	-	-
F2	99	99	99	99	-	-
Mean bodyweight at Day 21						
F0	40	45	43	43	-/38¥	-
F1	41	43	40	38	-	-
F2	36	38	38	36	-	-

* Fertility index: No. of pregnancies x 100/No. of matings

** Viability index: No. of pups alive at Day 5 x 100/No. of pups born alive

*** Lactation index: No. of pups alive at Day 21 x 100/No. of pups alive at Day 5

¥ data are given separately for first and second litter

Executive summary

Materials and Methods

The investigations addressed results from bromide studies present in the literature and provided additional data about the effects of bromide in the endocrine and reproductive systems. Estimation of an ADI is discussed in relation to the present residue situation. A three-generation reproduction study in rats was performed using sodium bromide concentrations within a range of 75-19200 mg/kg diet, examining the effects on reproductive system. In three successive generations, at least two litters per female rat were raised. In the first generation, a third litter was raised for the investigation of transplacental transport of bromide. Furthermore, an additional litter was bred with parent animals of the highest dosage group which were changed to control diet in order to investigate reversibility of effects.

Results and Discussion

In the present three-generation reproductive toxicity study it was shown that of the high dosed (19200

mg NaBr/kg diet) females mated with untreated males only 20% became pregnant, and none of the untreated females mated with high dosed males became pregnant. Therefore, the observed effect of reduced and absent fertility in the 4800 and 19200 mg/kg diet groups, respectively, were due to infertility of male as well as female rats. This conclusion is in accordance with the histopathological lesions found in the testes as well as in the ovaries in the 90-day studies. In addition, pup viability at the 4800 ppm dose levels was significantly reduced but survival was shown to be greater in the second when compared to the first litter.

All the young of the first litter alive on post-natal day 5 died before day 21 while all young alive on post-natal day 5 were still alive on day 21 in the second litter. No effects on breeding were observed at dose levels of 1200 ppm and below. For the group of animals treated at 19200 mg/kg diet, infertility was observed. After 7 month on the high dose level, the diet was changed to control diet for 3 month and rats were mated again. In contrast to the infertility observed before, animals showed fertility index of 62%, viability index of 61% and lactation index of 90%. From these results it is clear that the effects of bromide on reproduction system are reversible but could not be entirely compensated. No macroscopic anomaly in neither pup was observed throughout the investigation, although it is known that bromide easily crosses the placenta. Dams and F1 rats examined for bromide concentrations in internal organs showed equal amounts of bromide in kidney which provides that rats had been exposed to bromide in utero. Body and organ weight determinations did not reveal a clear dose-related pattern. A dose-dependent decrease in T4 levels in serum of parent animals of the F0 generation was observed. This finding is indicative of an inhibitory action of bromide on the synthesis of thyroid hormones, resulting in a physiological feedback mechanism of increased thyrotropic hormone (TSH) secretion by the pituitary gland causing an increased stimulation of the thyroid. This is in good agreement with the activation of the thyroid found by histological examination in the previous 90-day studies mentioned above. The decrease in thyroid hormones in animals treated at high dose levels was confirmed in an experiment on the time dependency of the effect of bromide on the thyroid. Using standard diets containing 4800 and 19200 mg NaBr/kg, significantly decreased thyroxine concentrations were found in both groups. From this experiment, it appeared that after only three days the thyroxine concentration in serum was significantly decreased and that it remained constant during an experimental period of 12 weeks.

In the present three-generation toxicity study, the decrease in thyroid hormones was the most sensitive criterion

Conclusion In this three-generation reproductive toxicity study it was demonstrated that administration of 4800 and 19200 ppm of NaBr caused a reduction in the fertility of both sexes of rats, an increased litter loss and pup mortality. A cross-mating experiment revealed that the effects of bromide on reproduction system are reversible as could be shown by one group of animals of the highest dosage level which were changed to control diet and mated again.

3.7.1.3 [Study 3] Repeat Dose (gavage) 90-Day Toxicity Study of sodium bromide in Rats, Including Recovery Assessments

Reference Study report, 2016b

See study details under 3.9.1. specific target organ toxicity – repeated exposure

3.7.1.4 [Study 4] No guideline study: 90-day oral repeated dose toxicity study of sodium bromide study in rat

Reference A6.4.1/04, Doc. No. 592-005

van Logten, M. J., et al., 1974. Semichronic Toxicity Study of Sodium Bromide in Rats. Toxicology 2, 257-267

See study details under 3.9.1. specific target organ toxicity – repeated exposure

3.7.1.5 [Study 5] No guideline study: 90-day oral repeated dose toxicity study of sodium bromide study in rat on a low chloride diet

Reference A6.4.1/05, Doc. No. 592-006

Van Logten M.J., Rauws A.G., Kroes R., Den Tonkelaar E.M. and van Esch G.J. 1976. Semichronic Toxicity Studies of Sodium Bromide in Rats on a Normal Diet and a Low Chloride Diet Author. Med. Fac. Landbouww Rijksuniv. Gent 41 (2), 1499-1507

See study details under 3.9.1. specific target organ toxicity – repeated exposure

3.7.1.6 [Study 6] Dose range-finder for OECD 416; 2-generation reproduction study

Reference A6.8.2/01, Doc. No.553-001

Study report, 2001.

Guideline Dose range-finder for OECD 416; 2-generation reproduction study. Performed to similar standard but with limited animal numbers and observations. Parental animals only dosed.

Reliability Klimisch 1: (reliable without restriction). GLP dose range-finding study conducted under conditions similar to OECD Guideline.

Species / strain Rat, Sprague-Dawley

Test material Ammonium bromide

CAS 12124-97-9

EC 235-183-8

Purity: 99.94%

Study design

Materials and method

The study was performed in rats to determine a maximum tolerated dose of ammonium bromide which can be used as the highest dose in subsequent reproduction studies, and to provide guidance in the selection of the lower dose levels.

Rats were treated via the diet with 0, 1600, 3200 or 6400 ppm of ammonium bromide (corresponding to 0, 120, 240, and 480 mg/kg bw/day with 1 ppm=0 0.075 mg/kg bw/day) for two weeks prior to mating, throughout the mating, gestation and lactation periods until termination after the first generation had been

weaned.

Route of administration: oral, feed

Details on mating procedure: Animals were paired on a one male to one female basis, with both animals being in the same treatment group. Mating was judged to have occurred if sperm was present in a vaginal lavage or if a copulatory plug was in situ. Vaginal lavage was examined early each morning. The Day of mating was designated Day 0 of gestation.

Seven days were allowed for mating. If no mating sign was observed during that time, the female was allowed a ´rest` period of two days before placed with a second male for additional seven days. If no mating considered to have occurred at the end of the seconnd mating period the female was transfer to an individual solid bottomed cage.

F0 animals were treated for 2 weeks prior to mating, throughout the mating, gestation and lactation periods until the first generation had been weaned.

Doses / Concentrations:

0, 1600, 3200, 6400 ppm (nominal in diet, corresponds to 0, 127/228, 242/454, 503/651 mg/kg bw/day for males/females)

10 animals/sex/dosage group

Estrous cyclicity (parental animals): Not examined; study is a dose range finder

Sperm parameters (parental animals): Not examined; study is a dose range finder

Litter observations: Number and sex of pups, stillbirths, live births, presence of gross anomalies, examination for presence of milk in stomach

Bodyweights: Pre-weaning F1 pups: by litter, en masse (sexes separate), on Days 1, 4, 7 and 14 of lactation, and individually by sex on Day 21 of lactation.

Postmortem examinations (parental animals): Parental animals were sacrificed after their litter had reached Day 21 of lacatation An external examination, macroscopic examination of tissues and organs of thoracic and abdominal cavities in situ, reproductive tract examined for signs of pregnancy, number of visible implantation sites

Postmortem examinations (offspring): Examinations at pre-weaning comprised: external examination of anomalies; presence of milk in stomach; gross necropsy of cranial, thoracic and abdominal cavities in situ (for pups found dead or killed on or after Day 14 of lactation)

Examinations at weaning comprised: external examination of anomalies, macroscopic examination of tissues and organs of cranial, thoracic and abdominal cavities in situ (animals killed on Day 21 of lactation)

Statistics: Not performed

Offspring viability indices:

Birth Index: Total number of pups born (live and dead)/Number of implantation scars

Live Birth Index: Number of pups live on Day 0 of lactation/ Total number born (live and dead)

Viability Index: Number of pups live on Day 4 of lactation/Number live on Day 0

Lactation Index: Number of pups live on Day 21 of lactation/ Number Live on Day 4

Overall Survival Index: Number of pups live on Day 21 of lactation/Total number of pups born (live and dead)

Findings Details on results (P0)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)

Mortalities:

There were no premature deaths during the study.

Clinical signs:

At 6400 ppm, rolling gait was noted in all animals and was generally noted following the first few days of treatment and persisted throughout the treatment period. In addition, piloerection and hunched posture accompanied this finding. In females, approximately half of the animals also showed hyperactivity. As a result of the generally ill condition of these animals, most developed staining on their body and an unkempt appearance to their coat.

At 3200 ppm clinical effects of treatment were similar to those noted for animals at 6400 ppm, but were of a lesser severity. Nine males and six females showed rolling gait, but the onset of this was around the fifth week of treatment and was generally evident throughout the treatment period. None of the animals at this level showed an unkempt coat.

At 1600 ppm, three females showed transient piloerection but it was uncertain if this finding was related to treatment due to the lack of rolling gait at this dose level.

Table: Group incidences of clinical signs in F0 males

Observation	Males			
	Group/Dose Level (p.p.m. Ammonium Bromide.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
Rolling gait	0	0	9	10
Piloerection	1	0	2	2
Hunched posture	0	0	0	1
Subdued behaviour	0	0	2	0
Unkempt coat	0	0	0	6
Agitated behaviour	2	1	0	0
Hair loss on sacral/neck region	1	2	0	2
Staining of coat on head/nasal/dorsal/forelegs/tail/ perigenital/abdominal region	1	2	5	8
Encrustation on toe	2	1	1	2
Scabbing on hindfoot/tail/abdominal/sacral/ thoracic region	2	0	3	1
Bleeding/swelling on forefoot	0	1	0	1
Lump on tail	0	2	0	0
Staining around eye(s)	0	0	3	9
Swelling in perigenital region	0	0	1	0
Swelling on hindfoot/toe	0	0	0	2
Abnormal coloured tail	0	0	0	2
Lesion on hindfoot	0	0	1	0

Table: Group incidences of clinical signs in F0 females

Observation	Females			
	Group/Dose Level (p.p.m. Ammonium Bromide.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
Rolling gait	0	0	6	10
Piloerection	2	3	2	5
Hunched posture	0	0	3	8
Subdued behaviour	0	0	1	0
Unkempt coat	0	0	0	8
Agitated behaviour	0	0	0	0
Hyperactive behaviour	0	0	0	4
Hair loss on forelegs/hindlegs/head/dorsal/ ventral region	6	0	4	2
Scabbing around mouth	1	2	0	0
Staining of coat on head/neck/nasal/forefeet/forelegs/ mouth/perigenital/ears/hindfeet/ ventral abdomen/wholebody	4	5	6	8
Encrustation on hindfoot	1	0	0	0
Abnormal coloured tail	0	0	1	0
Discharge from eye	0	0	1	1
Staining around eye(s)	0	0	0	4
Thin appearance	0	0	0	1
Swelling on hindfoot	0	0	0	1

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)

Body weights/body weight gain:

In males, a reduction in bodyweight gain throughout the first week of treatment was noted at 6400 ppm when compared to control. Thereafter, the bodyweight gain for these animals was essentially similar to controls; consequently there was a reduction in the overall bodyweight gain throughout the treatment period (-7.5% at week 8). At 3200 ppm, the mean bodyweight gain in males was slightly reduced (-6.7% at week 8) and at 1600 ppm there was an increase in bodyweight gain throughout the first weeks of treatment, which could not positively be attributed to treatment.

For females there was no obvious treatment related effect on bodyweight performance prior to mating at any dose level, and during gestation for animals treated at 1600 and 3200 ppm when compared with control. At 6400 ppm, the calculations during gestation for females were based on one animal as a result of poor pregnancy rate. For this animal, the bodyweight gain throughout the gestation period was less than any individual animal in the control group. The group mean bodyweights at the start of lactation were greater than control at 3200 and 1600 ppm. However, by Day 14 of lactation, the absolute weights were essentially similar in all dose groups.

Table: Body weight F0 males

Week of Treatment	Group/Dose Level (p.p.m.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
Pretrial	250 ± 9	248 ± 7	247 ± 7	244 ± 5
0	316 ± 17	303 ± 27	308 ± 13	310 ± 12
1	360 ± 21	360 ± 20	348 ± 24	339 ± 17
2	392 ± 27	398 ± 22	372 ± 24	368 ± 22
3	424 ± 28	434 ± 27	399 ± 24	396 ± 30
4	443 ± 30	451 ± 30	416 ± 29	409 ± 30
5	460 ± 32	477 ± 31	430 ± 29	425 ± 33
6	472 ± 31	495 ± 30	444 ± 38	439 ± 41
7	493 ± 33	513 ± 34	464 ± 45	456 ± 45
8	510 ± 33	532 ± 37	476 ± 44	472 ± 47
Weight Gain Weeks 0-8	194	229	168	162
% of Control	-	118	87	84

Table: Body weight F0 females

Week of Treatment	Group/Dose Level (p.p.m.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
Pretrial	170 ± 4	175 ± 5	174 ± 6	173 ± 6
0	194 ± 6	206 ± 8	204 ± 8	204 ± 10
1	206 ± 8	222 ± 9	221 ± 13	220 ± 14
2	221 ± 8	238 ± 10	234 ± 16	230 ± 19
Weight Gain Weeks 0-2	27	32	30	26
% of Control	-	119	111	96

	Group/Dose Level (p.p.m.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
Day of Gestation[a]				
0	224 ± 9	247 ± 16	236 ± 15	281
7	263 ± 7	285 ± 16	276 ± 20	302
14	303 ± 7	322 ± 19	314 ± 18	329
20	374 ± 12	396 ± 33	389 ± 19	381
Weight Gain Days 0-20	150	149	153	100
% of Control	-	99	102	67
Day of Lactation [b]				
1	258 ± 13	284 ± 19	285 ± 29	-
7	310 ± 19	325 ± 21	322 ± 16	-
14	338 ± 20	349 ± 15	340 ± 6	-
21	331 ± 15	340 ± 12	333 ± 11	-

Note: gestation value of group number 4 is based on only one animal

a. pregnant animals only
b. animals rearing young to day 21 only

Food consumption:

A reduction in group mean food consumption, compared with control, was noted for males towards the latter part of the treatment period at 6400 and 3200 ppm. Food consumption in males treated at 1600 ppm was similar to controls.

In females, food consumption during the pre-mating and gestation periods was similar in all dose groups. Slight differences in the food consumption during lactation were not obviously a result of treatment.

REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS)

At 6400 ppm, although seven females showed a positive mating sign, only one female became pregnant. Fertility indices in males and females were 10%.

At 3200 ppm, small differences in the mating performance, as assessed by the number of nights to positive mating sign and by the number of animals passing oestrus, and small differences in the fertility indices were considered too small to be positively attributable to treatment.

Mating performance and fertility indices at 1600 ppm were similar to control

Table: Mating performance and fertility indices

Number of Nights to Positive Mating Sign	Group/Dose Level (p.p.m.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
	Number of Animals (Number of these not becoming pregnant)			
1	6	4	0	0
2	1	1	3	1 (1)
3	1	1	1	3 (3)
4	2	3	3	1 (1)
5	0	0	0	0
6	0	0	1	0
7	0	0	0	0
8-14	0	0	1	0
Over 14	0	1	0	2 (1)
No clear indication of mating	0	0	1 (1)	3 (3)
Median number of nights to positive mating sign	1	2.5	4	>4
Number passing one oestrus	0	0	0	4
Number passing 2 or more oestruses	0	0	0	1
Number of males paired	10	10	10	10
Number of siring males	10	9	8	1
Male Fertility Index (%)	100	90	80	10
Number of females paired	10	10	10	10
Number pregnant	10	10	9	1
Female Fertility Index (%)	100	100	90	10

At 6400 ppm, the only litter produced did not survive to Day 4 of lactation.

At 3200 ppm, there was a reduced lactation index noted, but largely reflects the litter where all pups died and events leading to pup mortality had probably been established by Day 4 of lactation.

At 1600 ppm, no treatment-related effects on pup survival and lactation index was noted.

Mean duration of gestation was 21.6, 21.4, 22.1 and 22 days for animals treated with 0, 1600, 3200 and 6400 ppm ammonium bromide respectively, showing slight increase of duration of gestation with increasing amounts of test substance. The mean number of implant sites per pregnancy did not differ between the groups.

Table: Duration of gestation and overall litter performance

	Group/Dose Level (p.p.m.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
Number Pregnant	10	10	9	1
Duration of Gestation (Days)				
21	4	6	2	-
22	6	4	4	1
23	-	-	3	-
24	-	-	-	-
Mean Duration	21.6	21.4	22.1	22.0
Number of females producing a live litter	10	10	8	1
Gestation index as %	100	100	89	100
Mean number of implant sites [a] per pregnancy ± standard deviation	15.3 ± 1.2	15.4 ± 4.1	15.6 ± 4.3	-
Mean total number of pups [a] born	14.1 ± 1.9	13.3 ± 4.3	14.4 ± 4.3	-
Mean number of live pups [a] per litter ± standard deviation:				
Day 0 of lactation	14.0 ± 1.8	13.3 ± 4.3	13.6 ± 3.8	-
Day 1 of lactation	13.8 ± 1.6	13.2 ± 4.2	12.2 ± 4.2	-
Day 4 of lactation	11.6 ± 3.9	12.2 ± 4.4	12.0 ± 4.1	-
Day 7 of lactation	11.5 ± 3.8	12.0 ± 4.2	11.8 ± 4.5	-
Day 14 of lactation	11.5 ± 3.8	11.7 ± 4.1	11.0 ± 4.0	-
Day 21 of lactation	11.5 ± 3.8	11.7 ± 4.1	10.8 ± 4.4	-

GROSS PATHOLOGY (PARENTAL ANIMALS)

Necropsy findings of F0 females were limited to four animals, all treated at 6400 ppm, which showed a dilated uterus at necropsy; none of these animals were pregnant. In addition, one of these animals had dark foci on all lung lobes. These findings were not considered to be related to treatment. No adverse findings during gross necropsy were seen in F0 females dosed with 1600 and 3200 ppm.

DETAILS ON RESULTS (F1)

VIABILITY (OFFSPRING)

At 6400 ppm, the only litter produced did not survive to Day 4 of lactation.

At 3200 ppm, all pups in 4 out of 9 litters died before Day 21 of lactation and included one litter where all pups were born dead, resulting in a reduced gestation index at this level. Considering the litters where some pups survived, there was a slight increase in pup mortality compared to the other dose groups.

At 1600 ppm, there were no obvious effects on litter size or survival.

The viability index (number of pups live on Day 4 of lactation/number live on Day 0) and overall survival index (number of pups live on Day 21 of lactation/total number of pups born) for the control group was lower than might have been expected.

Three pups from two litters treated at 3200 ppm and one pup from one litter treated at 1600 ppm were killed due to their condition (cold, subdued behaviour, abnormal breathing) on or before Day 12 of lactation. It is not possible to indicate positively if these findings were attributable to treatment.

Table: F1 generation survival indices

		Group/Dose Level (p.p.m.)			
		1 (0)	2 (1600)	3 (3200)	4 (6400)
Birth Index	Mean Litter Index (%)	94	88	91	90
	Number Losing >2 pups	0	2	3	0
	Number of Litters	10	9	9	1
Live Birth Index	Mean Litter Index (%)	98	100	83	89
	Number Losing >1 pup	1	0	4	0
	Number of Litters	10	10	9	1
Viability Index Days 0-4	Mean Litter Index (%)	67	92	57	0
	Number Losing >3 pups	3	2	4	1
	Number of Litters	10	10	8	1
Lactation Index Days 4-21	Mean Litter Index (%)	99	97	72	-
	Number Losing >1 pup	0	2	4	-
	Number of Litters	8	10	6	-
Overall Survival Index Birth-21	Mean Litter Index (%)	66	89	40	0
	Number Losing >4 pups	3	1	6	1
	Number of Litters	10	10	9	1

CLINICAL SIGNS (OFFSPRING)

For three pups of the 3200 ppm group and one pup from the 1600 ppm group, cold, subdued behaviour and abnormal breathing were recorded but these findings could not be positively related to treatment.

Other occasional observations on the pups and litters were considered to be incidental and consistent with those usually seen in this type of study.

BODY WEIGHT (OFFSPRING)

Slight differences in pup weights at birth did not indicate any obvious adverse effect of treatment.

At 3200 ppm, mean weights of litter and pups were lower than control by Day 21 of lactation.

Slight differences in litter and pup weights at 1600 ppm compared to control were not positively related to treatment.

Since none of the pups of the only litter produced at 6400 ppm survived until Day 21 of lactation, pup body weights could not be determined in this dose group.

Table: Group mean litter and pupweight

Day of Lactation	Group/Dose Level (p.p.m.)			
	1 (0)	2 (1600)	3 (3200)	4 (6400)
LITTER				
Day 1	80 ± 8	79 ± 22	70 ± 22	-
Day 4	103 ± 36	103 ± 34	99 ± 36	-
Day 7	156 ± 54	151 ± 46	137 ± 54	-
Day 14	318 ± 109	294 ± 82	265 ± 110	-
Day 21	496 ± 157	481 ± 128	431 ± 181	-
Mean of Litter Mean Pup Weight				
MALES				
Day 1	6.1 ± 0.3	6.3 ± 0.7	6.0 ± 0.8	-
Day 4	8.9 ± 1.1	9.1 ± 1.5	8.4 ± 1.1	-
Day 7	13.6 ± 1.5	13.6 ± 2.0	11.9 ± 1.7	-
Day 14	29.0 ± 4.8	27.3 ± 3.9	25.0 ± 2.0	-
Day 21	44.5 ± 4.8	45.0 ± 7.1	42.1 ± 3.9	-
FEMALES				
Day 1	5.4 ± 0.6	5.9 ± 0.8	5.8 ± 0.8	-
Day 4	8.5 ± 1.2	8.1 ± 1.4	8.0 ± 1.3	-
Day 7	13.1 ± 1.8	12.1 ± 2.3	11.3 ± 1.9	-
Day 14	26.0 ± 2.0	24.4 ± 4.3	21.0 ± 6.4	-
Day 21	43.1 ± 4.9	39.4 ± 7.1	38.2 ± 3.2	-

RESULTS AND DISCUSSION

There were no premature parental deaths during the study.

Parental toxicity at 6400 ppm was demonstrated in males by reduced bodyweight gain over the treatment period and reduced food consumption after cohabitation in males. Clinical signs of reaction to treatment of both sexes at this dose level included rolling gait, piloerection, hunched posture and unkempt coat. In addition, females showed hyperactive behaviour. These clinical effects were consistent with those previously seen with this test material.

At 3200 ppm, slightly reduced bodyweight gain in males over the treatment period was noted. Clinical signs of reaction to treatment in both sexes were similar to those described for animals at the highest treatment group (6400 ppm), however no unkempt coat was noted and the effects were generally less severe.

Mating performance and fertility were obviously affected by treatment at 6400 ppm. For the one female which produced a litter, the litter was dead before Day 4 of lactation.

At 3200 ppm, there was an increased incidence of total litter loss in 4 out of 9 litter and a slight increase in pup mortality for surviving litters. Mean litter and pup weights were reduced by Day 21 of lactation when compared to control.

At the low dose level of 1600 ppm, no effects or findings which could be directly related to treatment with ammonium bromide were observed

Rats of the highest dosage group (6400 ppm) showed rolling gait, piloerection, hunched posture and unkempt coat in both sexes and hyperactive activity in females only. The clinical effects observed for the group treated at 3200 ppm were the same, however no unkempt coat was noted and the effects were less severe. At 1600 ppm, animals showed transient piloerection only. Reduced bodyweight gain was noted in males at 3200 (13%) and 6400 (16%) ppm. Reduced food consumption was also noted in males at ≥3200 ppm. A slight increase of duration of gestation was noted at 3200 and 6400 ppm (mean duration: 22.1 and 22 days, respectively, compared to 21.6 days in controls). The mean number of implant sites per pregnancy did not differ between the groups. Mating performance and female fertility index was reduced at 3200 ppm (female fertility index: 90%) and 6400 ppm (female fertility index: 10%). From the rats treated at 6400 ppm, only one became pregnant and the litter produced was dead before Day 4 of lactation. At 3200 ppm, there was an increased incidence of total litter loss and a slight increase in pup mortality for surviving litters. There were no obvious effects on litter size or survival in the lowest dosage group (1600 ppm).

Conclusion No NOEL parental was determined. The NOAEL parental was 1600 ppm based on clinical signs of neurotoxicity noted in both sexes at ≥3200 ppm, reduced bodyweight gain noted in males at ≥3200 ppm and reduced mating performance and female fertility index noted at ≥3200 ppm. Clinical signs of piloerection noted at the dosage level of 1600 ppm was in the absence of other clinical signs not considered as an adverse effect.

The NOEL offspring was 1600 ppm. The NOAEL offspring was 1600 ppm based on increased mortality noted in pups at ≥3200 ppm and reduced mean of litter mean pup weight noted at 3200 ppm (M: 5%; F: 11%).

3.7.1.7 [Study 7] Repeated Dose 90-Day Oral Toxicity of ammonium bromide in Rats (OECD TG 408)

Reference A6.4.1/01, Doc. No. 533-001

Study report, 2000a

See study details under 3.9.1. specific target organ toxicity – repeated exposure

3.7.1.8 [Study 8] No guideline study: 4 week dose range-finding study of ammonium bromide in rat

Reference A6.3.1/01, Doc. No. 532-001

Study report, 1999

See study details under 3.9.1. specific target organ toxicity – repeated exposure

ADVERSE EFFECTS ON DEVELOPMENT

3.7.1.9 [Study 9] Pre-natal developmental toxicity study (OECD TG 414) of sodium bromide in rat

Reference A6.8.1/05, Doc. No. 551-003

Study report, 1995

Guideline	EPA OPP 83-3 (Prenatal Developmental Toxicity Study) OECD Guideline 414 (Prenatal Developmental Toxicity Study) GLP compliance: yes
Reliability	1 (reliable without restriction)
Species / strain	Species: rat Strain: other: Crl: CD BR VAF/Plus
Test material	Details on test material: - Name of test material (as cited in study report): Sodium bromide ´technical grade` - Description: White crystalline solid - Analytical purity: 99.84% - Lot/batch No.: 940045 - Expiration date of the lot/batch: February 1996 - Storage condition of test material: at room temperature in the light Ammonium bromide is an inorganic salt that dissociates to its composite ions in aqueous solutions at environmental pH and temperature. Comparison of the available data on the various bromide salts have shown that the bromide ion is the relevant ion for determination of the toxicological profile with simple cations such as potassium, sodium or ammonium, that are ubiquitous in nature, having little or no influence on the bromide ion properties. It is therefore justified to read-across data from other inorganic bromide salts to ammonium bromide.

Route of administration: oral: gavage

Vehicle: water

Details on exposure:

PREPARATION OF DOSING SOLUTIONS:
Dosage volumes were calculated for individual animals on Day 6 of pregnancy and adjusted according to bodyweight on Day 8, 10, 12 and 14.

VEHICLE
- Concentration in vehicle: 0, 10, 30 and 100 mg/mL
- Amount of vehicle (if gavage): 10 mL/kg bw

Details on analytical verification of doses or concentrations:

The mean achieved concentrations of sodium bromide in formulations prepared on the first and last days of treatment were within 8% of nominal concentrations.

Duration of treatment / exposure: Days 6-15 post coitum

Frequency of treatment: daily

Duration of test: until Day 20 post coitum

Doses / concentrations

Doses / Concentrations:

0, 100, 300 and 1000 mg/kg bw/day

Basis: actual ingested

No. of animals per sex per dose: 25 females/group

Control animals: yes, concurrent vehicle

Examinations

Maternal examinations:

CAGE SIDE OBSERVATIONS: No data

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: daily

BODY WEIGHT: Yes
- Time schedule for examinations: on Days 0, 3, 6, 8, 10, 12, 14, 16, 18 and 20 of pregnancy

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Yes
- on Days 3, 6, 8, 10, 12, 14, 16, 18 and 20 of pregnancy

POST-MORTEM EXAMINATIONS: Yes
- only two animals were examined; one which died uring the study period and another one which had to be killed for humane reasons.

Ovaries and uterine content:

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes

Fetal examinations:

- External examinations: Yes: [all per litter]
- Soft tissue examinations: Yes: [half per litter]
- Skeletal examinations: Yes: [half per litter]

Statistics:

Significance tests, employing analysis of variance followed by an intergroup comparison with the control, were performed on the following parameters and results are presented in relevant table of this report:
bodyweight change, mean food consumption, litter data, sex ratio and foetal abnormalities and variants.
Depending on the heterogeneity of variance between treatment groups, parametric tests, analysis of variance followed by Williams`test or non-parametric tests, Kruskal-Wallis followed by Shirley`s test were used to analyse these data, as appropriate.
For litter data and foetal changes the basic sample unit was the litter, and, due to the preponderance of non-normal distributions, non-parametric analyses were routinely used. Analysis of foetal abnormalities was performed using a trend test on the number of litters affected, followed by a one-tail 2 sample permutation test. All significant (ie $p \leq 0.05$) intergroup differences from the control are reported only when supported by a significant analysis of variance ($p \leq 0.05$).
Where 75% or more of the values for a given variable were the same, a Fisher`s exact test was used.

Results and discussion

Results: maternal animals

Maternal developmental toxicity

Details on maternal toxic effects:

Treatment at 1000 mg/kg bw/day was associated with unsteady gait in all animals. This sign was first apparent following administration of the second dose on Day 7 post coitum. Thereafter, all animals showed this sign at daily examination prior to dosing on Day 8-11 inclusive and, after dosing on Days 8-15. From Day 11, although most animals showed unsteady gait prior to dosing on Days 12 and 13, the incidence was lower on Days 14 and 15 with only 12/24 animals showing this signs prior to dosing on Day 15. Although the final dose was administered on Day 15 post coitum, unsteady gait was apparent for 8/24 animals on Day 17.

As the treatment period progressed, additional abnormalities of movement became apparent: all animals showed feet falling through the cage grid floor during ambulation on at least one occasion, and 23 animals showed poorly coordinated movements on at least one occasion. Both of these signs were first apparent after dosing on Day 9 post coitum. In both cases, there was striking difference in the incidence of affected animals prior to dosing as opposed to after dosing: although only 1 or 2 animals showed these signs prior to dosing up to 24 animals showed feet falling through the cage grid floor during ambulation after dosing, and up to 16 animals showed poorly coordinated movements. It was noted that the incidence of poorly coordinated movements was highest towards the end of the

treatment period (days 13-15). Following administration of the final dose on Day 15, these signs were not apparent on Days 16-20.
Treatment at 1000 mg/kg bw/day was also associated with reduced bodytone in all animals. This sign was first apparent following administration of the second dose on Day 7 post coitum. During Days 8-15, there was a clear difference in the incidence of affected animals prior to dosing as opposed to after dosing. This difference was most pronounced towards the end of the treatment period (Days 13-15), when only 1 to 4 animals were affected prior to dosing in contrast to 18-24 animals after dosing. Although the final dose was administered on Day 15, reduced bodytone was apparent for 3/24 animals on Day 16 and 1/24 animals on Day 17.

Treatment at 1000 mg/kg bw/day was also associated with hair loss; 22/24 animals showed hair loss compared with 0/25 controls. In all cases, the hair loss was first noted between Day 14 and Day 19 post coitum and in 7 animals was apparent on Day 20 post coitum.

Occasional instances of increased lacrimation, brown staining on fur, periorbital staining and wet staining around the urogenital region were also observed at 1000 mg/kg bw/day.

No clinical signs considered to be attributable to treatment were observed at 100 or 300 mg/kg bw/day.
There was one mortality on the study which was considered to be related to treatment. One animal of the highest dosage group (1000 mg/kg bw/day), was sacrificed for humane reasons prior to dosing on Day 11 of pregnancy. Prior to sacrifice, reduced bodytone, unsteady gait, red periorbital staining, brown staining on fur, poorly coordinated movements, increased lachrymation, wet urogenital staining and bodyweight loss (bodyweight loss of 19 g were recorded between Days 6-8 of pregnancy) were evident. In addition, poorly coordinated movements were observed earlier than for the other animals in the same treatment group. Post mortem examination failed to establish any obvious cause for the physical condition. It is clear evidence that this animal showed a more severe response to treatment compared with the rest of the animals at the same dosage level, all of which survived to termination.

At 1000 mg/kg bw/day, mean bodyweight gain during the first six days of treatment was significantly lower than in controls. Thereafter, mean bodyweight gains during Days 12-16 were comparable to the controls. However, mean bodyweight gains during Days 16-20 were significantly lower than in controls, also after correction of body weight gains for gravid uterus weight.

At 300 mg/kg bw/day, bodyweight gain throughout Days 6-16 was comparable to the controls. However as at 1000 mg/kg bw/day, bodyweight gain during Days 16-20 was significantly lower than in controls. This was also true after correction for gravid uterus weight.

At 100 mg/kg bw/day, there was a slightly increased bodyweight gain compared to controls.

Food consumption of animals treated at 1000 mg/kg bw/day was higher than in controls during the first four days of treatment (differences attained statistical significance for Days 8 and 9), despite the fact that bodyweight gains were significantly lower than in controls during this period. This was reflected in higher food conversion ratios during this period, indicative of impaired efficiency of food utilisation. Food consumption at this dosage was noticeably higher than in controls during Days 14-15 and lower during Days 18-19.

At 100 and 300 mg/kg bw/day, there were no adverse effects on food consumption or food utilisation. Other than the previously mentioned increased incidence of hair loss in the 1000 mg/kg bw/day group compared with controls, the incidence of findings noted at macroscopic post mortem examination did not indicate any obvious adverse effect of treatment.

NOAEL: 100 mg/kg bw/day (actual dose received) (maternal toxicity)

LOAEL: 300 mg/kg bw/day (actual dose received) (maternal toxicity)

Results (fetuses)

Details on embryotoxic / teratogenic effects:

One female receiving 100 mg/kg bw/day showed total litter loss in utero (total resorption). In view of the absence of similar findings at higher dosages which indicates no dose-response relationship this finding is considered to be co-incidental and unrelated to treatment. The following assessment is based on the 23, 21, 24 and 22 females with live young at Day 20 in Groups 1-4, respectively.
Reproductive performance:

No effects on the reproductive performance, fetal deaths, fetal weight as well as on the sex ratio was evident on comparison of treated groups with concurrent controls.

Skeletal and visceral malformations:

There was a higher incidence of foetuses from the 1000 mg/kg bw/day group showing malformations. These malformations were principally visceral, affecting the urogenital system (i.e. absent left kidney and/or ureter, absent or narrow left uterine horn), and thoracic skeletal malformations manifest as abnormalities of the ribs. No similar malformations were observed in the controls.
At 100 and 300 mg/kg bw/day, the type and incidence of malformations did not indicate any adverse effect of treatment.

Skeletal anomalies and variants:

At 1000 mg/kg bw/day, the incidence and distribution within litter of foetuses with skeletal anomalies was significantly different from that of controls. Minimally distorted ribs were seen in 8 foetuses in 7 litters, another 5 foetuses in 4 litters showed more severe rib anomalies and were classified as malformed. There was and increased incidence of foetuses/litters showing irregular ossification of the thoracic vertebral centra, and shortened/absent 13th ribs. The latter finding being corroborated by a complete absence of foetuses showing supernumerary ribs, a highly unusual incidence. The percentage of foetuses/litters with reduced ossification of the cranial centres was statistically significantly higher than in controls.
At 300 mg/kg bw/day, the incidence and distribution within litters of foetuses with skeletal anomalies was also statistically significantly different from that of controls. The difference was principally due to an increased incidence of foetuses with reduced ossification. In addition, there was a slightly higher percentage incidence of foetuses with variant sternebrae, principally due to an increase in unossified sternebrae. It was noted that only one foetus showed supernumerary ribs. In view of the effects observed at 1000 mg/kg bw/day, these differences are considered to be related to treatment.
At 100 mg/kg bw/day, the type, incidence and distribution of skeletal anomalies and the percentage incidence of supernumerary ribs and variant sternebrae did not indicate any obvious adverse effects of treatment.

Visceral anomalies:

The type, distribution and incidence of visceral anomalies did not indicate any obvious adverse effects of treatment.

NOAEL: 100 mg/kg bw/day (actual dose received) (embryotoxicity)

LOAEL: 300 mg/kg bw/day (actual dose received) (embryotoxicity)

Table: Summary of Adult Performance

Category	**Group (Dosage [mg NaBr/kg/day])**			
	1 (0)	**2 (100)**	**3 (300)**	**4 (1000)**
No. of mated	25	25	25	25
No. of killed (Day 11 of pregnancy)	0	0	0	1
No. of non-pregnant	2	3	1	2
Total litter loss in utero	0	1	0	0
No. with live young at Day 20	23	21	24	22

Table: Bodyweights and bodyweight change during pregnancy - dams with live young, group mean values

Group ([mg NaBr/kg/day])	**No. of animals**	**Bodyweight [g] at Day of pregnancy And (bodyweight change [g]) from Day 6 of pregnancy**										**Corrected bodyweight (gain)**
		0	**3**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	***
1 (0)	23	222.7 (-47.0)	249.9 (-19.8)	269.7 (0)	281.8 (12.1)	293.4 (23.7)	310.9 (41.2)	325.5 (55.8)	346.0 (76.3)	376.5 (106.8)	414.9 (145.2)	333.4 (63.7)
2 (100)	21	221.1 (-46.8)	248.4 (-19.5)	268.0 (0)	280.5 (12.6)	294.1 (26.2)	312.0 (44.0)	327.9 (59.9)	347.4 (79.4)	379.1 (111.1)	417.9 (149.9)	336 (68)
3	24	224.2	249.0	269.8	282.5	296.2	312.8	325.5	345.3	369.3	403.7	328.2

(300)		(-45.6)	(-20.8)	(0)	(12.8)	(26.5)	(43.0)	(55.7)	(75.6)	(99.5) *	(133.9) *	(58.5)
4 (1000)	22	223.4 (-46.3)	248.5 (-21.2)	269.7 (0)	279.6 (9.9)	284.5 (14.9) **	298.1 (28.4) *	312.9 (43.2) **	333.0 (63.3) **	360.1 (90.4) **	390.7 (121.0) **	316.2 (46.5)

* statistically different from control with $p \leq 0.05$

** statistically different from control with $p \leq 0.01$

*** bodyweight and bw gain were corrected for gravid uterus weight (bw day 20- gravid uterus weight; bw gain - gravid uterus weight)

Table: Food consumption during pregnancy - Dams with live young, group mean values [g/rat/day]

Days of pregnancy	**Group (Dosage [mg NaBr/kg/day])**			
	1 (0)	**2 (100)**	**3 (300)**	**4 (1000)**
No. of animals observed:	**23**	**21**	**24**	**22**
3-5	27	26	25	26
6-7	27	28	28	30
8-9	28	28	29	32*
10-11	28	30	29	28
12-13	30	31	30	31
14-15	31	32	32	36**
16-17	34	34	32	34
18-19	33	34	31	30**

* statistically different from control with $p \leq 0.05$

** statistically different from control with $p \leq 0.01$

Table: Litter data - Group values

Group **Dosage [mg NaBr/kg/day])**	**1 (0)**	**2 (100)**	**3 (300)**	**4 (1000)**
Dams with live young				
No. of litters	23	21	24	22
Group mean values				
No. of corpora lutea	16.2	15.4	15.3	15.7
No. of implantations	14.9	14.6	14.0	14.1
No. of*in utero*death				
- early	0.6	0.6	0.7	0.8
- late	0.2	0.0	0.1	0.2
- early and late	0.8	0.6	0.8	1.0
No. of live young	14.1	14.0	13.1	13.1

Litter weight [g]	53.69	55.0	50.52	49.0
Foetal weight [g]	3.81	3.92	3.84	3.75
Graivid uterine weight [g]	81.48	81.88	75.35	74.46
Sex ratio [%]	48.0	54.0	47.5	48.0
Litter incidence (´n`)				
Number of *in utero* foetal death: early				
- 0	13	12	13	10
- 1	7	6	6	8
- 2	2	3	4	3
- 3	1		1	1
Number of *in utero* foetal death: late:				
- 0	19	21	21	19
- 1	4		3	2
- 2				1
Number of *in utero* foetal death: early and late:				
- 0	10	12	11	9
- 1	10	6	8	7
- 2	2	3	4	4
- 3				2
- 4	1		1	

Table: Foetal abnormalities - prevalence and distribution in litters

Category	**No. of affected foetuses/litter (n)**	**Group (Dosage [mg NaBr/kg/day])**			
		1 (0)	**2 (100)**	**3 (300)**	**4 (1000)**
		No. of litters with ´n` foetuses affected			
No. of litters examined		**23**	**21**	**24**	**22**
Malformation	0	20	21	20	14
	1	2	-	3	5
	2	-	-	1	2
	3	1	-	-	-
	7	-	-	-	1
Visceral anomaly	0	11	10	16	14
	1	11	6	7	3
	2	1	4	1	3
	3	-	1	-	1
	4	-	-	-	1
Skeletal anomaly	0	12	12	6*	4**

	1	6	4	4	3
	2	2	3	6	5
	3	1	2	6	3
	4	2	-	1	5
	5	-	-	1	-
	6	-	-	-	2
Mean foetuses affected per litter [%]					
Malformations		1.7	0.0	1.7	5.3
Visceral anomalies		7.8	11.4	5.7	13.2
Skeletal anomalies		13.9	11.9	29.1	40.6

* statistically different from control with $p \leq 0.05$

** statistically different from control with $p \leq 0.01$

Table: Skeletal Variants of Foetuses - Group values

Group (Dosage [mg NaBr/kg/day])	**Foetuses examined**	**Foetuses with**													
		13 ribs		**14 ribs**		**Normal sternebrae**		**Unossified sternebrae**		**Reduced sternebrae**		**Asym./bip. sternebrae**		**Total variant sternebrae**	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1 (0)	159	146	92.5	13	7.5	94	58.6	45	27.9	26	17.9	1	0.4	65	41.4
2 (100)	149	130	88.1	19	11.9	78	51.9	40	27.1	38	25.9	5	3.7	71	48.1
3 (300)	154	153	99.4	1	0.6	64	42.9	62	40.0	37	23.0	5	3.0	90	57.1
4 (1000)	137	137	100.0*	0	0*	27	20.3**	89	63.3**	51	38.8**	5	2.8	110	79.7**

* statistically different from control with $p \leq 0.05$

** statistically different from control with $p \leq 0.01$

TABLE 8

Skeletal and visceral malformations - incidence summary

	Foetuses				Litters			
Group	1	2	3	4	1	2	3	4
Dosage (mg/kg/day)	Control	100	300	1000	Control	100	300	1000
No. examined	324	295	315	289	23	21	24	22
No. affected	5	0	5	16	3	0	4	8
REGION/Description	Incidence							
CRANIAL								
Anophthalmia	-	-	1	1	-	-	1	1
THORACIC								
Distorted/minimally distorted/ossification irregularities ribs	-	-	-	5	-	-	-	4
Multiple sternebral/costal cartilage abnormalities	-	-	1	-	-	-	1	-
Duplicated inferior vena cava	1	-	-	-	1	-	-	-
LUMBAR/ABDOMINAL								
Umbilical hernia	-	-	1	-	-	-	1	-
Absent kidney	-	-	-	9[b-j]	-	-	-	3
Absent ureter	-	-	-	10[a-j]	-	-	-	4
Small kidney	-	-	-	1[a]	-	-	-	1
Absent uterine horn	-	-	-	2[f,g]	-	-	-	2
Narrow uterine horn	-	-	-	5[a-e]	-	-	-	3
Small indeterminate gonad	-	-	-	2[b,c]	-	-	-	2
SACROCAUDAL								
Interrupted vertebral column	1	-	-	-	1	-	-	-
OTHER								
Squat foetus syndrome	3	-	2	-	1	-	1	-

Superscripts indicate findings common to one foetus

TABLE 9

Visceral anomalies - incidence summary

	Foetuses				Litters			
Group	1	2	3	4	1	2	3	4
Dosage (mg/kg/day)	Control	100	300	1000	Control	100	300	1000
No. examined #	160	146	156	136	23	21	24	22
No. affected #	13	17	9	16	12	11	8	8
REGION/Description	Incidence*							
Subcutaneous haemorrhage:								
cranium	-	-	-	1	-	-	-	1
trunk	1	-	1	-	1	-	1	-
CRANIAL								
Haemorrhages affecting:								
brain	3	5	-	2	3	5	-	2
eyes	-	-	1	-	-	-	1	-
CERVICAL								
Small thyroid	-	-	-	1	-	-	-	1
Displaced oesophagus	-	-	-	1	-	-	-	1
THORACIC								
Interventricular septal defect (small)	2	2	3	-	2	2	3	-
Partially duplicated inferior vena cava	1	-	-	-	1	-	-	-
LUMBAR/ABDOMINAL								
Liver: abnormal lobation	2	3	3	4	2	3	3	4
haemorrhage within lobe	1	1	-	2	1	1	-	1
Intra-abdominal haemorrhage	1	2	-	3	1	2	-	2
Dilated renal pelvis/ureter	1	3	1	2	1	2	1	2
Displaced testis(es)	1	2	-	4	1	2	-	4
Dilated umbilical vein, minimal	-	-	-	1	-	-	-	1

* Individual foetuses may occur in more than one category

\# Excludes malformed foetuses

TABLE 10

Skeletal anomalies - incidence summary

	Foetuses				Litters			
Group	1	2	3	4	1	2	3	4
Dosage (mg/kg/day)	Control	100	300	1000	Control	100	300	1000
No. examined #	159	149	154	137	23	21	24	22
No. affected #	21	16	43	54	11	9	18	18
REGION/Description	Incidence*							
Reduced ossification of:								
one or more cranial centres	7	5	22	28	4	3	13	13
cervical vertebral arches	1	1	-	-	1	1	-	-
sacrocaudal vertebral arches	6	5	19	8	4	4	10	6
one or more centres pelvic girdle	2	2	7	1	2	2	7	1
digital centres	1	1	6	1	1	1	5	1
CRANIAL								
Sutural bone(s)	1	-	4	2	1	-	4	2
CERVICAL								
Cervical rib(s)	4	-	2	3	2	-	2	2
THORACIC								
Irregular ossification vertebral centra	5	6	6	12	3	5	5	8
Distorted rib(s),minimal	-	-	-	8	-	-	-	7
Shortened/absent 13th rib(s)	-	1	1	8	-	1	1	6
Misshapen sternebra(e)	-	1	1	-	-	1	1	-
LUMBAR/ABDOMINAL								
Irregular ossification vertebral elements	1	-	-	-	1	-	-	-
OTHER								
One less thoracolumbar vertebra	-	-	1	-	-	-	1	-
OBSERVATIONS AT AUTOPSY / EVISCERATION								
Minimally protruding tongue	-	-	1	-	-	-	1	-
Abnormal lobation liver	-	2	-	1	-	2	-	1

* Individual foetuses may occur in more than one category

\# Excludes malformed foetuses

Executive summary:

Materials and Methods

The potential developmental toxicity of sodium bromide was investigated in pregnant Crl: CD BR rats. Dosages of 0, 100, 300 and 1000 mg/kg bw/day were administered daily at a constant volume of 10 mL/kg bw in water as the vehicle by intragastric intubation, to groups of 25 rats each from Days 6-15 post coitum inclusive. On Day 20 post coitum, females were sacrificed and subjected to examination, litter values determined and foetuses subsequently sexed. Half of the foetuses were examined for visceral abnormalities; the remainder were observed for skeletal changes. During the study, clinical signs, body weights, body weight gains and food consumption of dams were regularly examined and reproduction parameters were determined after Cesarian section.

Results and Discussion

Treatment of pregnant dams with sodium bromide during gestation days 6 through 15 was associated with clear signs of maternal toxicity principally manifest as a lower rate of bodyweight gain during Days 6-12 of pregnancy, abnormalities of gait, reduced bodytone and poorly coordinated movements at the top dose level of 1000 mg/kg bw/day. No effects on the reproductive performance, fetal deaths, fetal weight as well as on the sex ratio was evident on comparison of treated groups with concurrent controls. Detailed examination of foetal morphology at this dose level revealed a higher incidence of foetuses/litters showing absent left kidney, absent left ureter, absent/narrow left uterine horn, distorted ribs, shortening/absence of 13th ribs, irregular ossification of the thoracic vertebral centra, reduced and/or unossified sternebrae and, reduced ossification of one or more cranial centres, than in the control group. Although it was noted that seven foetuses in one litter had no left kidney and ureter (3 of these foetuses also had an absent or narrow left uterine horn), these abnormalities were also apparent for one foetus in each of two further litters. In addition, one foetus in another litter had a small left kidney, absent left ureter and a markedly narrow left uterine horn. Therefore, an association with treatment of dams is considered likely, since the litter and not the foetus is the principal unit of assessment, but these effects are probably secondary to severe maternal toxicity at this dose level. It is noteworthy that there was no obvious reduction in mean foetal weight. Some of the observed skeletal abnormalities may reflect effects on maternal bodyweight gain and food consumption. In contrast, the defects observed in the urogenital system are extremely rare and considered more likely to reflect a selective effect on embryofoetal development than a secondary effect resulting from toxicity to the parent female. At 300 mg/kg bw/day, no adverse effects on the parent female were observed during the treatment period. However, following the withdrawal of treatment, bodyweight gain was statistically significantly lower than controls. This effect was also recorded at 1000 mg/kg bw/day on the present study. This is circumstantial evidence that the lower rate of bodyweight gain following the withdrawal of treatment reflects an earlier effect during the dosing period, which has been detected within the context of this screening study. Detailed examination of foetal morphology revealed a higher incidence of foetuses showing reduced ossification of various components of the skeleton compared with controls. It is noteworthy that there was no obvious reduction in mean foetal weight. At 100 mg/kg bw/day, there was no observeable maternal response to treatment and no obvious adverse effects on morphological development of the conceptus.

Conclusions:

Treatment of pregnant rats with sodium bromide through organogenesis caused severe maternal toxicity at the high dose level of 1000 mg/kg bw/day while at the mid dose level of 300 mg/kg bw/day, maternal toxicity was less pronounced as in the high dose group and was characterised by significantly depressed body weight gains. Taking into consideration the results of the fetal examinations it is concluded that sodium bromide treatment with concentrations of 1000 mg/kg bw/day leads to a higher incidence of foetuses/litters showing absent left kidney, absent left ureter and absent/narrow left uterine horn. Foetuses treated at 300 and 1000 mg/kg bw/day showed a higher incidence of reduced ossification of diverse components of the skeleton. There was no observable maternal or foetal effect related to treatment at 100 mg/kg bw/day while at the mid and high dose level, maternal toxicity as a consequence of treatment with sodium bromide was evident. Therefore, the no effect level for the parent female and in utero development of the foetus is determined to be 100 mg/kg bw/day.

3.7.1.10 [Study 10] Dose range finding study - developmental toxicity of sodium bromide, rabbit

Reference	A6.8.1/01, Doc. No. 551-004 Study report, 2008a
Guideline	equivalent or similar to EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study) Remarks: study is a dose range finder equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) Remarks: study is a dose range finder GLP compliance: yes
Reliability	1 (reliable without restriction)
Species / strain	Species: rabbit Strain: New Zealand White
Test material	Details on test material:

- Name of test material (as cited in study report): Sodium bromide
- Description: White crystalline material
- Analytical purity: 99.92%
- Lot/batch No.: 060258
- Stability under test conditions: considered to be stable under the storage conditions.
- Storage condition of test material: at room temperature in the dark

Ammonium bromide is an inorganic salt that dissociates to its composite ions in aqueous solutions at environmental pH and temperature. Comparison of the available data on the various bromide salts have shown that the bromide ion is the relevant ion for determination of the toxicological profile with simple cations such as potassium, sodium or ammonium, that are ubiquitous in nature, having little or no influence on the bromide ion properties. It is therefore justified to read-across data from other inorganic bromide salts to ammonium bromide.

Administration / exposure

Route of administration: oral: gavage

Vehicle: water

Details on exposure:

PREPARATION OF DOSING SOLUTIONS:
Samples of the test material formulation were analysed for concentration of sodium bromide. The accepted range was ± 10% of nominal concentration and the results indicated that the performed formulations were within ± 7% of the nominal concentration

VEHICLE
- Concentration in vehicle: 31.3, 62.5, 125 and 250 mg/mL for the non-pregnant phase and 25, 50 and 100 mg/mL for the pregnant phase of the study.
- Amount of vehicle (if gavage): 4 mL/animal/day

Analytical verification of doses or concentrations: yes

Details on mating procedure:

Samples of the test material formulation were analysed for concentration of sodium bromide. The accepted range was ± 10% of nominal concentration and the results indicated that the performed formulations were within ± 7% of the nominal concentration.

Duration of treatment / exposure:

please refer to "any other information on materials and methods".

Frequency of treatment:

Escalating dose animals: 3 consecutive days
Fixed dose animals: 13 consecutive days
Pregnant phase: Day 3 - Day 28 of gestation

Doses / concentrations

125, 250, 500 and 1000 mg/kg bw/day for the escalating dose regime and 500 mg/kg bw/day for the fixed dose for 13 days during the non-pregnant phase of the study. 100, 200 and 400 mg/kg bw/day for the pregnant phase of the study.
Basis: actual ingested

No. of animals per sex per dose:

6 females for the non-pregnant part of the study: 3 for the escalating dose part, 3 for the fixed dosage.
24 time-mated female rabbits were used for investigations on potential developmental effects of the test substance: 6 animals/dosage group and 5 control animals receiving vehicle only

Control animals: yes, concurrent vehicle

Examinations

Maternal examinations:

CAGE SIDE OBSERVATIONS: No data

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: All animals were examined for overt signs of toxicity, ill-health or behavioural change immediately before dosing, immediately post dosing and in addition one and five hours after dosing during the working week.

BODY WEIGHT: Yes
- Time schedule for examinations: Non-pregnant animals were weighed daily, pregnant animals were weighed on Days 3, 6, 9, 12, 15, 18, 21, 24, 27 and 29 of gestation.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
Individual food consumption was recorded for non-pregnant phase animals daily and for pregnant phase animals for the periods Days 3-6, 6-9, 9-11, 12-15, 15-18, 18-21, 21-23, 24-27 and 27-29 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: Yes / No / No data
- Number of implantations: Yes

Fetal examinations:

- External examinations: Yes
- Soft tissue examinations: Yes: [all per litter]
- Skeletal examinations: Yes: [all per litter]
- Head examinations: No data

Statistics: None performed due to the variability of the data and the small group size used in this dose range finding study.

Results and discussion

Details on maternal toxic effects:

Escalating dose animals:
Treatment of three animals at dose levels of 125, 250 and 500 mg/kg bw/day for three consecutive days did not reveal any overt signs of toxicity as assessed by clinical condition, bodyweight and bodyweight change or food intake. Treatment of the same animals at 1000 mg/kg bw/day on a single occasion resulted in notable clinical signs (decreased respiration and ataxia) and the following day this dosage was terminated. Necropsy did not show any cause for the clinical conditions.

Fixed dose animals:
Following treatment at 500 mg/kg bw/day for 13 days, one animal showed decreased respiration rate and ataxia on Day 8 and was euthanized for animal welfare reasons. There was an indication of reduced food intake in the days preceding this occurrence, but no corresponding effect on bodyweight gain. Necropsy finding of this animal did not reveal any cause for its condition. Treatment of the other two animals was not associated with evidence of toxicity as assessed by clinical condition, bodyweight, bodyweight change, food intake and necropsy findings were considered to be unremarkable.

Pregnant phase:
Treatment of pregnant rabbits at 400 mg/kg bw/day from day 3 through 28 of gestation demonstrated ataxia in two animals from Day 25 and 28 of gestation, respectively, and resulted in early termination of the first affected animal on Day 27. When ataxia was observed, the signs persisted to the next dosing occasion on the following day. Lower food intake was apparent for both of these animals from Day 24 of gestation but there was no obvious corresponding effect on bodyweight gain. Necropsy findings of either animal did not reveal any obvious cause for the clinical observations. Treatment of the other four animals at 400 mg/kg bw/day was not associated with marked evidence of toxicity as assessed by clinical condition, bodyweight, bodyweight change, food intake or necropsy findings. There were no obvious adverse effects observed at dosages of 100 and 200 mg/kg bw/day. Neither the type, incidence nor distribution of necropsy findings indicated any adverse effect of treatment with sodium bromide.

NOAEL: 200 mg/kg bw/day (actual dose received) maternal toxicity

LOAEL: 400 mg/kg bw/day (actual dose received) maternal toxicity

Results (fetuses)

Details on embryotoxic / teratogenic effects:

Only a small number of litters were available for the evaluation of treatment related effects. The numbers of implantations, pre and post implantation losses, live litter size, sex ratio, litter, placental and foetal weights and external foetal morphology did not indicate any adverse effect of treatment when administering sodium bromide doses of 100, 200 and 400 mg/kg bw/day during Days 3-28 of gestation.

NOAEL**:** 400 mg/kg bw/day (actual dose received) fetotoxicity

LOAEL: > 400 mg/kg bw/day (actual dose received) fetotoxicity

Any other information on results

One female dosed at 100 mg/kg bw/day gave birth to two of the offspring before necropsy on Day 29 of gestation. In the absence of any signs of early delivery for other animals at this or higher dosages, this single event was considered incidental and not related to treatment.

Table: Bodyweight, Gravid Uterus Weight and Bodyweight Performance during Gestation - Group Mean Values

Dose Level [mg/kg bw/day]	Bodyweight [kg]		Bodyweight Change Days 3-29 [kg]	Gravid UterusWeight	Adjusted Bodyweight Day 29 [kg]	Adjusted Bodyweight Change Days 3-29[kg]
	Day 3	Day 29				
0	3.83	3.68	0.3	0.445	3.24	-0.14
100	3.21	3.84	0.63	0.53	3.31	0.1
200	3.2	3.83	0.62	0.495	3.33	0.13
400	3.17	3.76	0.59	0.499	3.26	0.09

Table: Food Consumption during Gestation - Group Mean Values

Dose level [mg/kg bw/day]	Food Intake [g/animal/day] during day of gestation:								
	3-6	6-9	9-12	12-15	15-18	18-21	21-24	24-27	27-29
0	102	121	110	129	132	143	149	124	117
100	151	190	173	160	167	151	116	96	115
200	161	173	158	160	171	168	147	117	109
400	121	153	151	144	161	160	140	99	124

Table: Litter Data on Day 29 of Gestation - Group Mean Values

Dose Level [mg/kg bw/day]	No. of corpora lutea	No. of implants	No. embryonic/foetal deaths			Implantation loss [%]		No. live foetuses			% male foetus	Male foetal weight [g]	Female foetal weight [g]	Mean foetal weight [g]	Litter weight [g]	Mean placental weight [g]	Total placental weight [g]
			E	L	T	Pre	Post	M	F	T							
0	13.3	7.8	0.3	0.5	0.8	42.1	11.3	3.5	3.5	7	53	43.7	39.7	42.6	285.9	5	33.7
100	11.6	8.8	0.2	0	0.2	23.8	2.5	3.8	4.8	8.6	43.3	48.3	4	47.7	400.2	5.6	47.8
200	11.5	8.5	0.2	0.5	0.7	24.1	7	3.8	4	7.8	48.7	42.2	41.3	42	329.3	5.5	43.3

400	12	7.8	0.3	0	0.3	35.4	2.8	4.3	3.3	7.5	54.4	48	44.4	46.2	337.8	5.8	43

E early
L late
T total
M male
F female

Executive summary:

Materials and Methods:

The purpose of the study was to provide information for the selection of suitable dose levels for a pre-natal developmental toxicity study on sodium bromide in the rabbit. The study was designed to investigate effects of sodium bromide on the progress and maintenance of pregnancy in the rabbit, including early indication of potential effects on embryonic and foetal development. The test substance was administered orally by gavage, prior to implantation and throughout the period of organogenesis until the day prior to expected delivery. The study was divided in two parts: at first, the maximum tolerated dose was established using non-mated animals and applying an escalating dosing regime; then the potential effects on pregnancy were investigated in time-mated animals at a fixed dose level following administration for 13 days and at three dose levels during days 3 through 28 of gestation. Sodium bromide was administered once daily to three non-pregnant rabbits using an escalating dosing regime. An initial dosage of 125 mg/kg bw/day was investigated, followed by 250, 500 and 1000 mg/kg bw/day at intervals of 3 days. A further three non-pregnant animals were dosed once per day for 13 consecutive days at 500 mg/kg bw/day which were euthanized on Day 14. For investigation on the pre-natal development, two groups of six time-mated rabbits were dosed daily on Days 3-28 of gestation at 100, 200 and 400 mg/kg bw/day. A control group of 5 time-mated rabbits received vehicle only. All animals were euthanized on Day 29 of gestation. Clinical signs, bodyweights and food consumption were monitored during the investigation period and all animals were subjected to gross necropsy including examination of the uterus. In the offspring, foetal sex, external foetal appearance and foetal weight were determined.

Results and Discussion:

Treatment of non-pregnant animals at 1000 mg/kg bw/day on the escalating dose regime was associated with ataxia and decreased respiration rate following a single day of treatment which precluded this dosage from further investigation. While this effect may have been directly attributable to this dosage, it may also, in part, be a result of cumulative toxicity from the previous dosages applied (125, 250 and 500 mg/kg bw/day for three consecutive days each). Treatment of non-pregnant animals at 500 mg/kg bw/day for 13 consecutive days resulted in one animal being euthanized after showing similar signs following seven days treatment, although the remaining two animals survived termination (Day 14) without showing any adverse effects of treatment. Treatment of pregnant animals at 100, 200 and 400 mg/kg bw/day on Days 3-28 of gestation was not associated with any clear adverse effects on bodyweight, bodyweight gain or food consumption during gestation. There were no clinical signs observed after treatment with 100 and 200 mg/kg bw/day. At 400 mg/kg bw/day, two out of six animals showed ataxia towards the end of the study, with one animal being euthanized due to animal welfare reasons. Macroscopic necropsy observation did not indicate any adverse effects of treatment in the dams. No treatment-related effects were noted in the remaining 4 animals treated at 400 mg/kg bw/day. There was no pre- or post-implantation loss noticed and no adverse effects were observed regarding foetal weight, sex ratio or macroscopic findings. Although ataxia and decreased respiration as the only adverse clinical signs in dams were observed after treatment at 400 mg/kg bw/day and no adverse effects appeared at 100 and 200 mg/kg bw/day, the study author suggests to use 250 mg/kg bw/day as a high dose for the main teratology study in rabbits, since treatment at 400 mg/kg bw/day could lead to early termination for some animals. In addition, it was argued that the observed ataxia is a result of cumulative toxicity and therefore treatment should start at Day 6 of gestation to reduce the risk of early termination of animals. However, the applicant concludes that there were no adverse effects observed in four out of six animals dosed at 400 mg/kg bw/day and there were no treatment related changes in the foetal parameters examined (foetal weight, sex ratio, implantation loss) at this dose level. In addition, clinical signs observed in two rabbits treated at 400 mg/kg bw/day were not indicative for severe toxicity, were seen only towards the end of the study and the application period started already on day 3 vs. day 6 according to the guideline. Since dams did not show any treatment-related effects when dosed at 100 and 200 mg/kg bw/day, the dosing regime used for this dose range finding study (concentrations and duration of treatment) should have been used for the main study as well.

Conclusions:

Treatment of non-pregnant animals at 500 or 1000 mg/kg bw/day or pregnant animals at 400g/kg bw/day was associated with adverse clinical signs (ataxia and decreased respiration rate) in the absence of clear effects on bodyweight or food intake during the observation period. In the pregnant phase of the study, no treatment-related effects on the fetal parameters examined were noted up to the highest dose level studied.

The study author concluded that the observed clinical signs were resulting from cumulative toxicity with some animals being more susceptible than others. For the main developmental toxicity study on sodium bromide dose levels of 25, 75 and 250 mg/kg bw/day were selected and, in addition, the start of application was set on Day 6 of gestation. The main study was performed using the dosages recommended by the study author.

From the results obtained in this dose range finding study, the applicant, however, concludes that the same dosage levels than those applied in the dose-range-finding study should have been used for the main study since no severe maternal toxicity was seen at 400 mg/kg bw/day and no signs attributable to treatment were noticed at the lower dosage levels used (100 and 200 mg/kg bw/day).

3.7.1.11 [Study 11] Pre-natal developmental toxicity study (OECD TG 414) of sodium bromide in rabbit

Reference

A6.8.1/02, Doc. No. 551-006

Study report, 2008b

Guideline

Test guideline according to Guideline: EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)

equivalent or similar to Guideline: OECD Guideline 414 (Prenatal Developmental Toxicity Study)

equivalent or similar to Guideline: other: 12 NohSan No 8147 (Japanese Ministry of Agriculture, Forestry and Fisheries Testing guidelines for Toxicology studies) Deviations no

GLP compliance: yes

Reliability

1 (reliable without restriction)

Species / strain

Species: rabbit

Strain: New Zealand White

Test material

Details on test material:

- Name of test material (as cited in study report): Sodium bromide
- Description: White crystalline solid
- Analytical purity: 99.92%
- Lot/batch No.: 060258
- Stability under test conditions: considered to be stable under the storage conditions
- Storage condition of test material: at ambient temperatures in the dark

Ammonium bromide is an inorganic salt that dissociates to its composite ions in aqueous solutions at environmental pH and temperature. Comparison of the available data on the various bromide salts have shown that the bromide ion is the relevant ion for determination of the toxicological profile with simple cations such as potassium, sodium or ammonium, that are ubiquitous in nature, having little or no influence on the bromide ion properties. It is therefore justified to read-across data from other inorganic bromide salts to ammonium bromide.

Administration / exposure

Route of administration: oral: gavage

Vehicle: water

Details on exposure:

PPREPARATION OF DOSING SOLUTIONS:
Satisfactory homogeneity and stability of the test substance in aqueous formulations at concentrations of 1 and 250 mg/ml following storage at +4°C for up to 14 days had been confirmed. Formulations of test material used to dose the animals in this study were routinely analysed for achieved concentration and the results revealed that concentrations were within the acceptable range of ± 10%. Only on one analysis occasion, formulations at 6.25 mg/ml (25 mg/kg bw/day group) and 62.5 mg/ml (250 mg/kg bw/day group) formulation were ±12% of nominal concentration.

VEHICLE
- Concentration in vehicle: 6.25, 18.8 and 62.5 mg/mL
- Amount of vehicle (if gavage): Approximately 4 mL;
The dose volume given was calculated using the most current recorded bodyweight.

Analytical verification of doses or concentrations: yes

Details on analytical verification of doses or concentrations:

Satisfactory homogeneity and stability of the test substance in aqueous formulations at concentrations of 1 and 250 mg/ml following storage at +4°C for up to 14 days had been confirmed. Formulations of test material used to dose the animals in this study were routinely analysed for achieved concentration and the results revealed that concentrations were within the acceptable range of ± 10%. Only on one analysis occasion, formulations at 6.25 mg/ml (25 mg/kg bw/day group) and 62.5 mg/ml (250 mg/kg bw/day group) formulation were ±12% of nominal concentration.

Duration of treatment / exposure: Day 6-28 of gestation

Frequency of treatment: daily

Doses / concentrations

25, 75 or 250 mg/kg bw/day
Basis: actual ingested

No. of animals per sex per dose:

30 time-mated animals for the test groups and 29 time-mated animals as controls. One animal (number 101), allocated to the Control group, was rejected from the study prior to the commencement of dosing due to animal welfare considerations. The data for this animal are not presented within this report but is retained with the raw data for the study.

Control animals: yes, concurrent vehicle

Details on study design:

- Dose selection rationale:
The dose levels had been chosen based on the result of a dose-range finding study (please refer to Document IIIA, Section 6, Point 6.8.1/01). Because of ataxia being observed at treatment with 400 mg/kg bw/day which could have potentially led to the early termination of animals, thereby reducing the number of litters available for assessment, this precluded the use of 400 mg/kg/day as the high dosage for this main study and it was therefore necessary to choose a dosage where no maternal toxicity could be expected. Therefore, the study author decided to use 250 mg/kg bw/day as the high dose level to prevent potential early termination of animals.

Examinations

Maternal examinations:

CAGE SIDE OBSERVATIONS: No data

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: All animals were examined for overt signs of toxicity, ill-health or behavioural change immediately before dosing immediately post dosing and one hour after dosing every day and in addition, five hours after dosing during the working week.

BODY WEIGHT: Yes
- Time schedule for examinations: Animals were weighted on Days 3, 6, 9, 12, 15, 18, 21, 24, 27 and 29 of gestation.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption was recorded for the periods Days 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27 and 27-29 of

gestation.

WATER CONSUMPTION:
Formal gravimetric measurement of daily water intake was instigated after visual inspection of water bottle residues indicated a possible increase in water consumption for treated animals.

Ovaries and uterine content:

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes

Fetal examinations:

- External examinations: Yes: [all per litter]
- Soft tissue examinations: Yes: [half per litter]
- Skeletal examinations: Yes: [half per litter]

Statistics:

The following parameters were analysed statistically, using the test methods outlined below:
Bodyweight and bodyweight change, food consumption, foetal, placental and litter weights: Bartlett´s test for homogeneity of variance and one way analysis of variance, followed by Dunnet´s multiple comparison test or, if unequal variances were observed, an alternative multiple comparison test.
All litter data parameters: Kruskal-Wallis non-parametric analysis of variance, and a subsequent pairwise analysis of control values against treated values using the Mann-Whitney U-test, where significance was seen.
Foetal evaluation parameters, including skeletal or visceral findings: Kruskal-Wallis parametric analysis of variance and Mann-Whitney U-test.

Results and discussion

Details on maternal toxic effects:

CLINICAL SIGNS AND MORTALITY
Clinical observations did not indicate any obvious effects of treatment at any dose level used in the investigation. There were no unscheduled deaths during the study.

BODY WEIGHT AND FOOD CONSUMPTION
Bodyweight and bodyweight change during gestation did not indicate any adverse effects of treatment at the dosages investigated. At 250 mg/kg bw/day, overall bodyweight change was slightly superior to controls, due to higher bodyweight gains between Day 8 to 12 of gestation. At the lower dosages (25 and 75 mg/kg bw/day) bodyweight change was essentially similar to controls, although overall bodyweight gain at 75 mg/kg bw/day was superior to control following adjustment for the contribution of the gravid uterus.
Food intakes during gestation did not indicate any obvious adverse effects of treatment at any of the dosages investigated. At 250 mg/kg bw/day, food intake was slightly lower than control prior to treatment with sodium bromide. However, following the commencement on Day 6, food consumption was slightly higher than controls up to Day 24 of gestation. A similar pattern of higher food consumption, although to a lesser extent, was apparent for females receiving 75 mg/kg bw/day from Day 6 to Day 21 of gestation.

GROSS PATHOLOGY
The incidence of necropsy findings among adult animals on Day 29 of gestation did not indicate any obvious adverse effects of treatment.

OTHER FINDINGS
Animals treated at 75 and 250 mg/kg bw/day showed increased water intake. Water consumption of the lowest dosage group (25 mg/kg bw/day) was considered to be unaffected by treatment.

NOAEL: 250 mg/kg bw/day (actual dose received) maternal toxicity

LOAEL: > 250 mg/kg bw/day (actual dose received) maternal toxicity

Results (fetuses)

Details on embryotoxic / teratogenic effects:
VIABILITY
Intergroup differences in litter data did not indicate any adverse effect of maternal treatment on foetal survival at any of the dosages investigated.

BODY WEIGHT
Placental, litter and foetal weights were unaffected by maternal treatment at any of the dosages used in the investigation.

GROSS PATHOLOGY
Neither the type, incidence or distribution of findings observed for foetuses during necropsy, nor visceral head or skeletal examinations indicated any adverse effect of maternal treatment on foetal growth or morphological development. Similarly, the assessment of skeletal development parameters did not reveal any treatment-related trends in the proportion of foetuses (or litters) that indicated any effect of maternal treatment. At 250 mg/kg bw/day, there was a significant difference in the proportion of foetuses with 13 rib pairs. At 75 mg/kg bw/day, there was a significant difference in irregular ossification of more than one cranial bone.

NOAEL: 250 mg/kg bw/day (actual dose received) fetotoxicity

LOAEL: > 250 mg/kg bw/day (actual dose received) other: fetotoxicity

Any other information on results

At 25 mg/kg bw/day one female showed total litter lossin utero. In the absence of any increased post-implantation loss in remaining litters at this dosage or any similar incidences at the higher dosages applied (75 and 250 mg/kg bw/day) the total litter loss in on low dose female only was not considered to be of any toxicological relevance.

Table : Summary of female performance

Category	Dose Level [mg/kg bw/day]			
	0	24	75	250
Initial group size	29	30	30	30
Non-pregnant	3	4	4	3
Total litter loss in utero	0	1	0	0
With live young at Day 29	26	25	26	27

Table A6.8.1/02-2: Clinical Observations in Dams - Group Incidence

Parameter investigated	Number of animals affected at Dose Level [mg/kg bw/day]			
	0	25	75	250
Number of females	29	30	30	30
Prior to dosing				
Generalised fur loss	4	3	3	8
Generalised scabbing	-	-	2	-
Staining around ano-genital region	-	1	-	1
Scab on tail	1	-	-	-
Crooked gait	1	-	-	-
Broken tail	-	1	-	-
Days 6-12 #				

Generalised fur loss	3	4	2	7
Generalised scabbing	-	-	2	1
Staining around ano-genital region	-	1	-	1
Broken tail	-	1	-	-
Days 13-21				
Generalised fur loss	2	1	2	6
Generalised scabbing	-	-	1	1
Staining around ano-genital region	-	1	-	2
Red staining of ano-genital region and cage tray	1	-	-	-
Diarrhoea	-	-	1	2
Decreased respiration rate and noisy respiration	1	-	-	-
Broken tail	-	1	-	-
Days 22-29				
Generalised fur loss	1	-	-	3
Staining around snout	-	-	-	1
Fur staining	-	-	-	1
Staining around ano-genital region	1	1	-	5
Diarrhoea	-	-	1	3
Wound on upper lip	-	-	1	-
Broken tail	-	1	-	-

Table : Bodyweight Change - Group Mean Values

Dose level [mg/kg bw/day]	Bodyweight Change [kg] during days of gestation										
	3-6	6-7	7-8	8-9	9-12	12-15	15-18	18-21	21-24	24-27	27-29
0	0.07	0	0.02	0.01	0.05	0.08	-0.02	0.05	0.06	0.07	0.05
25	0.08	0.01	0	0.01	0.04	0.07	0.02*	0.01	0.07	0.05	0.05
75	0.04	0.02	0.01	0.02	0.06	0.1	0	0.04	0.06	0.05	0.05
250	0.05	0.03	0.02	0.07	0.09	0.07	0.02**	-0.01	0.05	0.06	0.05

* p0.05

** p0.001

Table: Gravid Uterus Weight and Adjusted Bodyweight Performance - Group Mean Values

Dose Level [mg/kg bw/day]	Bodyweight [kg]		Bodyweight Change Days 6-29 [kg]	Gravid UterusWeight [kg]	Adjusted Bodyweight Day 29 [kg]	Adjusted Bodyweight Change Days 6-29 [kg]
	Day 6	Day 29				
0	3.45	3.82	0.37	0.5	3.32	-0.13
25	3.52	3.84	0.32	0.47	3.37	-0.15
75	3.5	3.89	0.39	0.47	3.42	-0.08

250	3.47	3.91	0.44	0.51	3.4	-0.08

Table: Food consumption - Group Mean Values

Dose level [mg/kg bw/day]	Food Intake [g/animal/day] during day of gestation:								
	3-6	6-9	9-12	12-15	15-18	18-21	21-24	24-27	27-29
0	129	135	129	116	115	128	119	108	112
25	130	133	127	106	112	124	114	100	99
75	129	141	140	128	125	137	119	109	108
250	122	143	151	138	143*	142	126	109	109

* p0.05

Table: Litter Data on Day 29 of Gestation - Group Mean Values

Dose Level [mg/kg bw/day]	No. of corpora lutea	No. of implants	No. embryonic/foetal deaths			Implantation loss [%]		No. live foetuses			% male foetus	Male foetal weight [g]	Female foetal weight [g]	Mean foetal weight [g]	Litter weight [g]	Mean placental weight [g]	Total placental weight [g]
			E	L	T	Pre	Post	M	F	T							
0	10.8	8.6	0.1	0.6	0.7	20.2	7.6	4.4	3.5	7.9	56.1	43.2	42.2	43.0	334.7	5.3	41.4
25	9.8	7.8	0.4	0.2	0.6	20.4	6.9	3.8	3.5	7.3	50.9	44.7	45.2	45.4	326.1	5.6	40.1
75	10.4	7.5	0.3	0.2	0.5	27.2	5.6	3.9	3.0	7.0	56.4	46.9	46.4	46.8	315.1	5.9	38.6
250	11.0	8.3	0.1	0.6	0.7	26.7	7.4	4.0	3.6	7.6	53.9	45.9	45.0	46.1	331.6	6.1	41.7

E early
L late
T total
M male
F female

Table: Foetal Skeletal Development - Group Incidence

Skeletal Development	Dose Level [mg/ml]											
	0			25			75			250		
	Number of Foetuses (litters) affected											
	NF	NL	%	NF	NL	%	NF	NL	%	NF	NL	%
Number examined	205	26	-	182	25	-	181	26	-	204	27	-
Number of ribs:												
11/11	1	1	0.4	1	1	0.8	-	-	-	-	-	-
11/12	1	1	0.5	-	-	-	-	-	-	-	-	-
12/11	-	-	-	1	1	0.6	-	-	-	1	1	0.5
12/12	116	25	56.1	117	23	61.6	120	24	65.4	160	27	80
12/13	15	11	7.5	9	8	5.8	5	4	2.2	10	7	4
13/11	-	-	-	1	1	0.4	-	-	-	-	-	-
13/12	13	9	5.8	9	6	4.5	11	10	6.8	11	9	4.9
13/13	59	17	29.7	43	18	26.2	45	15	25.6	21	10	10.6*
Number of fully ossified sternebrae:	1	1	0.5	-	-	-	4	4	1.8	10	6	3.4
4	7	5	3.2	13	9	8.3	12	6	5	12	7	7.7

4 >4	197	26	96.3	169	25	91.7	165	26	93.2	182	26	88.9
Incomlete ossification of more than 1 cranial bone	1	1	1.3	8	4	10.4	5	4	4.0	2	2	1.4
Irregular ossification of more than 1 cranial bone	23	14	25.9	27	18	32.7	42	20	53.2**	31	19	35.1
Asymmetrical ossification of more than 1 sternebrae	1	1	0.5	2	2	1.4	3	3	1.3	10	6	3.5
Two or more sternabrae fused	-	-	-	3	2	1.6	2	2	0.8	5	5	2.1
Total number of affected	163	26	80.3	132	25	75.7	148	26	83.4	148	27	72.2

NF number of foetus

NL number of litters

% group mean %

- not applicable; no foetuses affected

* p0.05

** p0.01

Applicant's summary and conclusion

Conclusions:

The oral administration of sodium bromide during Days 6-28 of gestation at dose levels of 25, 75 and 250 mg/kg bw/day did not produce any adverse toxicological changes in the parameters investigated in neither maternal animals nor in foetuses. Therefore, the highest dosage used (250 mg/kg bw/day) can be regarded as a NOAEL both for maternal and embryotoxicity.

Executive summary:

Materials and Methods:

The study was designed to investigate the effects of sodium bromide on embryonic and foetal development of the rabbit. The test substance was therefore administered orally by gavage from implantation throughout the period of organogenesis until the day prior to expected delivery. Time-mated animals were dosed at 25, 75 or 250 mg/kg bw/day of sodium bromide or received distilled water as a control during Days 6-28 of gestation. Clinical signs, bodyweight and food consumption were monitored throughout the study and water consumption was instigated as the study progressed. All animals were euthanized on Day 29 of gestation and subjected to gross necropsy including examination of the uterine contents. Foetuses were subjected to macroscopic necropsy examination. From half of the foetuses from each litter the head was removed and preserved in Bouins fixative for visceral examination and the other half of the foetuses were eviscerated, skinned and processed for skeletal examination. The dose levels used in this main teratology study in rabbit were selected on the basis of a dose-range-finding teratology study where dose levels of 0, 100, 200 and 400 mg/kg bw/day were administered to pregnant rabbits from day 3 through 28 of gestation.

Results and Diskussion:

Treatment of rabbits on Days 6-28 of gestation at dosages of 25, 75 and 250 mg/kg bw/day was well tolerated by the rabbit and was not associated with any adverse toxicological changes. Increased water intake at 75 and 250 mg/kg bw/day was apparent throughout the treatment period and might be due to the high salinity of the dose formulations. While no adverse effects on bodyweight gain or food consumption was apparent, administration of 250 mg/kg bw/day resulted in higher bodyweight gain and food consumption compared to controls during the early part of the treatment period. At 75 mg/kg bw/day, a similar pattern of slightly higher food intake during the early dosing period was apparent, without bodyweight gains being obviously increased. Considering the higher bodyweight change normally observed for rabbits, the observed difference in the early treatment period might reflect normal biological variation. However, the increased food consumption was already seen during the first four days in a teratology study on rats, which were treated at 1000 mg/kg bw/day (please refer to Document IIIA, Section 6, Point 6.8.1/05). There was no obvious effect of maternal treatment

on the survival, growth or development of the offspring at dosages up to 250 mg/kg bw/day. There was a decrease in the proportion of foetuses with 13 ribs in the highest dosage group (250 mg/kg bw/day) with 10.6% of animals having 13 ribs compared to 29.7% in controls due to an increase of foetuses with 12 rib pairs. As the lower incidence of 13 rib pairs was not associated with any decrease in rib pairs (i.e. there were no foetuses with 11 rib pairs at this dosage) or any trend in other developmental parameters, the lower proportion of foetuses with 13 rib pairs at 250 mg/kg bw/day can not be considered to be a treatment-related effect and is therefore of no toxicological significance. Furthermore, this effect was seen in a small number of females in this group that had a relatively low number of implantations, with the number of animals with unilateral implantation being also higher at this dosage. As treatment did not start until Day 6 of gestation, it is considered that implantation had already been established and thus, no treatment-related effect for these incidences is taken into account. Likewise, the irregular ossification at 75 mg/kg bw/day of cranial bones was considered to be of no toxicological relevance in the absence of a dose-related response. In addition, the same as described for the high dose group is true, since reduced ossification of cranial bones was seen in litters from a few dams only with a low number of implantations.

3.7.1.12 [Study 12] Pre-natal developmental toxicity study (OECD TG 414) of ammonium bromide in rat

Reference	A6.8.1/03, Doc. No. 551-001 Study report, 2000b
Guideline	OECD Guideline 414 (Prenatal Developmental Toxicity Study) EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study) GLP compliance: yes
Reliability	1 (reliable without restriction)
Species / strain	Species: rat Strain: Sprague-Dawley
Test material	- Name of test material (as cited in study report): Ammonium bromide - Physical state: White crystalline solid - Analytical purity: 99.94% - Lot/batch No.: 980060 - Storage condition of test material: In the dark at ambient temperature
Study design	**Administration / exposure** **Route of administration:** oral: gavage **Vehicle:** water **Details on exposure:** PREPARATION OF DOSING SOLUTIONS: The test material was formulated as a solution in water for irrigation. Fresh suspensions were prepared daily on the day prior to use. For all formulations containing test material, the requisite quantity of test material was weighed, the necessary volume of vehicle added and the formulation mixed by manual inversion VEHICLE - Concentration in vehicle: 10 mg/mL, 30 mg/mL and 100 mg/mL - Amount of vehicle (if gavage): 10 mL/kg bw/day **Details on analytical verification of doses or concentrations:** Analysis of 5 mL-samples of dosing formulations revealed differences from nominal concentrations of less than 2% for all dose levels of ammonium bromide, and low coefficients of variations indicated that the formulations were homogeneous **Duration of treatment / exposure:** Mating procedure: 3 consecutive days

Duration of exposure: Days 6-19 inclusive of gestation (Day 0 being the day of detecting mating)
On Day 20 Caesarean Section was performed.

Frequency of treatment: once daily

Duration of test: 20 days

Doses / concentrations:

Doses / Concentrations:
100 mg/kg bw/day
300 mg/kg bw/day
1000 mg/kg bw/day

Basis: actual ingested

No. of animals per sex per dose: 24 females/group

Control animals: yes, concurrent vehicle

Maternal examinations:

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: Daily

BODY WEIGHT: Yes
- Time schedule for examinations: Individual bodyweights were recorded on Days 4 and 6-20 of gestation. Only those recorded on Days 4, 6, 9, 12, 15, 17 and 20 of gestation are presented in the report

FOOD CONSUMPTION: Yes
The weight of food consumed by each animal was recorded daily, commencing on Day 4 of gestation

WATER CONSUMPTION: No

POST-MORTEM EXAMINATIONS: No
- Sacrifice on gestation day 20
During necropsy of the first batch of animals, large variation in the size of placentae between dams was noticed. Therefore, for the necropsy of the second and third batch of rats, the placental weights were taken, where appropriate, for each animal. These data are not included in the report, but are maintained in the study files

Ovaries and uterine content:

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes

Fetal examinations:

- External examinations: Yes: all per litter
- Soft tissue examinations: Yes: half per litter
- Skeletal examinations: Yes: half per litter
- Head examinations: No data

Statistics:

Litter mean foetal weight data (sexes combined) were analyzed using the Kruskal-Wallis ANOVA. Maternal bodyweight gain over Days 6-12 and 6-20 of gestation, and food consumption over Days 7-20 of gestation were analysed by analysis of variance. Where significant heterogeneity occurred, log and/or square root transformations were applied but variations remained heterogeneous, and so the Kruskal-Wallis ANOVA was used.

Historical control data: No data

Findings

Results and discussion

Maternal developmental toxicity

Details on maternal toxic effects:

Treatment related clinical observations were noted for all animals treated at 1000 mg/kg bw/day and included rolling gait, animal limp when handled, hunched posture, subdued behaviour, piloerection, eyes dark and abnormal respiration indicative for neurological effects. These signs were noted following one day of treatment and generally persisted throughout the treatment period. One animal of this dosage group was sacrificed on Day 10 of gestation due to the severity of these signs. One animal of the medium dose group (300 mg/kg bw/day) showed piloerection on Days 8-12 of gestation, however in the absence of any of the other treatment related findings noted for the high dose group (1000 mg/kg bw/day) this was not considered to be directly related to treatment. Other occasional clinical signs such as hair loss and staining were considered to be unrelated to treatment. No clinical signs of toxicity were noted in the 100 mg/kg bw/day dose group. The group mean bodyweight gain over the dosing period was significantly reduced at 1000 mg/kg bw/day (-18%) when compared with the control group. This reduction was a consequence of the reduced body weight gain during the first six days of treatment (Days 6-12 of gestation). There was a notable weight loss for two of the animals of this dosage group during this period. Thereafter, bodyweight gain for animals treated at 1000 mg/kg bw/day was similar to control. Bodyweight performance at 300 and 100 mg/kg bw/day was essentially similar to control. The slightly but significantly increased weight over Days 6-20 was attributed to the increased mean uterus weight at that level. Slight differences in the group mean food consumption of the high dose group during the first half of the treatment, particularly around Days 10-13 of gestation, were considered to be related to the low food consumption of two of the animals for which a weight loss during the first six days of treatment was noted. Food consumption of the medium and low dose group was slightly but significantly greater than control, 107% and 105% of control values, respectively, when comparing food consumption from Days 7-20 in total (467, 457 and 435 g for medium, low and control group, respectively). These differences were considered too small to be positively attributed to treatment. At necropsy for one animal of the high dose group uterus filled with red fluid was noted. Other occasional maternal necropsy findings noted for the remaining animals were considered to be unrelated to treatment. There was no evidence of any effect of treatment with ammonium bromide on the pregnancies, in terms of intra-uterine mortality. The level of pre-implantation loss was higher than normally expected, with values of 24, 16, 26 and 21% for control, low, medium and high dose group, respectively. There was no indication of a treatment-related effect as it reflected events occurring prior to treatment. Parental toxicity might have been underestimated since effects on the endocrine system were not investigated. In the 3-generation reproductive study (NaBr Section 8.7.3 - Reproductive toxicity Van Leeuwen et al (1983)) maternal effects on the thyroid hormone was noted at 3800 ppm equivalent to 187 mg bromide/kg bw/day. Therefore, an effect at 300 mg/kg bw/d could be expected.

Dose descriptor: NOAEL: 300 mg/kg bw/day (actual dose received) for maternal toxicity

Dose descriptor: NOAEL: < 100 mg/kg bw/day (actual dose received) for developmental toxicity

Table 6.8.1/03-2: Group Mean Bodyweights, Bodyweight Gains and Food Consumption of Pregnant Female Rats Treated with Ammonium Bromide

Day of Gestation	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (100)	3 (300)	4 (1000)
0	222 ± 18	231 ± 25	223 ± 20	220 ± 14
4	234 ± 16	238 ± 21	234 ± 15	234 ± 14
6	249 ± 18	254 ± 20	250 ± 16	248 ± 14
9	264 ± 17	270 ± 22	267 ± 18	252 ± 18
12	284 ± 19	291 ± 24	287 ± 20	263 ± 21
15	303 ± 19	314 ± 25	307 ± 21	280 ± 20
17	326 ± 19	340 ± 26	328 ± 23	302 ± 21
18	357 ± 22	374 ± 29	365 ± 28	337 ± 28
Weight Gain Days 6-12	35 ± 5	38 ± 6	37 ± 7	15 ± 13***
% of Control	-	109	106	43
Weight Gain Days 6-20	108 ± 14	120 ± 16*	115 ± 20	89 ± 20***
% of Control	-	111	106	82
Weight Gain Days 6-20 Minus gravid uterus weight	52 ± 7	50 ± 6	59 ± 2	34 ± 3
% of Control	-	96	114	65
Food consumption Days 7-20	435	457**	467***	424
% of control	-	105	107	97

* significantly different from control, P<0.05
** significantly different from control, P<0.01
*** significantly different from control, P<0.001

Results (fetuses)

Details on embryotoxic / teratogenic effects:

Table 6.8.1/03-3: Pregnancy performance and foetal weight

Parameter investigated	Group (Dose level [mg Ammonium bromide/kg bw/day]) 1 (0)	2 (100)	3 (300)	4 (1000)
Number of animals mated	24	24	24	24
Number pregnant	22	22	22	23
Number of premature decedents	0	0	0	1
Number of decedents pregnant	0	0	0	1
Number pregnant at Day 20 necropsy	22	22	22	22
Pregnancy frequency [%]	92	92	92	96
Total corpora lutea graviditatis	287	305	288	287
Total number of implants	217	256	214	226
Pre-implantation loss [%]	24	16	26	21
Total live implants ([%])	191 (88)	247 (96)	188 (88)	208 (92)
Total dead implants ([%])	26 (12)	9 (4)	26 (12)	18 (8)
Total early embryonic deaths ([%])	21 (10)	5 (2)	22 (10)	17 (8)
Total late embryonic deaths ([%])	5 (2)	3 (1)	4 (2)	1 (0.4)
Total foetal deaths([%])	0	1 (0.4)	0	0
Mean corpora lutea graviditatis	13.0 ± 3.3	13.9 ± 3.3	13.1 ± 2.4	13.0± 3.8
Mean implants	9.9 ± 4.0	11.6 ± 3.6	9.7 ± 3.7	10.3 ± 4.2
Mean live implants	8.7 ± 36	11.2 ± 4.0	8.5 ± 4.0	9.5 ± 4.5
Mean dead implants	1.2 ± 2.2	0.4 ± 0.9	1.2 ± 1.9	0.8 ± 1.1
Mean early embryonic deaths	1.0 ± 2.0	0.2 ± 0.5	1.0 ± 1.9	0.8 ± 1.0
Mean late embryonic deaths	0.2 ± 0.9	0.1 ± 0.4	0.2 ± 0.5	0.05 ± 0.2
Mean foetal deaths	0	0.05 ± 0.2	0	0
Total live male foetuses ([%])	86 (45)	129 (52)	103 (55)	115 (55)
Total live female foetuses ([%])	105 (55)	118 (48)	85 (45)	93 (45)
Live foetal sex ratio [male:female]	1:1.21	1:0.94	1:0.83	1:0.81
Mean total uterus weight [g]	56 ± 21	70 ± 22	56 ± 22	55 ± 23
Mean litter mean foetal weight [g]:				
Males and females combined	3.92 ± 0.63	3.63 ± 0.65	3.7 ± 0.46	3.35 ± 0.4*
Males only	4.06 ± 0.14	3.69 ± 0.1	3.77 ± 0.13	3.36 ± 0.13
Females only	3.7 ± 0.1	3.61 ± 0.13	3.61 ± 0.14	3.31 ± 0.06

* Significantly different from control, p<0.001

Major foetal abnormalities:

Significantly reduced mean foetal weight at 1000 mg/kg bw/day (P<0.001; -14.5%) was noted when compared with control. At this dose level, 26 foetuses from 7 litters (out of 208 foetuses from 22 litters investigated) had abnormalities of the left kidney (absent/small/displaced/cystic); one of these foetuses also had an absent right kidney and right ureter. In 20 of these 26 foetuses the left ureter and/or left adrenal was also absent. Some of the affected foetuses also had narrowing of the left uterine horn (19 foetuses from 7 litters), and/or flattened/small spleen (18 foetuses from 6 litters). There was a dose-related increase in the incidence of foetuses with kinked ribs, with foetal incidence of 25% in the high dose group compared to 1.6% in controls and 4.5% and 9% in low and medium dose groups, respectively. The litter incidence was 82% in high dose group compared to 14% in controls and 32% in low and 41% in medium dose, respectively. Kinked ribs were often associated with incomplete ossification of ribs (foetal incidence of 16% in high dose and 0% in control group). At the high dose level, there was an increase in the number of foetuses with slightly kinked ribs (foetal incidence of 4.3% compared to 1.1% in controls) and curved scapula was observed in 18 foetuses from 8 litters (foetal incidence of 8.7% compared to 0.5% in control group). The latter finding generally occurred in foetuses with kinked ribs. Three foetuses from one litter had a short tail, but since this occurred in one litter this may have been an incidental finding. Other major foetal abnormalities were considered to have been incidental. For one small foetus of the low dose level a reduced spleen was seen, but there were no associated abnormalities of the kidneys, adrenals, ureters or uterus. For this reason this spleen finding was considered not to be of toxicological significance. Minor foetal abnormalities:

The minor visceral abnormalities did not provide any obvious indication of an adverse effect of treatment. The incidences of foetuses with dilated ureter, dilated renal pelvis and displaced testis were considered to be within the expected background frequency, although the possibility that the incidence at 1000 mg/kg bw/day represented a marginal increase could not be entirely discounted: 4.4% from the high dose group exhibited dilated renal pelvis compared to 1.6% in control group. There were 6.3% foetuses with dilated ureter in high dose, 5.9% in medium dose, 3.2% in low dose and 3.1% in control group. Displaced testis were seen with an incidence of 9.6% in the high dose group (1000 mg/kg bw/day) compared to 1.6% in the controls and 4.5% and 8% in low and medium dose groups (100 and 300 mg/kg bw/day), respectively. There was a slight increase in the incidence of foetuses at 1000 mg/kg bw/day with reduction in size of the 13th rib(s). The incidence of findings at 100 and 300 mg/kg bw/day were not obviously associated with treatment.

Skeletal ossification:

The ossification parameters provided no clear indication of any effects on skeletal ossification Although parameters like skull bones, sacral vertebral arches and unossified 5th metacarpals indicated treatment related decrease in ossification, other parameters (lumbar centra, 2nd and/or 4th metacarpal/metatarsal) showed no effect and some parameters indicated advanced ossification (anterior arch of atlas, cervical vertebral centra). Taking all these parameters into consideration, it was not possible to state that skeletal ossification had been affected by treatment with ammonium bromide.

Table A6.8.1/03-4: Group Incidence of Major Foetal Abnormalities

Abnormality	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (100)	3 (300)	4 (1000)
	Incidence of Foetuses (Litters)			
Total numbers examined	**5 (5)**	**17 (11)**	**23 (11)**	**85 (20)**
Number with major abnormality	**191 (22)**	**247 (22)**	**188 (22)**	**208 (22)**
Domed cranium/hydroencephaly/small foetus	0	1 (1)	0	0
Partially closed nasopharyngeal tract	0	0	0	1 (1)
Curved scapula	1 (0)	2 (2)	2 (2)	18 (8)
Incomplete ossification of scapula	0	1 (1)	0	0
Irregular ossification of clavicle	0	0	1 (1)	1 (1)
Interventricular septal defect	0	1 (1)	1 (1)	1 (1)
Partially duplicated inferior vena cava	0	1 (1)	0	0
Umbilical hernia	0	0	0	1 (1)
Kinked rib(s)	3 (3)	11 (7)	17 (9)	52 (18)
Slightly kinked rib(s)	2 (2)	2 (1)	4 (4)	9 (8)
Incomplete ossification of rib(s)	0	5 (3)	17 (8)	34 (14)
Left kidney absent/small/displaced, with/without absent left adrenal and absent left ureter	0	0	0	26 (7)
Cystic left kidney	0	0	0	1 (1)
Spleen flattened and/or reduced in size	0	1 (1)	0	19 (7)
Narrow left uterine horn with flattened ovarian end and displaced from ovary	0	0	0	14 (5)
Right kidney absent and absent right ureter	0	0	0	1 (1)
Short tail with/without thickening	0	0	0	3 (1)

Table A6.8.1/03-5: Group Incidence of Minor Foetal Abnormalities

Abnormality	Group (Dose Level 8mg/kg bw/day]) 1 (0)	2 (100)	3 (300)	4 (1000)
	Incidence of Foetuses (Litters)			
Total number examined (visceral)	**191 (22)**	**247 (22)**	**188 (22)**	**208 (22)**
Number with visceral abnormality	**58 (21)**	**79 (19)**	**72 (20)**	**97 (20)**
VISCERAL				
Haemorrhage affecting brain ventricles/surrounding tissue	1 (1)	3 (2)	3 (3)	9 (7)
Dilated brain ventricles	0	0	1 (1)	1 (1)
Domed cranium	0	0	0	1 (1)
Small eye(s)	1 (1)	1 (1)	2 (2)	3 (2)
Haemorrhage affecting eye(s)	1 (1)	1 (1)	0	1 (1)
Reduced/absent thyroid	1 (1)	1 (1)	0	8 (6)
Cervical remnant of thymus	20 (11)	15 (8)	16 (19)	5 (4)
Absent innominate artery	0	1 (1)	0	2 (1)
Small interventricular septal defect	0	1 (1)	0	3 (3)
Haemorrhage dorsal fat pad	0	1 (1)	1 (1)	1 (1)
Additiona lobe of liver within median cleft	1 (1)	0	0	1 (1)
Protrusion of median liver lobe with thinning of diaphragm	4 (4)	4 (2)	9 (5)	4 (4)
Minimal protrusion of median liver lobe with/without thinning of diaphragm	4 (4)	9 (6)	3 (3)	7 (5)
Hepatic haemorrhage	0	0	0	1 (1)
Enlarged spleen	0	0	0	1 (1)
Intra-abdominal haemorrhage	3 (2)	3 (3)	0	2 (2)
Dilated renal pelvis	3 (3)	0	2 (2)	9 (7)
Dilated ureter	6 (6)	8 (7)	11 (4)	13 (10)
Displaced testis	3 (3)	11 (7)	15 (10)	20 (10)
Left umbilical artery	0	2 (2)	0	4 (3)
Oedema on tail	0	0	0	1 (1)
Subcutaneous haemorrhage affecting:				
Cranium	13 (6)	25 (6)	6 (5)	24 (11)
Trunk	5 (4)	8 (3)	2 (2)	21 (11)
Limbs	1 (1)	3 (2)	0	11 (5)
Large foetus	4 (2)	0	0	0
Small foetus	4 (2)	16 (5)	16 (3)	51 (8)
Total number examined (skeleton)	**94 (21)**	**124 (22)**	**94 (21)**	**103 (21)**
Number with skeletal abnormality	**0**	**2 (2)**	**4 (3)**	**7 (5)**
SKELETAL				
Cervical rib	0	1 (1)	1 (1)	4 (2)
Additional area of ossification arising from 6th sternebra	0	1 (1)	2 (1)	0
Asymmetric alignment of costral cartilage elements	0	0	1 (1)	0
Asymmetric alignment of pelvic bones	0	0	1 (1)	0
Slight upward pelvic shift	0	0	0	3 (3)
NUMBER OF RIBS				
Vestigial rib(s) on 13th thoracic vertebra	0	0	1 (1)	1 (1)
Reduced rib(s) on 13th thoracic vertebra	0	0	2 (2)	7 (6)
13 complete ribs	79 (15)	119 (22)	89 (21)	94 (21)
Reduced supernumerary rib(s) on 14th thoracic vertebra	15 (11)	5 (3)	2 (2)	1 (1)

Table A6.8.1/03-6: Group Incidence of Skeletal Ossification Parameters

Abnormality	Group (Dose Level 8mg/kg bw/day]) 1 (0)	2 (100)	3 (300)	4 (1000)
	Incidence of Foetuses (Litters)			
Total Number examined	**94 (21)**	**124 (22)**	**94 (21)**	**103 (21)**
Incomplete ossification affecting:				
≥ 4 skull bones	5 (5)	21 (9)	29 (12)	52 (16)
≤ 3 skull bones	26 (13)	50 (21)	44 (19)	19 (13)
Cervical vertebral arch(es)	4 (3)	7 (4)	11 (9)	16 (10)
Thoracic centrum(a)	8 (7)	12 (9)	14 (9)	31 (18)
Thoracic vertebral arches	0	0	3 (2)	11 (9)
Pubis/es	3 (2)	27 (10)	17 (9)	17 (8)
Ischium/a	0	7 (4)	10 (6)	10 (6)
Lumbar vertebral arches	0	3 (2)	9 (6)	36 (14)
Lumbar centrum(a)	1 (1)	1 (1)	1 (1)	4 (3)
Sacral vertebral arch(es)	12 (6)	37 (15)	41 (17)	31 (13)
2nd and/or 4th metacarpal(s)/metatarsal(s)	1 (1)	17 (9)	33 (16)	34 (14)
Unossified:				
5th metacarpal(s)	40 (16)	84 (20)	79 (21)	95 (20)
5th metatarsal(s)	4 (3)	14 (7)	12 (9)	18 (10)
2nd and 4th metacarpal(s)/metatarsal(s)	0	2 (2)	3 (3)	2 (2)
Ossified:				
Anterior arch of atlas ossified	38 (13)	55 (17)	45 (19)	55 (16)
< 2 cervical vertebral centra ossified	6 (3)	33 (13)	36 (13)	45 (17)
Phalangeal elements ossified	7 (4)	3 (2)	0	0
Mean number of caudal centra ossified	3.8	3.5	3.6	3.6
Number of Sternebrae incompletely ossified:				
0	44 (16)	40 (13)	15 (6)	10 (8)
1	25 (15)	38 (14)	36 (14)	26 (12)
2	21 (19)	35 (15)	30 (11)	46 (17)
3	2 (2)	4 (4)	5 (4)	8 (6)
>3	1 (1)	7 (4)	8 (6)	13 (8)

Executive summary:

Ammonium bromide was tested in rats to detect effects of pregnancy when maternal exposure to the test material was confined to the period of major organogenesis. The study was performed in accordance with OECD guideline 414 and OPPTS 870.3700.

Mated rats were randomized into 4 treatment groups, each containing 24 animals. Rats were orally dosed by gavage daily over Day 6-19 inclusive of gestation, where Day 0 was the day of detecting mating. Ammonium bromide dose levels were 0, 100, 300 and 1000 mg/kg/day which were administered in water for irrigation as the vehicle at a constant volume of 10 mL/kg bw. Animals were monitored for clinical signs of toxicity, body weight and food consumption. The study was terminated on Day 20 of gestation. Status of each implantation was recorded and viable foetuses were examined. Half the foetuses from each uterus were fixed in methylated ethyl alcohol and examined by open dissection for abnormalities of the thoracic and abdominal viscera; kidneys and heart were cut and skeletons stained. These preparations were examined for the presence of skeletal abnormalities and for the extent of ossification. The other half of the foetuses were fixed in Bouin´s fluid for detailed analysis of soft tissue abnormalities using a freehand sectioning technique.

Maternal toxicity at 1000 mg/kg bw/day included abnormal clinical observations such as rolling gait, animal limp when handled, piloerection, subdued behaviour, hunched posture, eyes dark and abnormal respiration which were generally noted throughout the treatment period. These effects are consistent with those already described by other research groups after treatment with ammonium bromide. A reduction in bodyweight gain and slightly reduced mean food consumption was noted at 1000 mg/kg bw/day throughout the first 6 days of treatment.

There were no maternal effects at dose levels of 100 and 300 mg/kg bw/day.

Intra-uterine mortality was unaffected at all dose levels tested, but there was a statistically significant reduction in mean foetal weight (with $P<0.001$) at 1000 mg/kg bw/day.

Foetal abnormalities at 1000 mg/kg bw/day included reduced/absent left kidney: 26 from 208 investigated

foetuses, corresponding to a foetal incidence of 12.5% and a litter incidence of 31.8% (7 from 22 litters investigated) compared to a foetal incidence of 0% in controls. Kidney abnormalities were often associated with absence of the left adrenal and/or left ureter. 6.7% of foetuses treated with 1000 mg/kg bw/day also had narrowing of the left uterine horn and 9% showed abnormalities of the spleen. There was a slight increase in the foetuses with reduction in size of the 13thribs (6.8% in high dose group compared to 0% in controls).

A dose-related increase in the incidence of foetuses with kinked ribs was observed; foetal incidence of 1.6% in control, 4.5% in low dose, 9% in medium dose and 25% in high dose group animals were reported. The litter incidence of this abnormality was 14%, 32%, 41% and 82% for control, low, medium and high dose group, respectively. Kinked ribs were often associated with the appearance of incomplete ossification of ribs (foetal incidence of 0, 2, 9 and 16% for control, low, medium and high dose group, respectively). Treatment with 1000 mg/kg bw/day resulted in an increased incidence of foetuses with slightly kinked ribs (4.3% compared to 1.1% in controls). Within this dose group, 8.7% of foetuses showed curved scapula (0.5% in controls), 9% had abnormalities in spleen and 6,7% narrow uterine horn (in each case control values were 0%). Curved scapula generally appeared in animals which had also kinked ribs.

Kinked ribs, or wavy ribs, have been reported in rats by many researchers. Although kinked ribs have been classified as major abnormalities in this investigation, many researchers classify them as foetal variations. These findings have been reviewed by Kast (1994) who indicated that wavy ribs are often combined with fexures of limb bones, including the scapula. Wavy ribs are caused by numerous compounds with a large variety in their chemical structure and biological activity, and several modes of aetiopathology are discussed. Kast concluded that the wavy ribs developed in late gestation, and would have completely reversed within three weeks after birth, and therefore should be considered as a reversible pathological finding.

In this study, clinical signs (neurotoxic effects) and reduced bodyweight gain (18%) were noted in dams at 1000 mg/kg bw/day. Clinical signs (piloerection) were also noted in dams at 300 mg/kg bw/day. The clinical signs noted at the dose level of 1000 mg/kg bw/day consisted of rolling gait, animal limp when handled, hunched posture, subdued behaviour, piloerection, eyes dark and abnormal respiration. One animal of this dosage group was sacrificed on Day 10 of gestation due to the severity of these signs. Foetal effects were noted at all dose levels. There was a dose related increase in the incidence of foetuses with kinked ribs (4.5%, 9% and 25% after treatment with 100, 300 and 1000 mg/kg bw/day, respectively compared to 1.6% in controls). This effect was often associated with incomplete ossification of ribs (2%, 9% and 16% after treatment with 100, 300 and 1000 mg/kg bw/day, respectively compared to 0% in controls). A dose-related increased incidence of displaced testis was noted (4%, 8% and 10% after treatment with 100, 300 and 1000 mg/kg bw/day, respectively compared to 1.6% in controls). This finding, at least at low and mid dose, was within the background range of incidences for this strain of rats at the laboratory in question according to the study author. No historical control data is submitted to confirm this statement. At 1000 mg/kg bw/day, reduced mean foetal weight (15%), increased incidence of foetuses with slightly kinked ribs (4% compared to 1% in controls) and abnormalities (reduced/absent/displaced/cystic) of the left kidney, often associated with absence of the left adrenal and/or left ureter were noted (12.5% compared to 0% in controls). Some of the affected foetuses of this dose group also had narrowing of the left uterine horn (7% compared to 0% in controls) and flattened/small spleen (9% compared to 0% in controls). Moreover, increased incidence of reduced/absent thyroid was noted at 1000 mg/kg bw/day (3.8% compared to 0.5% in controls), and there was also an increased incidence of small foetus noted at this dose level (24% compared to 2% in controls). Also at 1000 mg/kg bw/day curved scapula (8.7% compared to 0.5% in controls) was observed, this finding generally occurred in foetuses with kinked ribs, and there was a slight increase in the incidence of foetuses with reduction in size of the 13th ribs (6.8% compared to 0% in controls). NOEL for maternal toxicity was determined at 100 mg/kg bw/day. NOAEL for maternal toxicity was determined at 300 mg/kg bw/day based on clinical signs of neurotoxicity and reduced bodyweight gain noted at 1000 mg/kg bw/day.

Parental toxicity might have been underestimated since effects on the endocrine system were not investigated. In the 3-generation reproductive study (NaBr Section 8.7.3 - Reproductive toxicity Van Leeuwen et al (1983)) maternal effects on the thyroid hormone was noted at 3800 ppm equivalent to 187 mg bromide/kg bw/day. Therefore, an effect at 300 mg/kg bw/d could be expected.

Signs of piloerection noted in dams at 300 mg/kg bw/day were in the absence of other effects not considered adverse. No NOEL/NOAEL was determined for developmental toxicity

Conclusion In this study, clinical signs (neurotoxic effects) and reduced bodyweight gain (18%) were noted in dams at

1000 mg/kg bw/day. Clinical signs (piloerection) were also noted in dams at 300 mg/kg bw/day. The clinical signs noted at the dose level of 1000 mg/kg bw/day consisted of rolling gait, animal limp when handled, hunched posture, subdued behaviour, piloerection, eyes dark and abnormal respiration. One animal of this dosage group was sacrificed on Day 10 of gestation due to the severity of these signs. Foetal effects were noted at all dose levels. There was a dose related increase in the incidence of foetuses with kinked ribs (4.5%, 9% and 25% after treatment with 100, 300 and 1000 mg/kg bw/day, respectively compared to 1.6% in controls). This effect was often associated with incomplete ossification of ribs (2%, 9% and 16% after treatment with 100, 300 and 1000 mg/kg bw/day, respectively compared to 0% in controls). A dose-related increased incidence of displaced testis was noted (4%, 8% and 10% after treatment with 100, 300 and 1000 mg/kg bw/day, respectively compared to 1.6% in controls). This finding, at least at low and mid dose, was within the background range of incidences for this strain of rats at the laboratory in question according to the study author. No historical control data is submitted to confirm this statement. At 1000 mg/kg bw/day, reduced mean foetal weight (15%), increased incidence of foetuses with slightly kinked ribs (4% compared to 1% in controls) and abnormalities (reduced/absent/displaced/cystic) of the left kidney, often associated with absence of the left adrenal and/or left ureter were noted (12.5% compared to 0% in controls). Some of the affected foetuses of this dose group also had narrowing of the left uterine horn (7% compared to 0% in controls) and flattened/small spleen (9% compared to 0% in controls). Moreover, increased incidence of reduced/absent thyroid was noted at 1000 mg/kg bw/day (3.8% compared to 0.5% in controls), and there was also an increased incidence of small foetus noted at this dose level (24% compared to 2% in controls). Also at 1000 mg/kg bw/day curved scapula (8.7% compared to 0.5% in controls) was observed, this finding generally occurred in foetuses with kinked ribs, and there was a slight increase in the incidence of foetuses with reduction in size of the 13th ribs (6.8% compared to 0% in controls). NOEL for maternal toxicity was determined at 100 mg/kg bw/day. NOAEL for maternal toxicity was determined at 300 mg/kg bw/day based on clinical signs of neurotoxicity and reduced bodyweight gain noted at 1000 mg/kg bw/day. Signs of piloerection noted in dams at 300 mg/kg bw/day were in the absence of other effects not considered adverse. No NOEL/NOAEL was determined for developmental toxicity

3.7.1.13 [Study 13] Pre-natal developmental toxicity study (OECD TG 414) of ammonium bromide in rat

Reference

A.6.8.1/04, Doc. No. 551-002

Study report, 2007

Guideline

OECD Guideline 414 (Prenatal Developmental Toxicity Study)

EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)

GLP compliance: yes

Reliability

1 (reliable without restriction). GLP Guideline study

Species / strain

Species: rat

Strain: Sprague-Dawley

Test material

Details on test material:

- Name of test material (as cited in study report): Ammonium bromide (CAS 12124-97-9)
- Physical state: White granular powder
- Analytical purity: 99.98%
- Lot/batch No.: 610060177
- Expiration date of the lot/batch: 2008-06-30
- Stability under test conditions: Formulations were used within the stability period of eight days
- Storage condition of test material: ambient temperature in the dark.

Study design

The objectives of this study were to indicate whether doses of 600 and 800 mg/kg/day were unequivocally maternally toxic and whether those doses produced the irreversible foetal malformations that were seen at 1000 mg/kg/day in the previous developmental toxicity study in rats. This study was also designed to demonstrate whether or not the kinked ribs observed at 300 mg/kg day were no longer present at weaning, and to attempt to identify a No Observed Effect Level.

In addition to the requirements of OECD guideline 414 two recovery groups were included in the study. Animals of these additional groups were allowed to litter and rear newborns until weaning.

Administration / exposure

Route of administration: oral: gavage

Vehicle: water

Details on exposure:

PREPARATION OF DOSING SOLUTIONS:
Formulations of each dose level were prepared independently. The required weight of test substance was weighed, and the appropriate volume of vehicle (water for irrigation) added. Solutions were mixed until visibly homogeneous.

VEHICLE
- Concentration in vehicle: 5 mg/mL, 30 mg/mL, 60 mg/mL and 80 mg/mL
- Amount of vehicle (if gavage): 10 mL/kg bw

Details on analytical verification of doses or concentrations:

Samples of dosing formulations were analyzed for concentration and homogeneity. The dosing solutions were analysed on 2 occasions, from those prepared for dosing on the first and second weeks of dosing. On each occasion, triplicate samples of approximately 1 mL were taken from each level containing the test item, and triplicate 2 mL samples from the Control.
Analysis of dosing formulations revealed concentrations within 2% of nominal and the low coefficients of variation (< 2%) indicated satisfactory homogeneity.

Details on mating procedure:

The objectives of this study were to indicate whether doses of 600 and 800 mg/kg/day were unequivocally maternally toxic and whether those doses produced the irreversible foetal malformations that were seen at 1000 mg/kg/day in the previous developmental toxicity study in rats. This study was also designed to demonstrate whether or not the kinked ribs observed at 300 mg/kg day were no longer present at weaning, and to attempt to identify a No Observed Effect Level.

Females were mated prior to acceptance onto the study. Mating procedure in accordance with OECD Guidelines for developmental toxicity. No more than 2 females used on the study were inseminated by any one male

Duration of treatment / exposure:

Mating period: 3 consecutive days

Duration of exposure: Days 6-19 of gestation

On Day 20 of gestation main study animals were sacrificed and Caesarean section was performed. Rats from the recovery group were allowed to litter; maternal animals and pups were killed for examination at weaning (Day 21 of lactation).

Frequency of treatment: Once daily

Duration of test: Main test: 20 days. Recovery groups: 41 days (The day on which parturition commenced was designated Day 0 of lactation)

Doses / concentrations

50 mg/kg bw/day
300 mg/kg bw/day
600 mg/kg bw/day
800 mg/kg bw/day

Basis: actual ingested

No. of animals per sex per dose: 22 females/group for both the main study and the additional two recovery groups.

Control animals: yes, concurrent vehicle

Details on study design:

- Dose selection rationale: The dose levels for Groups 4 and 5 are between the original Intermediate and High dose levels used in a previous developmental toxicity study, Irving and Hallmark (2000). The level for Group 3 was the original Intermediate dose level and was used again to allow comparisons between the 2 studies; it was also an appropriate level for the littering phase. The dose level for Group 2 is below the previous Low dose level, and was intended to be a No Observed Effect Level.

Examinations

Maternal examinations:

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: daily

BODY WEIGHT: Yes
- Time schedule for examinations: On Days 4 and 6-20 of gestation. Only those recorded on Days 4, 6, 12, 15, 17 and 20 are presented in this report.
In addition, for the animals that littered, maternal bodyweights were recorded on Days 1, 7, 14 and 21 of lactation (where the day of littering is Day 0 of lactation).

FOOD CONSUMPTION: Yes
- Time schedule for examinations: daily, commencing on Day 4 of gestation until Day 20 of gestation. For animals that littered, consumption was recorded over Days 0-7, 7-14 and 14-21 of lactation

WATER CONSUMPTION: No

POST-MORTEM EXAMINATIONS: Yes (females allowed to litter)
- Sacrifice on lactation day 21
- Organs examined: Dams were necropsied; external examination followed by macroscopic examination of the tissues and organs of cranial, thoracic and abdominal cavities in situ. Reproductive tracts were examined for signs of implantation; numbers of implantation sites were recorded.

OTHER: Observations on females with litters during lactation:
Females from the recovery groups (0 and 300 mg/kg bw/day) were allowed to litter normally. The day on which parturition commenced was designated Day 0 of lactation. The duration of gestation in days was evaluated. The number of live and dead pups born in each litter was recorded as soon as possible after completion of parturition on Day 0 of lactation.

Ovaries and uterine content:

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes

Fetal examinations:

- External examinations: Yes: all per litter
- Soft tissue examinations: Yes: half per litter
- Skeletal examinations: Yes: half per litter
- Head examinations: No data

OTHER:
In addition to the requirements of OECD guideline 414 two additional groups were included in the study. Animals were either treated daily over Days 6-19 of gestation with 300 mg ammonium bromide/kg bw/day or left untreated. These additional groups of rats were allowed to litter and rear newborns until weaning. Pups were necropsied and examined for abnormalities.

Pre-weanlings found dead or which had to be killed before Day 14 of lactation were sexed, checked for the presence of milk in the stomach and for any externally visible abnormalities. Pups were fixed for optional further examination. Weanlings at scheduled termination were necropsied, macroscopic examination of the tissues and organs of the thoracic and abdominal cavities in situ was performed. Pups were weighed prior to necropsy. Following examination, pups were fixed and stained for skeletal examination confined to the ribs and alignment of the pelvic girdle.

Statistics: None

Historical control data: No data

Results and discussion

Details on maternal toxic effects:

All animals receiving 600 or 800 mg/kg bw/day showed treatment-related clinical signs, which included staggering, rolling gait, subdued behaviour, slow/irregular respiration, body held low, hunched posture, piloerection, eyelids encrusted, stained eyes and stained fur. These signs are indicative for neurological effects. Other findings noted at these levels were considered to be either associated with the above findings or to have been incidental. One animal of the 600 mg/kg bw/day group was killed for humane reasons on Day 11 of gestation with signs that were similar but more marked than others at this level or at 800 mg/kg bw/day. Necropsy did not indicate a cause of death, but death was probably attributable to treatment. At 300 mg/kg bw/day one animal was subdued, with body held low on one occasion, and a second animal had rolling gait on one occasion. Although these signs were also observed at 600 and 800 mg/kg bw/day, the low incidence at 300 mg/kg bw/day could not be conclusively attributed to treatment. The other clinical signs observed at this level were considered to be incidental.
One animal of the recovery group that was treated with 300 mg/kg bw/day, was found dead on Day 1 of lactation. There were no clinical signs recorded prior to death, and it was considered that the death was incidental, possibly associated with parturition.

No clinical signs were observed after treatment with 50 mg/kg bw/day.

There were no necropsy findings at any dose level that were associated with treatment.

At 800 mg/kg bw/day, mean weight gain over Days 6-17 of gestation was lower than in control animals (-9.8%). Weight gain over Days 6-20 appeared similar to control when the comparison was made with those control animals sacrificed at Day 20 of gestation; however, when the weight gain was adjusted for gravid uterus weight or when compared with those control animals allowed to litter, it was considered that gain over Days 6-20 had been lower than control. At 600 mg/kg bw/day, mean gain over Days 6-20 of gestation was slightly greater than control (+ 14.8%), particularly after Day 15 of gestation; the difference became more noticeable when the gain over Day 6-20 was adjusted for gravid uterus weight. At 300 mg/kg bw/day, mean weight gain over Days 6-20 was slightly greater than control (+ 13.9%), including the gain after adjustment for gravid uterus weight. On Day 1 of lactation, recovery animals that had been treated at 300 mg/kg bw/day before, showed mean weight slightly greater than control, but on Days 7 and 14 of lactation, mean weights were similar to control rats. Over Days 14-21 of lactation, these animals had a slightly greater weight loss compared with control. In the 50 mg/kg bw/day dose group, mean bodyweight values were similar to control. In the 800 and 50 mg/kg bw/day dose groups, mean food consumption of dams was similar to control and in the 600 mg/kg bw/day dose group, mean food consumption was slightly greater than control. Animals that had been treated with 300 mg/kg bw/day during the gestation period consumed slightly more food than control during gestation, but showed slightly lower food consumption than control rats during lactation. Parental toxicity might have been underestimated since effects on the endocrine system were not investigated. In the 3-generation reproductive study (NaBr Section 8.7.3 - Reproductive toxicity Van Leeuwen et al (1983)) maternal effects on the thyroid hormone was noted at 3800 ppm equivalent to 187 mg bromide/kg bw/day. Therefore, an effect at 300 mg/kg bw/d could be expected.

Effect levels (maternal animals)

NOAEL: 300 mg/kg bw/day (actual dose received) for maternal toxicity

NOAEL: 50 mg/kg bw/day (actual dose received) for developmental toxicity

Table A6.8.1/04-1: Incidence of Clinical Observations and Necropsy Findings – Maternal Effects, Main Study

Observation/Finding	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (50)	3 (300)	4 (600)	5 (800)
Total number of females examined	22	22	22	22	22
Subdued	0	0	1	20	22
Rolling gait	1	0	0	22	22
Staggering	0	0	0	21	22
Body held low	0	0	1	16	21
Slow respiration	0	0	0	11	22
Irregular respiration	0	0	0	2	4
Piloerection	1	0	0	3	7
Hunched	0	0	0	9	12
Eyelid(s) encrusted	0	1	1	10	21
Eye(s) stained	0	0	0	0	10
Excess salivation	0	0	0	0	1
Staining on fur	0	0	0	0	5
Pale skin	1	0	0	0	1
Reddened skin	0	0	0	1	0
Hunched, subdued, limping	1	0	0	0	0
Nervous	0	0	0	1	0
Discharge from vagina	0	0	0	0	1
Prostrate; slow, wheezing respiration; discharge from eyes; excess salivation; subdued; killed prematurely	0	0	0	1	0
Swelling	1	0	0	1	0
Scabbing	0	1	0	0	0
Ear/tooth/hair damaged	0	0	0	1	2
Toe encrusted	0	0	0	1	0
Staining around nose	0	0	0	1	2
Thin	0	0	0	0	1
Uterus distended with brown fluid	1	0	0	0	0
Placentas fused	1	0	0	0	0

Table A6.8.1/04-2: Incidence of Clinical Observations and Necropsy Findings – Maternal Effects, Littering Phase

Observation/Finding	Group (Dose Level [mg/kg bw/day]) 1 (0)	3 (300)
Total number of females examined	22	22
Rolling gait	0	1
Piloerection	0	1
Eyelid(s) encrusted	1	1
Staining on fur	1	1
Toe encrusted	1	0
Discharge from vagina	0	1
Scabbing / bald areas / sparse hair	6	4
Tail bent/damaged	2	0
Died prematurely	0	1
Lungs dark and spongy	0	1

Table A6.8.1/04-3: Group Mean Bodyweight, Bodyweight Gain and Food Consumption – Main Study

Day of Gestation	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (50)	3 (300)	4 (600)	5 (800)
0	234 ± 15	231 ± 14	231 ± 15	231 ± 12	237 ± 18
4	241 ± 18	235 ± 16	237 ± 17	235 ± 13	246 ± 22
6	255 ± 20	249 ± 17	251 ± 18	248 ± 14	258 ± 24
9	270 ± 21	265 ± 18	270 ± 18	268 ± 17	270 ± 22
12	292 ± 22	286 ± 19	289 ± 19	286 ± 19	279 ± 23
15	313 ± 23	306 ± 22	312 ± 20	307 ± 20	300 ± 25
17	335 ± 29	330 ± 25	337 ± 22	334 ± 23	327 ± 27
20	370 ± 36	370 ± 29	382 ± 26	380 ± 30	374 ± 31
Weight Gain Days 6-20	116 ± 20	120 ± 17	132 ± 15	132 ± 20	116 ± 19
% of control	-	104	114	114	101
Corrected Weight Gain Days 6-20	46	49	51	59	42
Minus gravid uterus weight					
% of control	-	107	111	128	91
Mean Food Consumption Days 7-20	392	387	420	418	395
% of control	-	99	107	107	101

Table A6.8.1/04-5: Group Mean Bodyweight, Bodyweight Gain and Food Consumption – Littering Phase

	Group (Dose Level [mg/kg bw/day]) 1 (0)	3 (300)
Day of Gestation – Bodyweights		
0	240 ± 22	241 ± 12
4	245 ± 22	244 ± 14
6	257 ± 23	255 ± 16
9	273 ± 22	275 ± 17
12	294 ± 22	296 ± 18
15	318 ± 23	317 ± 19
17	340 ± 25	343 ± 23
20	379 ± 27	384 ± 29
Weight Gain Days 6-20	122 ± 12	129 ± 18
% of control	-	106
Mean Food Consumption Days 7-20	400	423
% of control	-	106
Day of Lactation – Bodyweights		
1	272 ± 26	279 ± 23
7	324 ± 25	321 ± 23
14	341 ± 14	340 ± 25
21	332 ± 20	321 ± 18
Mean Weight Gain	60	42
% of control	-	70
Mean Food Consumption	201	188
% of control	-	94

Results (fetuses)

Details on embryotoxic / teratogenic effects:

Details on embryotoxic / teratogenic effects:
At 300, 600 and 800 mg/kg bw/day, there was a increased incidence of foetuses with kinked ribs, with the incidence being similar in all three groups. There was also an increase at these levels of foetuses with the minor abnormalities of slightly kinked and incompletely ossified ribs. Additionally, 14 foetuses at 800 mg/kg bw/day, and 5 foetuses each at 300 and 600 mg/kg bw/day had curved scapulae of which one foetus at 800 mg/kg bw/day also had incomplete ossified scapula.
At 600 and 800 mg/kg bw/day, there were increased numbers of foetuses with fewer than 13 complete ribs. At 300 mg/kg bw/day, the incidence of foetuses with fewer than 13 complete ribs was considered too small to be attributed to treatment.

Incidences of other major and minor abnormalities at 300, 600 and 800 mg/kg bw/day were considered to be essentially similar to controls.

There was no indication of any treatment related effect on the incidence of foetal abnormalities at 50 mg/kg bw/day.

At 300, 600 and 800 mg/kg bw/day, there was an increased incidence of foetuses with many parameters that tend to indicate delayed ossification of skull bones and lumbar vertebral arches, unossified 5th metacarpals and the number of sternebrae retarded. However, there was also an increase in ossification of cervical vertebral centra, a parameter that tends to indicate advanced ossification. Other parameters such as ossified anterior arch of atlas (increased at 300 and 800 mg/kg bw/day but not at 600 mg/kg bw/day) and incomplete ossification of pubes (increased at 300 and 600 mg/kg bw/day but not at 800 mg/kg bw/day) showed no clear relationship to dose. Taking the parameters together, it was considered that there had been some effect on foetal ossification at these dose levels.
Animals treated with 50 mg/kg bw/day showed skeletal ossification parameters similar to control.

Pregnancy data
There were no obvious effects on embryo-foetal mortality at any level tested. Slight intergroup differences in pregnancy performance and foetal weight were considered too small to be attributed to treatment.
Females that were allowed to litter and had been treated with 300 mg/kg bw/day showed a mean duration of gestation less than control, with a higher proportion of females having a 21 day gestation period, compared to controls.

Littering phase:
Mean values for litter size and survival at 300 mg/kg bw/day were similar to control. There was no indication of a treatment-related effect on maternal care or feeding. Mean pup weights were slightly lower in pups from treated rats compared with controls, particularly on Day 21 of lactation and more noticeably among males. Mean litter weights at 300 mg/kg bw/day were also lower than control. It was considered that these effects were related, to some extent, to the shorter duration of gestation after treatment.
Among weanlings, the incidences of abnormalities and variants at 300 mg/kg bw/day were similar to control. With the exception of one pup which had minimally kinked ribs, there were no pups with kinked ribs at 300 mg/kg bw/day. Other minor findings such as mild bruising, swelling or cold, that are typical for pre-weaning pups were observed but have not been reported.

Table A6.8.1/04-4: Pregnancy Performance and Foetal Weight, Main Study

Parameter investigated	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (50)	3 (300)	4 (600)	5 (800)
Number of animals mated	22	22	22	22	22
Premature decedents	0	0	0	1	0
Number pregnant at Day 20 necropsy	21	21	22	19	21
Total corpora lutea graviditatis	280	264	292	239	277
Total number of implants	261	256	285	232	271
Pre-implantation loss [%]	7	3	2	3	2
Total live implants ([%])	234 (90)	242 (95)	277 (97)	224 (97)	255 (94)
Total dead implants ([%])	27 (10)	14 (5)	8 (3)	8 (3)	16 (6)
Total early embryonic deaths ([%])	22 (8)	13 (5)	8 (3)	8 (3)	16 (6)
Total late embryonic deaths ([%])	4 (2)	1 (0.4)	0	0	0
Total dead foetuses ([%])	1 (0.4)	0	0	0	0
Mean corpora lutea graviditatis	13.3 ± 2.9	12.6 ± 2.1	13.3 ± 1.3	12.6 ± 3.1	13.2 ± 1.9
Mean implants	12.4 ± 2.3	12.2 ± 2.4	13.0 ± 1.3	12.2 ± 3.0	12.9 ± 2.2
Mean live implants	11.1 ± 3.3	11.5 ± 2.3	12.6 ± 1.5	11.8 ± 3.0	12.1 ± 2.2
Mean dead implants	1.3 ± 2.7	0.7 ± 1.1	0.4 ± 0.6	0.4 ± 0.5	0.8 ± 1.1
Mean early embryonic deaths	1.0 ± 2.6	0.6 ± 1.0	0.4 ± 0.6	0.4 ± 0.5	0.8 ± 1.1
Mean late embryonic deaths	0.2 ± 0.9	0.05 ± 0.2	0	0	0
Mean dead foetuses	0.05 ± 0.2	0	0	0	0
Total live male foetuses ([%])	113 (48)	126 (52)	130 (47)	115 (51)	130 (51)
Total live female foetuses ([%])	121 (52)	116 (48)	147 (53)	109 (49)	125 (49)
Live foetal sex ratio (male:female)	1:1.07	1:0.92	1:1.13	1:0.95	1:0.96
Mean to8tal uterus weight g]	69 ± 18	72 ± 13	80 ± 7	73 ± 16	74 ± 13
Mean litter mean foetal weight [g]:					
Males and females combined	3.62 ± 0.41	3.88 ± 0.3	3.87 ± 0.24	3.85 ± 0.39	3.7 ± 0.21
Males only	3.76 ± 0.44	3.95 ± 0.28	3.98 ± 0.22	3.95 ± 0.39	3.8 ± 0.22
Females only	3.5 ± 0.44	3.79 ± 0.33	3.76 ± 0.26	3.68 ± 0.3	3.6 ± 0.21

Table A6.8.1/04-6: Group Incidence of Major Foetal Abnormalities

Abnormality	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (50)	3 (300)	4 (600)	5 (800)
	Incidence of Foetuses (Litters)				
Eye open and reduced (in size), tongue protruding, subcutaneous cervical oedema, omphalocele, hindlimbs shortened and maltorated, fore and hind paw digit(s) shortened with claws absent, right hindpaw with no recognisable digits, tail shortened, anus not patent	0	0	0	1(1)	0
Scapula/ae curved	0	0	5(3)	5(3)	14(5)
Scapula/ae incompletely ossified	0	0	0	0	1(1)
Right subclavian artery retro-oesophageal	0	1(1)	0	0	0
Partially duplicated inferior vena cava	1(1)	0	0	1(1)	0
Kinked ribs	1(1)	0	15(9)	19(10)	17(10)
Right forepaw, left hindpaw 5th digits reduced (in size)	0	0	0	0	1(1)
Number with major abnormality	2(2)	1(1)	18(9)	22(12)	22(10)
Total number examined	234 (21)	242 (21)	277 (22)	224 (19)	255 (21)

Table A6.8.1/04-7: Group Incidence of Minor Foetal Abnormalities and Variants – Visceral

Abnormality/Variant	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (50)	3 (300)	4 (600)	5 (800)
	Incidence of Foetuses (Litters)				
Subcutaneous haemorrhage – head	4(3)	4(3)	2(2)	6(5)	3(2)
- trunk	0	2(2)	0	1(1)	1(1)
- limbs/tail	1(1)	0	1(1)	0	0
Haemorrhage orbital socket	0	0	0	0	1(1)
Minimal haemorrhage eye vitreous chamber	0	0	0	0	1(1)
Eye(s) oval (in shape)	1(1)	0	0	4(2)	1(1)
Thyroid(s) markedly reduced (in size)	0	0	0	1(1)	1(1)
Haemorrhage dorsal fat pad	1(1)	1(1)	0	0	0
Cervical remnant of thymus	6(6)	7(5)	2(2)	2(2)	4(3)
Innominate artery absent	2(1)	1(1)	0	0	1(1)
Small interventricular septal defect	0	0	0	1(1)	0
Tendinous region of diaphragm locally thinned with minimal protrusion of median liver lobe	2(2)	3(3)	5(3)	2(2)	1(1)
Tendinous region of diaphragm locally thinned with protrusion of median liver lobe	1(1)	2(1)	0	0	0
Hepatic haemorrhage	0	0	1(1)	0	1(1)
Additionaö liver lobe with median cleft	16(11)	11(10)	17(12)	9(5)	4(4)
Kidney minimally reduced (in size)	0	0	0	0	1(1)
Kidney(s) caudal displacement/minimal caudal displacement	0	1(1)	1(1)	2(2)	4(3)
Renal pelvis dilated	(1)	0	0	0	1(1)
Ureter(s) dilated	0	0	1(1)	0	0
Intra-abdominal haemorrhage	0	0	0	1(1)	0
Testis(es) not fully descended to pelvic position	3(2)	1(1)	1(1)	3(3)	5(4)
Testis(es) medially displaced	0	1(1)	1(1)	3(3)	4(4)
Umbilicial artery left sided	5(5)	4(3)	1(1)	7(5)	383)
Small foetus	1(1)	3(3)	0	0	0
Number with minor visceral abnormality/variant	38(14)	33(17)	33(17)	38(15)	34(15)
Number examined by Wilson sectioning	116(21)	121(21)	138(22)	112(19)	128(21)
Total number examined	234(21)	242(21)	277(22)	224(19)	255(21)

Table A6.8.1/04-8: Group Incidence of Minor Foetal Abnormalities and Variants – Skeletal

Parameter	Group (Dose Level [mg/kg bw/day]) 1 (0)	2 (50)	3 (300)	4 (600)	5 (800)
	Incidence of Foetuses (Litters)				
Cranial bone(s) additional ossified area(s)	0	0	0	2(1)	0
Cervical rib(s)	0	1(1)	1(1)	1(1)	3(3)
Additional ossified area arising from sternebra 6	0	1(1)	0	0	0
Rib(s) minimally kinked	7(5)	4(3)	35(11)	39(16)	46(17)
Rib(s) incompletely ossified	4(4)	0	27(11)	33(13)	30(15)
Rib costal cartilage(s) asymmetrically aligned at point of attachment to sternum	0	0	1(1)	1(1)	0
Unilateral/bilateral rib 7 costal cartilages not attached to sternum	1(1)	1(1)	2(2)	0	3(3)
Xiphoid cartilage bifurcated	0	0	0	1(1)	0
Thoracic vertebral centrum unossified	0	1(1)	0	0	0
Sacral vertebral centrum unossified	0	1(1)	0	0	0
Number with minor abnormality/variant	**9(6)**	**9(7)**	**38(12)**	**46(17)**	**49(18)**
Total number examined	**118(21)**	**121(21)**	**139(22)**	**112(19)**	**127(21)**
Number of ribs: 12 complete ribs	0	0	0	1(1)	1(1)
13^{th} vestigial rib(s)	0	0	3(3)	4(3)	19(10)
13^{th} reduced rib(s)	0	1(1)	1(1)	8(7)	10(7)
13 complete ribs	108(21)	112(21)	133(22)	97(18)	97(20)
Vestigial supernumerary rib(s) on 1^{st} lumbar vertebra	10(6)	8(5)	2(2)	2(2)	0
Incomplete ossification affecting:					
≥4 skull bones	14(5)	10(6)	51(18)	48(14)	52(19)
≤3 skull bones	27(12)	35(17)	44(19)	31(17)	30(17)
Cervical vertebral arch(es)	7(3)	3(3)	10(6)	9(8)	8(6)
Thoracic centrum(a)	13(8)	13(8)	11(7)	19(12)	25(14)
Thoracic vertebral arch(es)	0	0	3(2)	5(4)	8(6)
Lumbar centrum(a)	0	1(1)	0	0	0
Lumbar vertebral arch(es)	0	0	21(9)	34(14)	29(15)
Pubis(es)	7(3)	4(4)	36(14)	19(10)	8(7)
Ischium(a)	2(2)	1(1)	11(5)	10(6)	6(5)
Sacral vertebral arch(es)	21(7)	19(10)	69(19)	54(16)	48(19)
2^{nd} to 4^{th} metacarpal(s)	1(1)	1(1)	45(17)	41(14)	37(18)
5^{th} metatarsal(s)	2(2)	2(2)	5(4)	6(5)	5(3)
Unossified:					
5^{th} metacarpal(s)	43(13)	50(18)	97(22)	77(18)	92(20)
5^{th} metatarsal(s)	9(4)	1(1)	1(1)	3(2)	2(2)
Ossified:					
Anterior arch of atlas	43(13)	47(17)	77(20)	53(15)	85(21)
>2 cervical vertebral centra ossified	9(4)	21(7)	53(17)	61(17)	60(19)
One or more sacrocaudal vertebra with connection between centrum and arch(es)	31(13)	37(16)	55(18)	40(14)	49(17)
Phalangeal elements	6(4)	2(1)	0	1(1)	0
Mean number of caudal vertebral centra	3.9	4.0	4.6	4.3	4.2
Number of sternebrae retarded: 0	53(14)	50(17)	43(15)	43(15)	18(12)
1	32(15)	48(19)	50(21)	50(21)	45(19)
2	31(9)	21(11)	33(14)	33(14)	52(20)
>2	2(2)	3(2)	13(8)	13(8)	12(9)
Total number examined	234(21)	242(21)	277(22)	224(19)	255(21)

Table A6.8.1/04-9: Littering Phase – Duration of Gestation

Duration of gestation [days]	Group/Dose Level [mg(kg bw/day] 1 (0)	3 (300)
Number of pregnant	20	19
21	6	14
22	13	4
23	1	1
Mean duration	21.8	21.3

Table A6.8.1/04-10: Group Mean Litter and Pup weight

Day of Lactation	Group/Dose Level [mg(kg bw/day] 1 (0)	3 (300)
LITTER		
Day 1	83 ± 11	77 ± 17
Day 4	122 ± 17	111 ± 23
Day 7	181 ± 22	166 ± 33
Day 14	365 ± 33	339 ± 62
Day 21	591 ± 51	529 ± 93
Mean of Litter Mean Pup Weight		
MALES		
Day 1	6.8 ± 0.6	6.7 ± 0.8
Day 4	10.1 ± 1.3	9.7 ± 1.4
Day 7	15.0 ± 1.9	14.5 ± 1.9
Day 14	30.2 ± 3.7	29.5 ± 3.7
Day 21	48.7 ± 6.4	46.5 ± 7.0
FEMALES		
Day 1	6.5 ± 0.5	6.3 ± 0.8
Day 4	9.6 ± 1.1	9.5 ± 1.5
Day 7	14.3 ± 1.6	14.2 ± 2.3
Day 14	29.3 ± 3.2	29.1 ± 4.1
Day 21	47.4 ± 5.0	45.6 ± 7.1

Table A6.8.1/04-11: Group Incidence of Minor Abnormalities and Variants among Weanlings

Abnormality/Variant	Group/Dose Level [mg(kg bw/day] 1 (0)	3 (300)
	Incidence of Weanlings (Litters)	
Visceral:		
Testis(es) not fully descended to pelvic position	0	2(2)
Number with minor visceral abnormality/variant	0	2(2)
Total number examined	249(20)	213(18)
Skeletal:		
Rib(s) thickened area	1(1)	1(1)
Rib(s) minimally kinked	0	1(1)
Pelvic girdle bilateral caudal displacement	1(1)	0
Number of ribs:		
13 reduced ribs	1(1)	4(4)
13 complete ribs	245(20)	209(18)
14 vestigal ribs	3(3)	0
Number with minor skeletal abnormality/variant	2(2)	2(2)
Total number examined	249(20)	213(18)

Executive summary:

A developmental toxicity study was performed with ammonium bromide in rats, applying concentrations of 50-800 mg/kg bw/day of the substance by gavage during the period of organogenesis, to detect effects on pregnancy (in accordance with OECD guideline 414). The study was also designed to demonstrate whether or not the kinked ribs observed at 300 mg/kg bw/day in a previous study and also in this study, were no longer present and were, thus, reversible at weaning and to attempt to identify a NOAEL for embryotoxic/teratogenic effects.

Mated female rats were randomised into 4 test groups and one vehicle group. Two additional groups were assigned to control and 300 mg/kg bw/day groups to serve as recovery animals (littering phase). Rats were treated once daily over Days 6-19 of gestation, where the day of detection of mating was assigned Day 0.

Animals were monitored for clinical signs of toxicity, bodyweight and food consumption. Main study animals were killed on Day 20 of gestation and status of each implantation was recorded. Viable foetuses were examined for visceral and skeletal abnormalities, including the state of skeletal ossification. Animals from the recovery group were allowed to litter and rear their young to weaning. Pups were necropsied and skeletons stained and examined for abnormalities with particular emphasis on the changes seen during organogenesis

Maternal toxicity at 600 and 800 mg/kg bw/day was characterized by clinical signs of toxicity (including staggering, rolling gait, subdued behaviour, slow/irregular respiration, body held low, hunched posture and piloerection). One animal at 600 mg/kg bw/day was killed for humane reasons. Necropsy findings did not indicate a cause of death, but the death was probably attributable to treatment. Bodyweight gain at 800 mg/kg bw/day was lower than control, but gain at 600 mg/kg bw/day was slightly greater than control. Food consumption at 800 mg/kg bw/day was similar to control, but consumption at 600 mg/kg bw/day was slightly greater than control.

Maternal effects at 300 mg/kg bw/day during gestation were limited to a slightly greater bodyweight gain and slightly greater food consumption. Among females with litters, there was a slightly greater weight loss than control during the third week of lactation, and lower food consumption during lactation.

There were no indications of maternal toxicity at 50 mg/kg bw/day.

There were no obvious effects on embryo-foetal mortality or foetal weights at any level tested.

At 300, 600 and 800 mg/kg bw/day, there were increased incidences of foetuses with kinked and/or slightly kinked ribs (5.4%, 8.5% and 6.6% of rats showing kinked ribs after treatment with 300, 600 and 800 mg/kg bw/day, respectively, compared to 0.4% in controls), and of foetuses with curved scapulae (1.8%, 2.2% and 5.5% after treatment with 300, 600 and 800 mg/kg bw/day respectively, compared to 0% in controls). At 600 and 800 mg/kg bw/day, there were increased numbers of foetuses with fewer than 13 complete ribs. At 300, 600 and 800 mg/kg bw/day, there were indications of effects on foetal ossification, although some parameters tended to indicate delayed ossification whilst others would be expected to show advanced ossification. In a previous teratogenicity study with ammonium bromide (dose levels: 100, 300 and 1000 mg/kg bw/day), there was a similar pattern of findings amongst the ossification parameters and it was concluded that no statement can be made regarding the influence of ammonium bromide treatment on ossification parameters.

Among the females treated at 300 mg/kg bw/day that were allowed to litter, the period of gestation was somewhat less than in controls, with a mean duration of 21.3 days for treated rats compared to 21.8 days in control animals, and the mean pup weights were slightly lower suggesting a consequence of the shorter gestation. Litter size and survival were not obviously affected.

The most important finding is, that among weanlings from rats treated at 300 mg/kg bw/day that were allowed to litter, the incidences of abnormalities of the ribs and pelvic girdle were similar to controls. This indicates that the kinked ribs and curved scapulae seen in the foetuses from rats treated at the same dose level are reversible effects which resolve after birth and are, thus, considered to be a transient change only.

The present study was designed to supplement the information from the standard developmental toxicity study in rats performed previously (Section 8.7.2 – Developmental toxicity AmBr Irvine and Hallmark (2000) Key). In that study, a dose of 1000 mg/kg bw/day was associated with maternal toxicity (clinical signs and reduced weight gain) and irreversible foetal kidney malformations which were observed in 7 out of 9 females that had shown the lowest weight gains over the first days of dosing. In the present study, doses of 600 and 800 mg/kg bw/day showed unequivocal maternal toxicity but the irreversible foetal malformations were not seen. From the findings in these two studies, it was considered that the threshold dose for unequivocal maternal toxicity was lower than the threshold dose for the irreversible foetal malformations in soft tissue, because maternal toxicity was seen at dose levels where no adverse effects on soft tissue had been noted. The previous study also indicated an increased incidence of foetuses with kinked ribs at 100, 300 and 1000 mg/kg bw/day, and with curved scapulae at 1000 mg/kg bw/day. Based on a review by Kast (1994) it was considered that the kinked ribs and the curved scapulae would have completely reversed within 3 weeks after birth and should therefore be considered as a reversible pathological finding. In the present study examination of weanlings treated at 300 mg/kg/day (dosing discontinued after Day 19 of gestation) showed that the kinked ribs were no longer present at weaning and confirmed this hypothesis also for ammonium bromide that the kinked ribs and curved scapulae should be regarded as a reversible pathological finding.

In the previous report it was considered by the original author that there were no indications of maternal effects at 100 and 300 mg/kg/day which could be confirmed in the present study. A review of the data from the previously performed teratology study indicated a slightly increased weight gain over Days 6-20 of gestation at 300 mg/kg/day when the

effect of gravid uterine weight was included in the consideration which cannot be considered to be a toxicologically relevant nor adverse effect. However, a review of the data from that study indicated a slightly increased weight gain over Days 6-20 of gestation at 300 mg/kg/day when the effect of gravid uterine weight was included in the consideration.

Since the time of that study, a 13 week toxicity study and this current study have been conducted, and both of these studies have indicated a tendency for increased weight gain at lower doses (increased weight gain over Days 6-20 of gestation at 300 and 600 mg/kg bw/day in this study; increased weight gain over early weeks of treatment in females at 225 mg/kg/day in a 13-week study but a decreased weight gain at higher levels (decreased weight gain over Days 6-12 and 6-20 of gestation at 1000 mg/kg bw/day in the previous teratogenicity study). There has also been a tendency, at some dose levels, for an increased weight gain in the early part of the dosing period, then decreased weight gain in the latter part of the dosing period (increased weight gain over first week of treatment, followed by overall reduced weight gain in females at 750 mg/kg/day during a 13 week toxicity study).

The authors of the present study have set the maternal and foetal NOAEL at 50 mg/kg bw/day. However, the maternal effects seen at 300 mg/kg bw/day are increased weight gain and increased food consumption which usually are not determined as adverse or toxic effects. Therefore, the maternal NOAEL should be derived at 300 mg/kg bw/day. Parental toxicity might have been underestimated since effects on the endocrine system were not investigated. In the 3-generation reproductive study (NaBr Section 8.7.3 - Reproductive toxicity Van Leeuwen et al (1983)) maternal effects on the thyroid hormone was noted at 3800 ppm equivalent to 187 mg bromide/kg bw/day. Therefore, an effect at 300 mg/kg bw/d could be expected. Since for the foetus effects (e.g. kinked ribs, curved scapula) were detected after dams had been treated at 300 mg/kg bw/day, foetal NOAEL should be at 50 mg/kg bw/day for the period of organogenesis and at 300 mg/kg bw/day post-weaning.

In this study, clinical signs (neurotoxic effects) were noted in dams at 600 and 800 mg/kg bw/day. The clinical signs consisted of staggering, rolling gait, subdued behaviour, slow/irregular respiration, body held low, hunched posture and piloerection. One animal at 600 mg/kg bw/day was sacrificed due to the severity of these signs. Bodyweight gain at 800 mg/kg bw/day was reduced (9%) when compared to controls (no statisticaly analysis was performed). Bodyweight gain at 300 and 600 mg/kg bw/day was increased (11% and 28%) when compared to controls (no statistically analysis was performed). There were no obvious effects on embryo-foetal mortality or foetal weights at any dose level tested. At 300, 600 and 800 mg/kg bw/day, there were increased incidences of foetuses with kinked ribs (5.4%, 8.5% and 6.7% of rats showing kinked ribs after treatment with 300, 600 and 800 mg/kg bw/day, respectively, compared to 0.4% in controls), and of foetuses with curved scapulae (1.8%, 2.2% and 5.5% after treatment with 300, 600 and 800 mg/kg bw/day, respectively compared to 0% in controls). There was also an increase at these dose levels of foetuses with incompletely ossified ribs (19%, 29% and 24% after treatment with 300, 600 and 800 mg/kg bw/day, respectively compared to 3% in controls). At 600 and 800 mg/kg bw/day, there were indications of effects on foetal ossification although it was concluded that no statement could be made regarding the influence of ammonium bromide treatment on ossification parameters. At 600 and 800 mg/kg bw/day there were increased numbers of foetuses with fewer than 13 complete ribs (incidence 13 complete ribs was 87% and 76% for the 600 and 800 mg/kg bw/day group, respectively compared to 92% in controls). Among the females treated at 300 mg/kg bw/day that were allowed to litter, the period of gestation was somewhat less than in controls, with a mean duration of 21.3 days for treated rats compared to 21.8 days in control animals. Litter size and survival were not obviously affected. Incidences of abnormalities of the ribs and pelvic girdle for weanlings from these rats were similar to those seen in controls, which indicates that the kinked ribs and curved scapulae seen in the foetueses from rats treated at the same dose level are transient in nature and are reversible effects which resolve after birth. NOEL for maternal toxicity was determined at 300 mg/kg bw/day. NOAEL for maternal toxicity was determined at 300 mg/kg bw/day (corresponding to 246 mg bromide/kg bw/day) based on clinical signs of neurotoxicity noted at ≥600 mg/kg bw/day. Parental toxicity might have been underestimated since effects on the endocrine system were not investigated. In the 3-generation reproductive study (NaBr Section 8.7.3 - Reproductive toxicity Van Leeuwen et al (1983)) maternal effects on the thyroid hormone was noted at 3800 ppm equivalent to 187 mg bromide/kg bw/day. Therefore, an effect at 300 mg/kg bw/d could be expected.

NOEL for developmental toxicity was determined at 50 mg/kg bw/day. NOAEL for developmental toxicity was determined at 50 mg/kg bw/day (corresponding to 41 mg bromide/kg bw/day) based on abnormalities of the ribs and scapulae and effects on foetal ossification noted at ≥300 mg/kg bw/day.

Conclusions:

In this study, clinical signs (neurotoxic effects) were noted in dams at 600 and 800 mg/kg bw/day. The clinical signs consisted of staggering, rolling gait, subdued behaviour, slow/irregular respiration, body held low, hunched posture and piloerection. One animal at 600 mg/kg bw/day was sacrificed due to the severity of these signs. Bodyweight gain at 800 mg/kg bw/day was reduced (9%) when compared to controls (no statisticaly analysis was performed). Bodyweight gain at 300 and 600 mg/kg bw/day was increased (11% and 28%) when compared to controls (no statistically analysis was performed). There were no obvious effects on embryo-foetal mortality or foetal weights at any dose level tested. At 300,

600 and 800 mg/kg bw/day, there were increased incidences of foetuses with kinked ribs (5.4%, 8.5% and 6.7% of rats showing kinked ribs after treatment with 300, 600 and 800 mg/kg bw/day, respectively, compared to 0.4% in controls), and of foetuses with curved scapulae (1.8%, 2.2% and 5.5% after treatment with 300, 600 and 800 mg/kg bw/day, respectively compared to 0% in controls). There was also an increase at these dose levels of foetuses with incompletely ossified ribs (19%, 29% and 24% after treatment with 300, 600 and 800 mg/kg bw/day, respectively compared to 3% in controls). At 600 and 800 mg/kg bw/day, there were indications of effects on foetal ossification although it was concluded that no statement could be made regarding the influence of ammonium bromide treatment on ossification parameters. At 600 and 800 mg/kg bw/day there were increased numbers of foetuses with fewer than 13 complete ribs (incidence 13 complete ribs was 87% and 76% for the 600 and 800 mg/kg bw/day group, respectively compared to 92% in controls). Among the females treated at 300 mg/kg bw/day that were allowed to litter, the period of gestation was somewhat less than in controls, with a mean duration of 21.3 days for treated rats compared to 21.8 days in control animals. Litter size and survival were not obviously affected. Incidences of abnormalities of the ribs and pelvic girdle for weanlings from these rats were similar to those seen in controls, which indicates that the kinked ribs and curved scapulae seen in the foetueses from rats treated at the same dose level are transient in nature and are reversible effects which resolve after birth. NOEL for maternal toxicity was determined at 300 mg/kg bw/day. NOAEL for maternal toxicity was determined at 300 mg/kg bw/day (corresponding to 246 mg bromide/kg bw/day) based on clinical signs of neurotoxicity noted at ≥600 mg/kg bw/day. NOEL for developmental toxicity was determined at 50 mg/kg bw/day. NOAEL for developmental toxicity was determined at 50 mg/kg bw/day (corresponding to 41 mg bromide/kg bw/day) based on abnormalities of the ribs and scapulae and effects on foetal ossification noted at ≥300 mg/kg bw/day.

ADVERSE EFFECTS ON OR VIA LACTATION

3.7.1.14 [Study 14] Study of milk production, elemental composition, bromide transfer via mothers milk and effects in suckling rats

Reference A6.8.1/07, Doc. No. 592-015

Vobecký M., Pavelka S. and Babický A. (2005). Bromide transfer through mother's milk and its impact on the suckling rat. Biological Trace Element Research, Vol. 103, 37.

3.7.1.15 [Study 15] Study of milk production, bromide transfer via mothers milk and effects on suckling rats

Reference A6.8.1/08, Doc. No.: 592-056

Pavelka S., Babický, A., Lener, L. Vobecký, M (2002) Impact of high bromide intake in the rat dam on iodine transfer to the sucklings. Food and Chem Toxicol 40, 1041-1045.

3.7.2 Human data

ADVERSE EFFECTS ON DEVELOPMENT

3.7.2.1 [Study 1] Case study: Effects of bromide exposure in mother and infant

Finken R. L. and Robertson W. O. (1963). Transplacental Bromism. American Journal of Diseases of Children, Vol. 106, p. 224 - 226.

3.7.2.2 [Study 2] Case study: Effects of bromide exposure in mother and infant

Pleasure J.R and Blackburn M.G. (1975). Neonatal bromide intoxication: prenatal ingestion of a large quantity of bromides with transplacental accumulation in the fetus. Pediatrics 55(4):503-6.

3.7.2.3 [Study 3] Case study: Effects of bromide exposure in mother and infant

Mangurten H.H. and Ban R., 1974. Neonatal hypotonia secondary to transplacental bromism. J. Pediatr. 85(3):426-8.

3.7.2.4 [Study 4] Case study: Transplacental bromide exposure and potentially related effects in childhood

Opitz J.M., Grosse F.R., Haneberg B. (1972). Congenital effects of bromism? Lancet 1(7741):91-92.

3.7.2.5 [Study 5] Case study: Transplacental bromide exposure and potentially related effects in infancy and childhood

Rossiter E.J.R. and Rendle-Short T.J. (1972). Congenital effects of bromism? Lancet 2(7779):705

ADVERSE EFFECTS ON OR VIA LACTATION

3.7.2.6 [Study 6] Case report: Bromide exposure to infants via mothers' milk

Tyson R.M. et al. (1938). Drugs transmitted through breast milk. III. Bromides J. Pediatr. 13:91-93

3.7.3 Other data (e.g. studies on mechanism of action)

ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

3.7.3.1 [Study 1] 4 or 12 weeks feeding study of sodium bromide to investigate alterations in the endocrine system

Reference	A6.10/09, Doc. No. 592-036 Loeber J. G., Franken M. A. M. and van Leeuwen F. X. R. (1983). Effect of Sodium Bromide on Endocrine Parameters in the Rat as Studied by Immunocytochemistry and Radioimmunoassay. Fd. Chem. Toxic. 21 (4), 391-404
Guideline	Guideline study No, there is no guideline available for this special investigation and study was performed according to good experimental practice. GLP No Study is a publication and was performed according to good experimental practice Deviations Not applicable; no guideline available for mechanistic investigations
Reliability	Reliability 2 Deficiencies Not applicable; no guideline available for mechanistic investigations
Species / strain	Rat Wistar
Test material	Sodium bromide

Purity 99.5 % (since the study is a publication there is no certificate of analysis to confirm this statement).

Stability Not examined

Study design

Test Animals
Sex male
Age/weight at study initiation: Animals were within a weight range of 60-100 g and approximately 3 weeks of age.
Number of animals per group: 10/group
Control animals Yes
Administration/Exposure
Oral via the diet
Duration of treatment: 4 or 12 weeks
Frequency of exposure: daily
Postexposure period: No postexposure period. Animals were decapitated and exsanguinated at the end of the 4- or 12-week treatment period.
Concentration Sodium bromide concentrations of 0, 20, 75, 300, 1200 and 19200 mg/kg diet, corresponding to 1.5, 5.6, 22.5, 90 and 1440 mg NaBr/kg bw/day
Vehicle None
Concentration in vehicle: Not applicable
Total volume applied: Diets and water were available ad libitum.
Controls plain diet
Examinations
Observations
Clinical signs Not examined
Mortality Not examined
Body weight Yes
Food consumption No; food was available ad libitum.
Water consumption No; water was available ad libitum.
Ophthalmoscopic examination: Not examined
Haematology Not examined
Clinical Chemisty Yes; Serum hormone levels (thyroxine, testosterone, corticosterone, thyroidstimulating hormone, follicle-stimulating hormone, adrenocorticotropic hormone, insulin and growth hormone) were established by radioimmunoassay.
Urinalysis Not examined
Sacrifice and pathology
Organ Weights Yes; Weight was taken from pituitary gland, thyroid and testes only.
Gross and histopathology: Yes; Pituitary gland, thyroid and testes were examined by histopathological and immunocytochemical techniques
Other examinations None
Statistics: Significance of differences in the radioimmunoassay between groups was analyzed by Student´s t test.
Further remarks: In a separate 12-week experiment, five animals receiving 19200 mg NaBr/kg diet and live control animals were submitted to a release test using thyrotropinreleasing hormone (TRH) in a dose of 1 µg/kg bodyweight. Five minutes after intravenous injection of the TRH the rats were decapitated and exsanguinated.

Findings

Observations
Clinical signs There were no clinical signs reported in this investigation.
Mortality No mortalities were reported in this investigation
Body weight gain: Compared to the control animals, the group on the 19200 mg NaBr/kg diet showed growth retardation which was noticeable after 4 weeks (9%) and more pronounced after 12 weeks (21%).
Food consumption and compound intake: No analysis for food consumption or compound intake was performed in this investigation.
Ophtalmoscopic examination: No ophthalmoscopic examination was performed in this investigation.
Blood analysis
Haematology: No haematological analysis was performed in this investigation.
Clinical chemistry: Serum hormone levels (thyroxine, testosterone, corticosterone,

thyroidstimulating hormone, follicle-stimulating hormone, adrenocorticotropic hormone, insulin and growth hormone) were established by radioimmunoassay.
There was a statistically significant decrease of T4 both after the 4- and the 12- week treatment with the 19200 mg NaBr/kg diet. Also in the 1200 mg/kg diet group, the T4 level was significantly reduced after the 4-week exposure period. On the other hand, TSH levels were significantly increased in the highest dose group. TRH had no effect on the T4 level, but as might be expected, it caused an increase in the TSH levels both in the control group and in the bromide group. With regard to the gonadotropic hormones LH and FSH there are a number of striking observations. In both series of experiments FSH increased significantly after exposure to a high level of bromide. This did not hold for LH. After 4 weeks a statistically significant decrease of the LH level in the highest dose group was seen compared to untreated animals, but this effect had disappeared after 12 weeks. The level of both LH and FSH was higher in the 4-week groups treated with 20- 1200 mg NaBr/kg diet than in those killed at 12 weeks. Since all animals were 3 weeks old at the beginning of the experiment, the 4-week groups were younger when killed than were the 12-week groups. With regard to the LH and FSH findings in the TRH test, it is surprising that only LH rises both in the control and in the bromide animals.
A statistically significant decrease in testosterone was observed after treatment with the 19200 mg NaBr/kg diet for 4 and 12 weeks. The results of the GH assay are difficult to interpret. The large variation within each dosage group may have been due to rapid fluctuations in GH secretion. Episodic bursts of GH secretion are responsible for the 10-20-folg increase in the GH concentration in the serum within 15-30 minutes. Nevertheless, it appears that with the highest dietary level of 19200 mg/kg there was a significant decrease after 12 weeks. Insulin levels were significantly increased by bromide treatment only in the highest dosage group but after 4 as well as after 12 weeks.
Corticosterone showed a tendency to decline in the sodium bromide-treated rats, particularly in the highest dosage group.
Urinalysis: No urinalysis was performed in this investigation.
Sacrifice and pathology
Organ weights Compared to control animals, the group on the 19200 mg NaBr/kg diet showed growth retardation, which was noticeable after 4 weeks and more pronounced after 12 weeks. There was a distinct increase in the thyroid weight in the 1200 mg/kg group after 4 weeks but not after 12 weeks. In the 19200 mg/kg group, however, a statistically significant increase was observed after both 4 and 12 weeks.
Gross and histopathology
Pituitary gland:
No histopathological changes could be detected in the haematoxylin/eosinstained sections of the pituitary glands of rats exposed to sodium bromide for 4 or 12 weeks. Using the immunoperoxidase techniques, selective immunocytochemical staining could be localized in different types of cell.
Immunoreactive GH cells were seen to be scattered throughout the anterior pituitary gland, frequently forming small clusters or strings. The cytoplasm was strongly stained and the cells had round to oval shapes. The thyrotropic cells revealed with the anti-TSH serum appeared to be polygonal, and were mainly located in the central part of the anterior pituitary gland. The staining reaction was again strong. Many stellate cells with cytoplasmic processes lying between other cells were positive for ACTH, and were preferentially localized in the lateral part of the anterior lobe. No immunocytochemical reaction was observed when the anti-hormone sera were substituted to normal sera.
Immunostaining procedures for GH, TSH and ACTH in the different types of cell in the anterior pituitary gland of the control rats and of animals treated with sodium bromide yielded different results. The average number of size of the immunoreactive cells and the intensitiy of the staining reaction in each procedure were first ascertained to serve as a base-line against which any change could be judged. In the pituitary gland of rats treated with 19200 mg NaBr/kg diet for 4 and 12 weeks, only a slight tendency towards less GH immunoreactivity was observed in comparison with the control animals. On the other hand there was distinctly more immunoreactivity for TSH and ACTH, but only after 12 weeks.
Thyroid:
After exposure of rats to 19200 mg NaBr/kg diet for four weeks, remarkable histopathological changes in thyroid were observed, characterized by an increase of follicles and a decrease in their size. The follicular epithelium was greatly heightened while the colloid was decreased in amount and more granular in appearance. This was also seen after an exposure time of 12 weeks.
No marked changes in the histological picture of the thyroid were detected in the lower dosage

groups, irrespective of the exposure time. Using the PAP (peroxidase-antiperoxidase) method, T4 was generally detected particularly in the follicular colloid but also in the follicular epithelium of normal rat thyroid tissue, but its distribution within the follicular colloid and the intensity of the reaction between follicles varied. In comparison with the reaction in the control animals, the follicles of the rats treated with 19200 mg NaBr/kg diet for 4 or 12 weeks were less intensely stained. Moreover, in the latter groups there was less variation in the intensity of the reaction within the follicular colloid.
Testes:
A decreased spermatogenesis and a reduction of tubule diameter were observed in the rats of the highest dosage group after 12 weeks of treatment. No immunocytochemical staining for testosterone could be achieved by the PAP method whatever fixation procedure was carried out. The spontaneous loss of testosterone from the Leydig cells during the fixation and embedding procedures is well recognized.

APPLICANT'S SUMMARY AND CONCLUSION
Materials and methods
The present study was initiated to ascertain whether alterations detected during a semichronic feeding study in rats (increase of relative weights of thyroid and adrenals and decrease in relative prostate weights; activation of the thyroid; decreased spermatogenesis) could be detected in male rats after exposure to high dietary concentrations of sodium bromide, and whether histopathological and immunocytochemical findings could be correlated with serum-hormone levels. Furthermore, a range of lower dietary concentrations of sodium bromide was studied to investigate whether a previously observed decrease in the serum-thyroxine level could be confirmed.
Inbred male Wistar rats within a weight range of 60-100 g were used throughout. Sodium bromide was mixed with the diet to give final concentrations of 0, 20, 75, 300, 1200 and 19200 mg/kg (corresponding to about 0, 1.5, 5.63, 22.5, 90 and 1440 mg/kg bw/day based on a default conversion of 1 ppm = 0.075 mg/kg bw/day (mean of 0.05 mg/kg bw/day and 0.1 mg/kg bw/day for older and young rats, respectively)). Separate controls were used for the group on the latter diet. Each test and control group for each exposure period consisted of 10 rats. Diets and tap-water were available ad libitum. After 4 or 12 weeks, animals were decapitated and exsanguinated.
After macroscopic inspection, the pituitary gland, thyroid and testes were weighed. Pituitary gland was fixed in 8 % (w/v) formaldehyde solution containing 4.5 % (w/v) mercuric chloride (sublimate) and 0.5 % (w/v) sodium chloride. Thyroid and testes were fixed in 4 % (w/v) formaldehyde in 0.067 M Sörensen buffer, pH 6.9, and in Bouin Holland´s solution, respectively.
Embedding was performed in paraplast and 5 µm sections were prepared. The sublimate was removed by treatment with lugol and sodium thiosulphate. The sections were stained with haematoxylin and eosin.
Two immunoperoxidase techniques were used for the localization of the hormones. For thyroid-stimulating hormone (TSH), growth hormone (GH) and adrenocorticotropic hormone (ACTH), immunocytochemical staining was carried out in accordance with the indirect peroxidise-labelled antibody method using 3,3´-diaminobenzidine and hydrogen peroxidise as substrates for peroxidase. The antisera used were the following: rabbit antiserum to rat TSH, specific anti-rat-GSH serum prepared in monkey and specific anti-pig-ACTH serum prepared in rabbit. Peroxidase-labelled secondary antibodies were used for the detection of bound specific primary immunoglobulins.
All sera were diluted in phosphate-buffered saline (PBS), pH 7.2. For thyroxine (T4) and testosterone, the unlabelled antibody method of peroxidaseantiperoxidase (PAP) was applied, using Immulok Histoset Immunoperoxidase Staining Kits with 3-amino-9-ethylcarbazole in N,N-dimethylformamid and hydrogen peroxide as substartes for the peroxidise reaction. Counterstaining with haematoxylin was sometimes performed. The hormone concentrations in the sera were measured by radioimmunoassay.
For TSH, GH, luteinising hormone (LH) and follicle-stimulating hormone (FSH) determinations, materials supplied by NIAMDD were used. Iodination was carried out using the Chloramine-T technique with slight modification. For TH, LH and FSH, separation of antibody-bound and free hormone was achieved by addition of sheep-anti-rabbit-γ-globulin coupled to a solid phase.
For GH, liquid-phase goat-anti-monkey- γ-globulin was used. Counting data were evaluated using a computer programme. Testosterone and corticosterone were measured I ethylene glycol diethyl ether and ethylene glycol-toluene extracts, respectively. For T4 determinations the Corning Immophase T4 Kit was used, slightly modified for rat serum.
In a separate 12-week experiment, five animals receiving 19200 mg NaBr/kg diet and live control animals were submitted to a release test using thyrotropinreleasing hormone (TRH) in a dose of 1

µg/kg bodyweight. Five minutes after intravenous injection of the TRH the rats were decapitated and exsanguinated.
Following clotting of the trunk blood, the serum was harvested and kept at - 20°C prior to use for the hormone assay.
Results and discussion
Compared to control animals, the group on the 19200 mg NaBr/kg diet showed growth retardation, which was noticeable after 4 weeks and more pronounced after 12 weeks. There was a distinct increase in the thyroid weight in the 1200 mg/kg group after 4 weeks but not after 12 weeks. In the 19200 mg/kg group, however, a statistically significant increase was observed after both 4 and 12 weeks.
No histopathological changes could be detected in the haematoxylin/eosinstained sections of the pituitary glands of rats exposed to sodium bromide for 4 or 12 weeks. Using the immunoperoxidase techniques, selective immunocytochemical staining could be localized in different types of cell.
Immunoreactive GH cells were seen to be scattered throughout the anterior pituitary gland, frequently forming small clusters or strings. The cytoplasm was strongly stained and the cells had round to oval shapes. The thyrotropic cells revealed with the anti-TSH serum appeared to be polygonal, and were mainly located in the central part of the anterior pituitary gland. The staining reaction was again strong. Many stellate cells with cytoplasmic processes lying between other cells were positive for ACTH, and were preferentially localized in the lateral part of the anterior lobe. No immunocytochemical reaction was observed when the anti-hormone sera were substituted to normal sera.
Immunostaining procedures for GH, TSH and ACTH in the different types of cell in the anterior pituitary gland of the control rats and of animals treated with sodium bromide yielded different results. The average number of size of the immunoreactive cells and the intensitiy of the staining reaction in each procedure were first ascertained to serve as a base-line against which any change could be judged. In the pituitary gland of rats treated with 19200 mg NaBr/kg diet for 4 and 12 weeks, only a slight tendency towards less GH immunoreactivity was observed in comparison with the control animals. On the other hand there was distinctly more immunoreactivity for TSH and ACTH, but only after 12 weeks.
After exposure of rats to 19200 mg NaBr/kg diet for four weeks (corresponding to 1440 mg/kg bw/day), remarkable histopathological changes in thyroid were observed, characterized by an increase of follicles and a decrease in their size.
The follicular epithelium was greatly heightened while the colloid was decreased in amount and more granular in appearance. This was also seen after an exposure time of 12 weeks. No marked changes in the histological picture of the thyroid were detected in the lower dosage groups, irrespective of the exposure time. Using the PAP (peroxidase-antiperoxidase) method, T4 was generally detected particularly in the follicular colloid but also in the follicular epithelium of normal rat thyroid tissue, but its distribution within the follicular colloid and the intensity of the reaction between follicles varied. In comparison with the reaction in the control animals, the follicles of the rats treated with 19200 mg NaBr/kg diet for 4 or 12 weeks were less intensely stained.
Moreover, in the latter groups there was less variation in the intensity of the reaction within the follicular colloid. A decreased spermatogenesis and a reduction of tubule diameter were observed in the rats of the highest dosage group after 12 weeks of treatment. No immunocytochemical staining for testosterone could be achieved by the PAP method whatever fixation procedure was carried out. The spontaneous loss of testosterone from the Leydig cells during the fixation and embedding procedures is well recognized. Nevertheless, further studies to demonstrate testosterone in the rat testis will be performed.
There was a statistically significant decrease of T4 both after the 4- and the 12- week treatment with the 19200 mg NaBr/kg diet. Also in the 1200 mg/kg group, the T4 level was significantly reduced after the 4-week exposure period.
On the other hand, TSH levels were significantly increased in the highest dose group. TRH had no effect on the T4 level, but as might be expected, it caused an increase in the TSH levels both in the control group and in the bromide group.
With regard to the gonadotropic hormones LH and FSH there are a number of striking observations. In both series of experiments FSH increased significantly after exposure to a high level of bromide. This did not hold for LH. After 4 weeks a statistically significant decrease of the LH level in the highest dose group was seen compared to control animals, but this effect had disappeared after 12 weeks. The level of both LH and FSH was higher in the 4-week groups (20 -1200 mg NaBr/kg) than in those killed at 12 weeks. Since all animals were 3 weeks old at the beginning of the experiment, the 4-week groups were younger when killed than were the 12-week groups. Similar differences in

LH and FSH levels in the sera of animals of these ages were observed before. With regard to the LH and FSH findings in the TRH test, it is surprising that only LH rises both in the control and in the bromide animals. Since FSH does not change, contamination of the TRH preparation with the LH/FSH-releasing hormone (LHRH) can be excluded. There may be two explanations for this phenomenon: (a) the observed rise of LH is due to cross-reacting TSH, although the antiserum is supposed to be specific for LH. Nevertheless, pituitary hormones have been shown to exist in different molecular forms and it is possible that the TSH form released by the pituitary gland after TRH treatment is different from that released after sodium bromide treatment. (b) extra handling stress is caused by the injection of TRH. It is well known that acute stress affects the level of a number of hormones, e.g. ACTH and GH. LH does not seem to be very sensitive in this respect, but this possibly cannot be excluded completely. In this respect the slight elevation of the corticosterone levels in both the control and the bromide groups after TSH injection should be noted. A statistically significant decrease in testosterone was observed after treatment with the 19200 mg NaBr/kg diet for 4 and 12 weeks. The results of the GH assay are difficult to interpret. The large variation within each dosage group may have been due to rapid fluctuations in GH secretion. Episodic bursts of GH secretion are responsible for the 10-20-fold increase in the GH concentration in the serum within 15-30 minutes. Nevertheless, it appears that with the highest dietary level of 19200 mg/kg there was a significant decrease after 12 weeks. Insulin levels were significantly increased by bromide treatment only in the highest dosage group but after 4 as well as after 12 weeks.

Corticosterone showed a tendency to decline in the sodium bromide-treated rats, particularly in the highest dosage group. In the present study attention was focused mainly on the pituitary gland, the thyroid and the testes. Several sites of action of sodium bromide can be distinguished. One of them is a direct effect on the thyroid. After treatment with sodium bromide, T4 production was hampered, as evidenced by immunocytochemistry and radioimmunoassay. Due to feedback regulation the pituitary gland was stimulated to produce and release TSH. In the TRH experiment the increase of TSH in the control group and the bromide group were of similar magnitude. However, only for the control group was this increase statistically significant. From this it might be concluded that in bromide-treated animals the pituitary gland has little capacity for releasing even more TSH. Marked histopathological and immunocytochemical changes in the thyroid tissue were induced, indicative of a typical hypothyroidism. In a hitherto unresolved way, at least two other hormones are affected by changes in the T4 level, GH and insulin. The observation that a decrease in T4 concentration was accompanied by a decrease in GH concentration in the serum is in agreement with similar findings reported previously. Moreover, T4 was shown to exert a diabetogenic effect. It is, therefore, conceivable that a decrease in the T4 level results in an increase in the insulin level. The changes in serum levels of both GH and insulin are in accord with the observed growth retardation of the rats. Finally, since GH may act as a thymotropic hormone, a decrease in GH level may also be connected with the reduction in relative thymus weight found earlier. The effect of sodium bromide on the adrenals seems to parallel that on the thyroid. A decrease in the corticosterone level in the serum, as assessed by radioimmunoassay, results in an increase in the production of ACTH by the pituitary gland, as found by immunocytochemical staining. Also the testes are affected by sodium bromide. Histopathological findings now and in the past have shown an inhibition of spermatogenesis indicating a deterioration of the Sertoli-cell function. It has been shown that the Sertoli cell produces a substance called inhibin, which hampers FSH release by the pituitary gland. This may be an explanation for the rise in FSH level observed in the bromide-treated rats. In addition, the Leydig cells may be affected, resulting in a decreased production of testosterone and consequently in a loweing of the secretory activity of the prostate. An expected rise in LH level triggered by the decreased testosterone level could not be detected.

Rather, after 4 weeks a slight fall was observed. It is conceivable that the damage caused in the Leydig cells is small compared to that in the Sertoli cells, thus giving rise to changes in FSH but not in the LH levels.

Conclusions

In conclusion it may be postulated that sodium bromide acts directly on certain endocrine organs such as the thyroid, adrenals and testes, thereby inducing alterations in the pituitary gland by feedback mechanisms.

LO(A)EL 1200 mg NaBr/kg diet, corresponding to 93.6 mg bromide/kg bw/day based on a distinct increase in thyroid weight after four weeks of treatment.

NO(A)EL 300 mg NaBr/kg diet, corresponding to 22.5 mg bromide/kg bw/day

ADVERSE EFFECTS ON DEVELOPMENT

3.7.3.2 [Study 2] Pre-natal developmental toxicity (no guideline)

Reference A6.9.2/02, Doc. No. 592-017

Harned et al., 1944

Guideline No guideline available for the special type of investigations performed; the study was performed according to good experimental practice

GLP: No; GLP was not mandatory at the time of study conduct

Deviations: Not applicable; there is no guideline available for the special type of investigations performed and the study was performed according to good experimental practice

Reliability Reliability 2

Deficiencies Not applicable; there is no guideline available for the special type of investigations performed and the study was performed according to good experimental practice

Species / strain Rat

Wistar

Test material Sodium bromide

Study design **Test Animals**

Sex: male and female

Age/weight at study initiation: Not indicated; according to the descriptions in the study, adult rats were used as treatment occurred during gestation.

Number of animals per group: Investigation was focused on the offspring, treatment was performed on the dams:

Treatment of dams [mg NaBr/kg bw/day]	No. of offspring investigated	
	male	female
120	16	18
80	18	12
40	20	13
0	17	13

Control animals Yes

Exposure

Administration oral by gavage once daily from Day 3 to Day 20 of gestation

Dose Levels 40, 80 and 120 mg/kg bw/day

Vehicle water

Concentration in vehicle: Not indicated

Total volume applied: Not indicated

Postexposure period: Approximately 85 days after pups were born.

Controls Vehicle

Examinations

Body Weight Yes; in frequent intervals

Signs of Toxicity Yes

Observation schedule: daily observation

Clinical Chemistry Yes; Blood bromide levels were determined in newborn and at Day 62 after birth. No other clinical-chemical examinations were performed.

Pathology Not performed

Histopathology Not performed

Further remarks From the age of 57-60 days the rats were prepared for learning in the maze. From the age of 61-85 days each animal was given two trials per day in a five cul-de-sac u-maze.

Bromide concentration was determined in addition to blood in dry tissue.

Findings

Body Weight: Bodyweight analyses of pregnant rats showed that the group treated at 40 mg/kg bw/day was heavier than the control group and the two higher dosage groups were heavier than the 40 mg group. When animals were allowed only 15 minutes of feeding, they all lost weight and put on weight again when feeding time was extended. This weight loss showed the same relationship among all groups whereas for the 80 and 120 mg groups the weight gain was more slowly compared to the lowest dosage group. All treated animals were heavier at the end of the investigation compared to controls. Pups from the groups treated at 80 and 120 mg/kg bw/day, the growth was subnormal for a few days, but this handicap was temporary and of a minor order. Weight curves of these animals reveal no evidence of any physical handicap.

Clinical signs of toxicity: Pregnant bromide treated rats offered less resistance to the passage of the stomach tube than did the normal rats, but no evidence of depression could be distinguished.

The mortality among the newborns was high and paralleled the doses given the mothers. Pups that died before 20 days of age were 2.3%, 27%, 42% and 58% from animals treated at 0, 40, 80 and 120 mg/kg bw/day, respectively (please refer to table 6.9.2/02-3).

Clinical Chemistry: Multiplying the relative concentrations of blood bromide levels by the mean value in the control group (3.8 mg/100 ml), one calculates that at parturition the values for bromide in the blood serum of the mothers were 152 mg%, 80 mg% and 42 mg% for animals treated at 120, 80 and 40 mg/kg bw/day based on the ratios of bromide content of the control and of the experimental groups in dried tissues which were 1:40 for the group treated at 120 mg/kg bw/day, 1:21 for the 80 mg/kg bw/day group and 1:11 for the 40 mg/kg bw/day group, respectively (please refer to table 6.9.2/02-2).

Serum bromide levels of pups from bromide treated dams at Day 62 of age revealed bromide concentrations being all in the same order of magnitude which were approximately 1 mg/100 ml less than that of the control group.

Newborn rats were analysed for bromide concentration 4 hours after birth. These analyses showed that the bromide concentrations in the treated groups bore approximately the same quantitative relationship to each other as the dosages administered to the mothers (please refer to table 6.9.2/02-1).

Table 6.9.2/02-1: Bromide content of newborn rats

Group [mg NaBr/kg bw/day]	No. of pups investigated	Bromide content [mg bromide/100 g] Wet tissue		Dry tissue	
		Mean	S.E. mean	Mean	S.E. mean
0	3	1.22	0.08	11.83	0.95
40	6	18.9	1.94	132.22	14.03
80	8	32.19	3.11	250.4	21.47
120	5	61.58	2.09	469.0	27.15

Table 6.9.2/02-2: Bromide content of blood in 62 day old rats

Group [mg NaBr/kg bw/day]	No. of pups investigated	Serum bromide [mg bromide/100 g]	
		Mean	S.E. mean
0	9	3.77	0.2
40	5	2.96	0.47
80	4	2.7	0.4
120	5	2.5	0.38

Table 6.9.2/02-3: Effect of sodium bromide on infant mortality

Group	No. of litters	Total No. born	Total No. lost before weaning	% lost	S.D. percentage
Control	6	43	1	2.3	2.29
Control from colony*	24	168	37	22.0	3.2
40 mg NaBr/kg bw/day	7	63	17	27.0	5.59
80 mg NaBr/kg bw/day	22	155	65	41.9	3.96
120 mg NaBr/kg bw/day	14	119	69	58.0	4.52

* rats born in colony during period of experimentation. These rats were given only routine care.

Pathology: No pathological examination was performed in this investigation.

Histopathology: No histopathological examination was performed in this investigation.

Bromide concentration: Bromide determination in dried tissues revealed that the ratios of bromide content of the control and of the experimental groups are 1:40 for the group treated at 120 mg/kg bw/day, 1:21 for the 80 mg/kg bw/day group and 1:11 for the 40 mg/kg bw/day group.

Newborn rats were analysed for bromide concentration 4 hours after birth. These analyses showed that the bromide concentrations in the treated groups bore approximately the same quantitative relationship to each other as the dosages administered to the mothers.

Learning in the maze: The criterion of errors shows a positive relationship between the number of errors and the strength of the dosage. The group at the highest dose level (120 mg/kg bw/day) made a significantly greater number of errors than each of the other groups. The performance of the animals treated at 80 mg/kg bw/day was reliably worse than the one from the control group. Other differences were not significant (please refer to table 6.9.2/02-4).

Table 6.9.2/02-4: Group means and standard deviations of the two criteria of maze learning

Group [mg NaBr/kg bw/day]	No. of rats	Errors		Time [sec]	
		Mean	S.D.	Mean	S.D.
0	30	172.5	57.78	896.59	387.3
40	33	190.1	87.07	825.76	670.57
80	30	199.9	70.8	878.48	628.0
120	34	274.0	148.79	1314.03	1226.53

The criterion of time shows that the 120 mg/kg bw/day group was virtually significantly slower than each of the other groups, but the other groups do not differ among themselves. The error and time curves show that all groups reached essentially the same level of performance before the end of the run. The standard deviations of the groups show, in general, an increase with increasing dosage. The standard deviations computed on the time scores show the same relationship as those for error scores; moreover, all groups differ significantly from one another, with the exception of the 80 and

40 mg/kg bw/day groups.

Conclusions APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

The purpose of the investigation was to study the effects of prenatal administration of sodium bromide to rats by means of tests designed to detect functional damage in the central nervous system of the offspring by studying the learning ability after birth.

Pregnant rats were treated at 40, 80 or 120 mg NaBr/kg bw/day from Day 3 to 20 of gestation. Pups born on Day 22 received bromide only by the milk of their mothers and were weaned until 20 days of age.

From Day 20-34 after birth, the drinking water was replaced by a 0.2% solution of sodium chloride, and from 35 days of age on, pups received a 0.5% NaCl solution.

Blood bromide concentration in dams, newborns (Day 4 after birth) and pups being 62 days old, was determined, as well as bromide content in dried tissues of the newborn rats.

From the age of 57-60 days the rats were prepared for learning in the maze. From the age of 61-85 days each animal was given two trials per day in a five cul-de-sac u-maze.

Results and discussion

The reduced serum bromide levels observed in the pups from bromidetreated dams at 62 days of age compared to control rats (approximately 1 mg/100 ml less than in controls) might be explained by a higher consumption of drinking water, which was supplemented with 0.2% and 0.5% NaCl from Day 20 and 35 on, respectively and was available ad libitum. Chloride at higher concentration is known to accelerate the elimination of bromide.

The criterion of errors in the maze learning experiment shows a positive relationship between the number of errors and the strength of the bromide-dosage. The criterion of time shows that the highest dosage group (120 mg/kg bw/day) was significantly slower than each of the other groups but the other groups did not differ among themselves. All groups reached the same level of performance before the 25th day of the test. This fact suggests that in the maze test the deleterious effects of bromide appear in the rate of learning rather than in the performance finally attained.

Previous studies had shown that there is a relationship between the amount of intact cerebral area and the maze learning. Correlation coefficients of 0.3-0.86 were reported most of them from 0.8 to 0.86, between the size of the cerebral lesion and the errors in maze tests.

Another publication reported a higher degree of relationship between the magnitude of the cerebral lesions and the errors in maze learning. This would appear to be provided with the assumption that sodium bromide would interfere with the development of the central nervous system.

Conclusion The criterion of errors in the maze learning experiment shows a positive relationship between the number of errors and the strength of the bromide-dosage. The criterion of time shows that the highest dosage group (120 mg/kg bw/day) was significantly slower than each of the other groups. Since all groups reached the same level of performance before the 25th day of the test, it can be suggested that in the maze test the effects of bromide appear in the rate of learning rather than in the performance finally attained. The results of this study demonstrate also a dose-related post-natal pup mortality.

LOAEL 80 mg/kg bw/day (corresponding to 62.4 mg bromide/kg bw/day), since these animals made a significantly greater number of errors than each of the other groups.

NOAEL 40 mg/kg bw/day (corresponding to 31.1 mg bromide/kg bw/day) based on the significantly greater number of errors made by the animals on the next higher dose level.

3.7.3.3 [Study 3] Postnatal Growth and Brain Development (no guideline)

Reference A6.8.1/06; Doc. No. 592-014

Disse et al., 1996

Guideline Guideline study No; no guideline available for the special investigations performed and study was performed according to good experimental practice.

GLP No; study was performed according to good experimental practice.

Deviations Not applicable, no guideline available for the special investigations performed.

Reliability Reliability 3

Deficiencies No investigation of maternal parameters such as body weight gain, corrected body weight gain, food consumption and clinical signs; only one dose applied; no skeletal and visceral examinations performed in pups. Therefore, the publication serves as supportive information as the investigations performed are not in compliance with OECD guideline 414 for the conduct of developmental toxicity studies in rats.

Species / strain Rat

Sprague Dawley

Test material Sodium bromide

Study design **Test Animals**

Sex: female

Age/weight at study initiation: Not applicable

Number of animals per group: 18 per group in treated groups, 14 in the control group. Fourteen animals received tap water throughout their pregnancy served as controls. Four other control rats were given an aqueous solution of 250 mg % of sodium chloride (saline) during the same period of pregnancy.

Mating period not indicated

Administration/ Exposure

Oral in drinking water

Duration of exposure: Days 5-15 of gestation

Postexposure period: Until postnatal Day 90

Concentration 250 mg % (corresponding to 0.25 g/100 ml or 200 mg/kg bw assuming a bodyweight of 250 g per rat throughout the study period with water consumption of 20 ml per day)

Vehicle aqueous solution

Concentration in vehicle: 250 mg % (corresponding to 0.25 g/100 ml or 200 mg/kg bw)

Drinking water with test substance was available ad libitum.

Controls: Tap water or 250 mg % NaCl (probably corresponding to 0.25 g/100 ml or 200 mg/kg bw)

Examinations

Body weight Yes

Food consumption Not determined

Clinical signs Not examined

Examination of uterine content: Not performed

Examination of foetuses

General Litter size, foetal weight

Skeleton Not examined

Soft tissue Yes; brain was examined only

Further remarks - Bromide concentrations in blood and brain homogenate were determined between postnatal day (PD) 1 and 90.

- Immunohistochemistry was used to visualize lysosomal acid phosphatase in frozen parasagittal sections of the brain.

- The size of various brain regions was measured on parasagittal sections using image analysis techniques

Statistic: Statistical differences were tested by ranking data and using the U-test to avoid dependency of test results on the frequency distribution of actual values.

Findings

Maternal toxic Effects: No maternal effects were described in this investigation.

Teratogenic / embryotoxic effects: Compared to controls, a smaller bodyweight was observed in bromidetreated rats from birth onwards (statistically significant from PD19 onwards). Brain weight was reduced. Differences became statistically significant from PD8 onwards and remained about 10% lower than normal brain weight in adults.

To estimate the deficits in development, measurements were performed and compared between bromide-treated and control rats of the same age. The delay in development was expressed as the difference between ages at which equal values were recorded in controls and bromide treated rats and secondly, bodyweight and brain weight difference of rats of the same age give incidence if treatment effects development.

Analysis of these data revealed that bromide does not monotonously interfere with development. Three phases have been identified for the parameters measured, although the separation was less obvious for the bodyweight than for the brain parameters. During PD1-10 the developmental delay was small; bromide contents of blood and brain

decreased towards control values. The next phase (PD10-40) started with a marked increase in deficit until PD20. During the following weeks the developmental deficit did not further increase. From PD 40 on, bromide-treated rats asymptotically reached maximal values (bodyweight, brain weight, protein content in brain) which however remained at subadult levels.

Bromide application specifically interfered with the development of olfactory system. In both control and bromide-treated rats, increasing numbers of olfactory glomeruli were established before and after birth.

Compared to controls, bromide caused a delay of a few days for the time required at which acid phosphatase activity first appeared in glomeruli after the latter had been formed as shown with synaptophysin immunohistochemistry. Thereafter, the acid phosphatase-staining pattern soon became similar to that of untreated controls or salinetreated animals.

The protein content was consistently lower in brains of bromide-treated animals than in controls from birth onwards. The difference was 11% of the mean control value at PD 90.

Synaptophysin immunohistochemistry revealed, however, that the size of glomerular profiles was on average 30% larger in treated than in control rats. A quantitative evaluation confirmed the visual impression of the existence of a subpopulation of large glomeruli. Additionally, this figure shows that a small shift to larger diameters was already present at PD8 and that the size difference increased for more than one month after birth, although the average size of glomerular profiles increased further in both experimental and control animals for at least three months after birth.

Other effects Blood samples of control pups showed approximately 1 mM bromide from the first postnatal day onward, whereas in bromide-treated rats values about 4.5 higher than in controls were determined indicative for a blood-placental in utero transfer of bromide from the dams to the offspring. During the following postnatal days, bromide concentration continuously decreased in blood as a consequence of its elimination in weaning rats and reached control level by about postnatal Day 20.

In brain homogenates prepared from control rats, bromide content was always lower than in blood of the same animals again indicative for a blood-placental in utero transfer of bromide from the dams to the offspring.. Immediately after birth, the concentration of bromide was three-fold higher in treated rats than in controls. At postnatal Days (PD) 2, 4, 6 and 8 bromide content decreased continuously and reached control values by PD 10. During the postnatal period, bromide concentration in brain decreased in absolute values, but the ratio brain/blood increased from 30 to about 50%.

Conclusions APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

The study was designed to investigate the potential effects of sodium bromide on the sodium bromide. Rat embryos were exposed to sodium bromide by providing an aqueous solution of 250 mg % NaBr (corresponding to 200 mg/kg bw) in the drinking water, ad libitum to their dams. Controls received either tap water or 250 mg % NaCl (corresponding to 200 mg/kg bw). Application was restricted to Days 5-15 of gestation.

Results and discussion

It had been demonstrated previously that enteral administration of sodium bromide may mimic some of the neurotrophic or neuroplastic effects of locally applied γ-aminobutyric acid (GABA). In adult rats, prolonged NaBr administration produced essentially the same dendritic changes in principal ganglion cells of the superior cervical ganglion as GABA. This effect included the formation of paramembraneous densities. NaBr was found to inhibit acetylcholine release and synaptic transmission in this ganglion. Corresponding changes in acetylcholinesterase were noted after long-lasting NaBr treatment indicating a differential modulation of various molecular forms of this enzyme. Like GABA, NaBr finally promoted synapse formation of principal ganglion cells with foreign cholinergic nerves implanted into the superior cervical ganglion.

The data presented in this investigation suggest that intake of sodium bromide by pregnant rats might interfere with general growth and brain development in the offspring. This effect apparently depends on bromide ions, because sodium chloride treatment did not induce any comparable changes. The present experiments provide further evidence that bromide ions may influence neuroplasticity under variable conditions. For example NaBr has turned out to affect different parts of the nervous system (sympathetic ganglia, visual system, and cerebral cortex) at various ages.

For the dams, the period of bromide administration was restricted to the 5th and 15th day of pregnancy. This period was apparently not identical with the time of bromide exposure to the offspring. The beginning of action may have been delayed, because blood concentration of bromide rises slowly and may not have reached sufficient levels until the dams fat stores were possibly saturated with bromide although other studies demonstrated that bromide is preferentially distributed in the extracellular space. After the end of treatment, the bromide content in blood and brain tissue remained significantly higher than in control pups until 10 and 20 days after birth, respectively. Thus, bromide may have directly influenced the offspring’s development during a period of three to four weeks, i.e. at some time between the last 6-9 days of gestation and 10-20 days after birth. The higher blood bromide content in pups during post-natal days 10 – 20 may be explainable by an exposure of the pups during the lactation period via breast milk of the maternal animals.

Bromide-dependent retardation of pup development is apparently not based on a uniform mechanism of interaction with developmental process. The kinetics of retardation rather suggests a distinction of three phases (PD1-10, PD10-40, PD40-90). Developmental delays were small and were more or less completely compensated, while bromide concentrations decreased to control levels in blood and brain.

Surprisingly, developmental deficits strongly increased thereafter. This finding indicates that that direct bromide actions on body growth and brain development if existing at all, were almost completely compensated, while bromide decreased and approached control level in blood. That

developmental retardation occurred thereafter, suggests that bromide must have affected some mechanisms involved in the regulation of postnatal development. Thus, indirect influences of bromide on development have to be taken into account.

Also during the following phases (PD10-40 and PD40-90), development was not uniformly influenced. Developmental retardation became prominent during two periods of development (PD10-17 and PD40-60), while deficits became smaller during intervening time. What appeared as a transient improvement indicates that there might have been a second trial to compensate deficits in development between PD20 and PD40 that is after weaning. Since the time course was similar for bodyweight and brain parameters, partial recovery was probably based on regulatory mechanism which do not act specifically on the organ level but more generally, e.g. like any hormonal mechanism.

In view of the complex set of deficits found after bromide treatment, one hardly expects bromide to have a uniform action mechanism or to modify a single type of molecular process indirectly influencing various other aspects of development. However, the fact that the reduction in brain weight is accompanied by a general deficit in body growth indicates that bromide may generally affect some metabolic regulation.

One suggestion how bromide could interfere with general metabolism has been given by Buchberger (Buchberger et al, 1990; please refer to section 6.4.1/12). The authors investigated the effect of bromide on the thyroid gland in rats and showed that with increasing serum bromide concentrations T4 and T3 levels decreased and that there was an increased thyroid weight in the treated groups compared to controls. Treatment with 200 mg sodium bromide/kg bw/day already showed these effects, possibly via iodide replacement in the thyroid gland and with that induction of changes regarding the thyroid hormones. Further on, in a review by Kast (Kast, 1994; please refer to section 6.8.1/04) it is supposed that endocrine modulations might affect the rats foetus Another possibility would be that bromide caused a general intoxication.

In the present experiments, however, the application time was probably too short to provide enough bromide for intoxication. After providing adult rats for 10 days with 250 mg% of bromide in drinking water (which is corresponding to 200 mg/kg bw), the maximum blood concentration reached a level of 10 mM bromide in blood, which is approximately 1/7th of those blood levels found in mice during bromism.

Not all bromide effects seem to cause a deficiency in development. In the olfactory bulb, the glomerular synaptic complexes were on average larger in bromide-treated rats than in controls, although in both groups of animals the size of glomeruli increased with postnatal age. Reasons for this discrepancy are at present unknown. On the one hand, some partial processes of brain development may be promoted rather than retarded by bromide and by its direct effects. On the other hand, bromide might also inhibit regressive processes, which are continually ongoing during replacement of olfactory receptor cells and their synapses in glomeruli. Bromide might have affected such degrading functions in neurons, e.g. by interfering with acidification in endosomes or lysosomes. Apart from interfering with general regulatory mechanisms of development, bromide may also exert some specific action on neural systems. As already mentioned, some GABA effects can be mimicked by sodium bromide. Another research group found that both GABA and NaBr can induce the formation of low affinity GABA receptors on cultured cerebellar neurons. Therefore, bromide-dependent hyperpolarisation may also interfere with some steps in the cascade of development reactions characterizing the trophic action of GABA. Bromide ions may hyperpolarize cells in various ways either by activating chloride influxes or by binding to synaptic membranes and thereby modifying their surface charge. By whatever mechanism, sodium bromide can reduce the release of certain but not all neurotransmitter substances from presynaptic nerve endings. Some reports indicate that strengthening GABA-like effects during development, by benzodiazepines, may affect brain development including olfactory functions. In addition, bioelectrical activity exerts trophic influences on normal brain development. As far as developmental deficits are concerned, future search for mechanisms will therefore have to distinguish among the several different possible modes of bromide action.

Summarizing the results of this investigation, the effects seen in the pups from bromide-treated rats compared to control animals regarding bodyweight performance, brain weight and protein content in brain are only minor in degree. Bodyweights from bromide-treated dams between postnatal Day 60 and 90 were about 40 g less compared to controls (200-240 g compared to 240-280 g in treated and control animals, respectively), but there was a high standard deviation within the bromide-treated

group. The differences in brain weight were negligible and the mentioned reduction in protein content of brain of about 11% can be explained by the slightly lower brain weight. It is important to note that in a study by Buchberger and colleagues (Buchberger et al, 1990; please refer to section 6.4.1/12) it was shown, that treatment with 200 mg sodium bromide/kg bw/day induces changes in the thyroid gland in the absence of adverse clinical signs. Changes of parameters of the endocrine system of dams were discussed to be the cause of modifications within the foetus, when treatment was performed during pregnancy (review by Kast, 1994; please refer to Document IIIA, Section 6, point 6.10/06). Since approx. the same amount of sodium bromide was applied to the rats in the present investigation, it is justified to assume that the same effects on the thyroid were induced by treatment and that these effects influenced foetal development. However, the effects seen within the present investigation were only minor in degree and should therefore not be regarded as an adverse effect.

Conclusion Treatment with bromide during Days 5-15 of gestation resulted in slightly lower bodyweight gain, brain weight and protein content of brain in the offspring, but the differences compared to control were only minor in degree and should therefore not be regarded as an adverse effect. During onset of development of pups of dams which have been treated through gestation, bromide levels continuously declined and were comparable to controls at later time points. Furthermore, the results of this investigation demonstrated that bromide-mediated developmental delays were completely compensated.

LO(A)EL maternal toxic effects: Not applicable

NO(A)EL maternal toxic effects: Not applicable

LO(A)EL embryotoxic /teratogenic effects: Not applicable

NO(A)EL embryotoxic /teratogenic effects: Not applicable

3.8 Specific target organ toxicity – single exposure

3.8.1 Animal data

3.8.1.1 [Study 1] Acute oral toxicity study of calcium bromide

Reference	Study report, 1996
Guideline	EPA OTS 798.1175 (Acute Oral Toxicity) Deviations: No GLP: Yes
Reliability	1 (reliable without restriction)
Species / strain	Species: rat Strain: Sprague-Dawley Sex: male/female
	Details on test animals or test system and environmental conditions: The animals were housed in groups of up to five by sex in solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately 2 hours after dosing, free access to mains drinking water and food was allowed throughout the study. The animal room was maintained at a temperature of 19-22C and relative humidity of 50-55%. The rate of air exchange was approximately 15 changes per hour and the lightning was controlled by a time switch to give 12 hours light and 12 hours darkness
Test material	Reference substance name: Calcium bromide EC Number: 232-164-6 /Cas Number: 7789-41-5 Molecular formula: CaBr2 IUPAC Name: calcium bromide Details on test material: White powder, Stored at room temperature over silica gel. For the purpose of the study the test material was freshly prepared, as required, as a solution at the appropriate concentration in distilled water.
Administration / exposure	Route of administration: oral: gavage Vehicle: water Details on oral exposure: All animals were dosed once only by gavage using metal cannula attached to a graduated syringe. Doses: 2000, 2646 or 3500 mg/kg BW No. of animals per sex per dose: 5 Control animals: no Details on study design: No further details
Results and discussion	Preliminary study: A range finding study was performed to establish a dosing regime, using 5000, 2000, 1000 and 500 mg/kg. Animals treated with 5000 mg/kg were found dead one day after dosing. Common signs of systemic toxicity were ataxia, hunched posture, lethargy, decreased respiratory rate, and laboured respiration with additional signs of pilo-erection and ptosis. Based on this information, dose levels of 2000, 2646 and 3500 mg/kg bw were selected for the main study.
	Effect levels: Dose descriptor: LD50

Based on: test mat. /CaBr2

Effect level (male/female): 2 210 mg/kg bw (95% CL: > 1 854 - < 2 635)

Effect level (male): 2 573 mg/kg bw (95% CL: > 2 243 - < 2 951)

Effect level (female): 1 512 mg/kg bw (95% CL: > 642 - < 3 561)

Clinical signs, mortality and gross pathology

Mortality: Deaths were noted during the day of dosing and one day after dosing.

Clinical signs: Common signs of systemic toxicity noted in all dose groups were ataxia, hunched posture, lethargy, decreased respiratory rate and laboured respiration with additional signs of pilo-erection and ptosis and isolated incidents of red/brown staining around the eyes. Isolated incidents of splayed or tiptoe gait were noted in animals treated with 2646 or 3500 mg/kg. Isolated incidents of loss of righting reflex were noted in animals treated with 2646 mg/kg. Dehydration was noted in one male treated with 3500 mg/kg.

Gross pathology: Abnormalities noted at necropsy of animals that died or were killed in extremis during e study were haemorrhagic lungs, dark liver, dark kidneys, haemorrhage, severe haemorrhage and sloughing of the gastric mucosa, haemorrhage, severe haemorrhage and sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small intestine. No abnormalities were noted at necropsy of animals that were killed at the end of the study.

Other findings: No further details

Registrant's summary and conclusions

Interpretation of results: practically nontoxic

Remarks: Migrated information Criteria used for interpretation of results: other: US EPA

Conclusions:

The acute oral median lethal dose (LD50) and 95% confidence limits of the test material, calcium bromide, in the Sprague-Dawley rat were calculated by the method of Thompson W.R to be:

All animals: 2210 (1854-2635) mg/kg bw

Males only: 2573 (2243-2951) mg/kg bw

Females only: 1512 (642-3561) mg/kg bw

3.8.1.2 [Study 2] Acute oral toxicity study of calcium bromide

Reference

Study report, 1980

Guideline

According to: EPA Federal Register Volume 43 (163): 37355-37356, 1978
GLP: yes

Reliability

2 (reliable with restrictions) (according to REACH registration)

Species / strain

Rat, Sprague-Dawley

Test material

Reference substance name: Calcium bromide
EC Number: 232-164-6
Cas Number: 7789-41-5
Molecular formula: CaBr2

Administration / exposure

Oral: gavage
Vehicle: Tween 80 1.5%

Doses range finding: 1000, 2500, 3000, 4000 and 5000 mg/kg
Groups range finding: 2 rats/sex/group

Doses LD50: 3000, 3446, 3959, 3243, 4547, 5223 and 6000 mg/kg

	Groups LD50: 5 rats/sex/group Control animals: no
Study design	Details on test animals and environmental conditions: body weight: 140-200 grams age: 4-6 weeks old Source: Bio-breeding Laboratories of Canada Ltd. 2224 Innes Road, Ottawa. caging: Shoebox bins Bedding material: Wood shavings No. of animals per bin: range finding - 2/sex/bin; LD50 - 5/sex/bin Ambient temp: 70-75 degree F Humidity: 40-50% Phtoperiod: 12/12h Acclimation period: 1 week Diet: pelleted diet, purina certified rodent chow 5002 water: from bottles, ad libitum Observations/examinations: - Duration of observation period following administration: 14 days - Frequency of observations and weighing: 0, 7 and 14 days - Necropsy of survivors performed: yes Statistics: From the accumulated mortality data the LD50 value and its 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon, Journal of Pharmacology and Experimental Therapeutics Vol. 96, 99-113, 1949.
Results and discussion	**Range finding study:** For the range finding, five dose levels (two rats/sex/dose) of 1000, 2500, 3000, 4000 and 5000 mg/kg were selected producing mortality of 0%, 0%, 0%, 25% (one male), 75% (two males, one female) respectively. **LD50 study:** LD50 with 95% confidence interval: All animals: 4400 (4037 – 4795) mg/kg bw Males only: 4450 (3788 - 5227) mg/kg bw Females only: 4100 (3789 - 4436) mg/kg bw Mortality: Mortality generally occurred within 72 hours post-dosing with incidences of delayed mortality at day 5 (4547 mg/kg) and day 7 (3446, 3959 mg/kg) post-dosing Clinical signs: Pharmacotoxic signs were observed in all test groups and were characterized by decreased activity, decreased muscle tone, decreased reflexes, ataxia, lacrimation, piloerection, tip toe gait. Signs of acute toxicity generally persisted for a period of seven days post-dosing with the exception of the 4243 mg/kg group which presented with signs of toxicity up until the 12th day post-dosing. Gross pathology: At necropsy lesions most frequently observed consisted of gastric distension with irritation or hemorrhage of gastric mucosa and free floating amorphous material in urinary bladder.
Registrant's summary and conclusion	Interpretation of results: slightly toxic Conclusions: Test article calcium bromide when administered to Sprague-Dawley Albino rats by oral gavage appears to be slightly toxic to both males and females with a slight predisposition in toxicity towards the females. LD50 – 4400 (4037-4795) mg/kg

3.8.1.3 [Study 3] Acute oral toxicity of calcium bromide

Reference	Study report, 1978a
Guideline	No guideline. No GLP.
Reliability	2 (reliable with restrictions) (according to REACH registration)
Species / strain	Rat, Charles River CD Body weight: 200 to 294 grams. Housed by sex in groups of 5 rats per cage, in hanging wiremesh cages in temperature and humidity-controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication No. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Laboratory Chow were available ad libitum, except for an overnight period preceding oral administration during which food, but not water, was withheld.
Test material	Reference substance name: Calcium bromide EC Number: 232-164-6 / Cas Number: 7789-41-5 Molecular formula: CaBr2
Administration / exposure	Oral: gavage Vehicle: distilled water Doses : 508.4, 807.1, 1281, 2034, 3229 and 5126 mg/kg. Dosage levels used were based upon the total formulation as received from the client. Groups: 5/sex/dose Controls: no
Study design	Observations: All rats were observed for mortality, only, during the first four hours after dosing, at 24 hours and daily thereafter for a total of 14 days. Body weights were recorded initially (control weight) and at 14 days
Results and discussion	LD50 with 95% confidence interval: All animals: 2447 (2231-2684) mg/kg bw Males: 2562 (2218-2960) mg/kg bw Females: 2336 (1942-2811) mg/kg bw
Registrant's summary and conclusion	Based upon the LD50 values derived, the test material would not be considered a toxic substance by the oral route of administration

3.8.1.4 Study [4] Acute oral toxicity study of calcium bromide

Reference	Study report, 1978b
Guideline	No guideline. No GLP.
Reliability	2 (reliable with restrictions) (according to REACH registration)
Species / strain	Rat, Charles River CD Body weight: 200 to 298 grams. The rats were housed by sex in groups of 5 rats per cage, in hanging wiremesh cages in temperature and humidity controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication Xo. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Laboratory Chow were available ad libitum, except for an overnight period preceding oral administration during which food, but not water, was withheld.

Test material	Constituent 1: Reference substance name: Calcium bromide EC Number: 232-164-6 / Cas Number: 7789-41-5 Molecular formula: CaBr2 Constitutent 2: Reference substance name: calcium bromide solution 53.5%
Administration / exposure	Oral: gavage Vehicle: distilled water Doses: 508.4, 1281, 2034, 3229, 5126 and 8137 mg/kg. a.i. - 71.99, 685.34, 1088.19, 1727.52, 2742.41, 4353.3 mg/kg. Groups: 5/sex/dose Control: no
Study design	Observations: All rats were observed for mortality, only, during the first four hours after dosing, at 24 hours and daily thereafter for a total of 14 days. Body weights were recorded initially (control weight) and at 14 days.
Results and discussion	LD50 with 95% confidence interval: male/female: 4068 (3521-4700) mg/kg bw
Registrant's summary and conclusion	Based upon the LD50 values obtained, the test material would not be considered a toxic substance by the oral route of administration.

3.8.1.5 [Study 5] Acute Inhalation Toxicity study of calcium bromide in Rats

Reference	Study report, 1978c
Guideline	No guideline. No GLP.
Reliability	2 (reliable with restrictions) (according to the REACH registration)
Species / strain	Rat, Charles River CD Male/female Body weights: male rats 210 - 226 grams, females rats 200 to 210 grams. The rats were housed individually in wire-mesh cages and were kept throughout the pre-and postexposure periods in a temperature and humidity controlled room in accordance with standards outlined in the "Guide For the Care and Use of Laboratory Animals; DHEW No. (N.I.H . 74-23) 1974". Purina Laboratory Chow and water were supplied ad libitum except when the rats were in the exposure chamber.
Test material	Reference substance name: Calcium bromide EC Number: 232-164-6 / Cas Number: 7789-41-5 Molecular formula: CaBr2 Calcium Bromide solid, white (fine) powder
Administration / exposure	Inhalation, whole body Vehicle: not specified Concentrations: 204 mg/l Analytical verification of test atmosphere concentrations: no Duration of exposure: ca. 1 h No. of animals per sex per dose: 5 Control animals: no

	Details on inhalation exposure: During exposure, the rats were caged individually in compartmented wire-mesh exposure cages. The cages were placed in a 160-liter cubical stainless steel and glass chamber. A constant chamber airflow was maintained by means of a rotary centrifugal air pump located at the exhaust side of the chamber. The chamber exhaust was filtered with an activated charcoal filter and a Cambridge Absolute filter before being discharged outside of the laboratory.
Results and discussion	**Mortality:** There was no death occurring among the rats. **Clinical signs:** The immediate response of the rats to the experimental atmosphere was an increase of activity in preening. After 10 minutes of exposure, the rats began to settle down. There was no sign of toxicity observed during the 1-hour exposure. At the termination o [*text missing in registration]* **Body weight:** The body weight gain of the rats appeared to be normal throughout the 14-day observation period. All rats gained weight. The initial and final mean body weights were 212±1 0 and 269±36 grams, respectively.
Registrant's summary and conclusion	Inhalation exposure of rats for 1 hour to a dust atmosphere of Calcium Bromide at calculated concentration of 204 mg/l did not result in any observable toxic effects during or after the exposure.

3.8.1.1 [Study 6] Acute Inhalation Toxicity study of calcium bromide in Rats

Reference	Study report, 1978d
Guideline	No guideline. No GLP.
Reliability	2 (reliable with restrictions) (according to the REACH registration)
Species / strain	Rats, Charles River CD Male/female Body weight: males 220-248 grams, females 204-224 grams. The rats were housed individually in wire-mesh cages and were kept throughout the pre-and postexposure periods in a temperature and humidity-controlled room in accordance with standards outlined in the "Guide For the Care and Use of Laboratory Animals; DHEW No. (N.I.H. 74-23) 1974". Purina Laboratory Chow and water were supplied ad libitum except when the rats were in the exposure chamber.
Test material	Reference substance name: Calcium bromide EC Number: 232-164-6 Cas Number: 7789-41-5 Molecular formula: CaBr2 Calcium Bromide solution, clear liquid
Administration / exposure	Route of administration: inhalation (aerosol) Type of inhalation exposure: whole body Vehicle: not specified Concentrations: 2.5 and 203.3 mg/l Analytical verification of test atmosphere concentrations: no Duration of exposure: ca. 1 h Groups: 5/sex/dose Control animals: no Details on inhalation exposure: During exposure, the rats were caged individually in

compartmented wire-mesh exposure cages. The cages were placed in a 160 - liter cubical stainless steel and glass chamber. A constant chamber airflow was maintained by means of a rotary centrifugal air pump located at the exhaust side of the chamber. The chamber exhaust was filtered with an activated charcoal filter and a Cambridge Absolute filter before being discharged outside of the laboratory.

Results and discussion

Mortality: None of the rats died during the 1 hour exposure period or the 14-day observation period.

Clinical signs: Exposure Concentration, 203.3 mg/l: The immediate response of the rats to the experimental atmosphere was an increase of activity in preening. After several minutes of exposure, the rats began to settle down. Signs of toxicity seen during and immediate [*text missing in registration*]

Body weight:
Exposure Concentration, 203.3 mg/l:
The rats continued to gain weight inspite of their Transient lethargic conditions. The mean body weights and their standard deviations before exposure and at 14 days postexposure were 226 ± 1 9 and 279 ± 42 grams, respectively.
Exposure Concentration, 2.5 mg/l:
The rats gained weight throughout the entire experimental period. The mean body weights and their standard deviations before exposure and at 14-day postexposure were 223 ±12 and 273 ± 36 grams, respectively.

Gross pathology: There was no death among the animals. Therefore, no necropsy was conducted.

Registrant's summary and conclusion

Rats were exposed to the aerosols of Calcium Bromide Solution (417-130A), H-1510 at calculated concentrations of 203.3 and 2.5 mg/l respectively. The duration of exposure was 1 hour which was followed by a 14-day observation period. At 203.3 mg/l, all of the rats became lethargic for 4 days. Complete recovery was seen at 8 days postexposure. None of the rats exhibited adverse effects after exposure to 2.5 mg/l.

3.8.1.2 [Study 7] Acute dermal toxicity study of calcium bromide in rats

Reference

Study report, 1978a

Guideline

No guideline. No GLP.

Reliability

2 (reliable with restrictions) (according to the REACH registration)

Species / strain

Rabbit, New Zealand White
Male/female

Weight at beginning: 2304 - 2934 grams.
They were individually housed in hanging wire-mesh cages in temperature and humidity-controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication No. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Rabbit Chow were available ad libitum. Body weights were measured initially (control weight) and at 14 days.

Test material

Reference substance name: Calcium bromide
EC Number: 232-164-6 / Cas Number: 7789-41-5
Molecular formula: CaBr2

Administration / exposure

Type of coverage: occlusive
Vehicle: physiological saline

Test site:

- Area of exposure: back
- % coverage: 20-30% of the body surface
- Type of wrap if used: The area of application was wrapped with gauze bandaging and occluded with Saran Wrap

Removal of test substance:
- Washing: Twenty-four hours later the bandages were removed and the backs were washed with tepid tap water.
- Time after start of exposure: 24 hours

Doses:
- Amount(s) applied: 200 and 2000 mg/kg

Duration of exposure:
24 hours
No. of animals per sex per dose: 2
Control animals: no

Details on study design:
The hair was removed from the back of each rabbit (20 - 30% of the body surface) with an electric clipper. The rabbits were divided into 2 groups of 2 male and 2 female rabbits each. The skin of one male and one female in each group was abraded with a scalpel blade. The abrasions penetrated the stratum corneum but were not deep enough to cause bleeding. The test material was applied once only to the backs of the rabbits, using 0.9% physiological saline as the wetting agent, at the following dosage levels: 200 and 2000 mg/kg. The area of application was wrapped with gauze bandaging and occluded with Saran Wrap. Twenty-four hours later the bandages were removed, and the backs were washed with tepid tap water. They were observed at 24 hours and daily thereafter for a total of 14 days for mortality only.

Results and discussion

Mortality: One of 4 rabbits at the 2000 mg/kg dosage level died. None of the rabbits at the 200 mg/kg dosage level died during the observation period.

Registrant's summary and conclusion

The minimum lethal dose by the dermal route of administration was found to be less than 2000 mg/kg but greater than 200 mg/kg.
In accordance with the requirements of the Federal Hazardous Substances act this test material would not be considered a toxic substance by the dermal route of administration.

3.8.1.3 [Study 8] Acute dermal toxicity study of calcium bromide in rats

Reference

Study report, 1978b

Guideline

No guideline. No GLP.

Reliability

2 (reliable with restrictions) (according to the REACH registration)

Species / strain

Rabbit, New Zealand White
Male/female
Body weights at beginning of stud: 2300 - 2545 grams.
They were individually housed in hanging wire mesh cages in temperature and humidity-controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication No. 74-23 (N.T.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Rabbit Chow were available ad libitum. Body weights were measured initially (control weight) and at 14 days.

Test material

Reference substance name: Calcium bromide
EC Number: 232-164-6 / Cas Number: 7789-41-5
Molecular formula: CaBr2

Administration / exposure	Type of coverage: occlusive Vehicle: unchanged (no vehicle) Duration of exposure: 24 hours Doses: 200 and 2000 mg/kg No. of animals per sex per dose: 2 Control animals: no Details on study design: The hair was removed from the back of each rabbit (20-30% of the body surface) with an electric clipper. The rabbits were divided into 2 groups of 2 male and 2 female rabbits each. The skin of one male and one female in each group was abraded with a scalpel blade. The abrasions penetrated the stratum corneum but were not deep enough to cause bleeding. The test material was applied once only to the backs of the rabbits, as received, undiluted, at the following dosage levels: 200 and 2000 mg/kg. The area of application was wrapped with gauze bandaging and occluded with Saran Wrap. Twenty-four hours later the bandages were removed, and the backs were washed with tepid tap water. They were observed at 24 hours and daily thereafter for a total of 14 days for mortality only.
Results and discussion	**Mortality:** None of the rabbits died during the 14-day observation period.
Registrant's summary and conclusion	The minimum lethal dose by the dermal route of administration was found to be greater than 2000 mg/kg. Based upon the results obtained and the requirements of the Federal Hazardous Substances Act, the test material would not be considered a toxic substance by the dermal route of administration.

3.8.1.4 [Study 9] Acute oral toxicity study of potassium bromide

Reference	Study report, 1992
Guideline	EPA OPP 81-1 (Acute Oral Toxicity) GLP: Yes
Reliability	1 (reliable without restriction)
Species / strain	Species: rat Strain: CD strain (remote Sprague-Dawley origin) Sex: male/female

Details on test animals and environmental conditions:

- Source: Charles River (UK) Ltd., UK
- Age at receipt: 5-7 weeks
- Weight at study initiation: Males: 220-276 g; Females: 152-219 g
- Fasting period before study: Animals were fasted for an overnight period of approximately 17 h before dosing, but had free access to water. Food was given back immediately after administration of the test item.
- Housing: Animals were housed in high density polypropylene RC1 cages measuring 56x38x18 cm, with stainless steel grid floors and tops.
- Diet: Commercially-available rodent diet (Altromin 1324N), ad libitum
- Water: Tap water, ad libitum
- Acclimation period: 17 days

ENVIRONMENTAL CONDITIONS:

- Temperature: 21 ± 2 °C
- Humidity: 55 ± 15 %
- Air changes: 17 complete air changes per hour without recirculation
- Photoperiod: 12 hours dark / 12 hours light

IN-LIFE DATES: From: 1990-04-08 To: 1990-05-02

Test material

Reference substance name: Potassium bromide

EC Number: 231-830-3 / Cas Number: 7758-02-3

Molecular formula: BrK

IUPAC Name: potassium bromide

Test material form: solid: particulate/powder

Remarks: migrated information: powder

Details on test material:

- Name of test material (as cited in study report): Potassium bromide
- Physical state: White powder (crystalline)
- Lot/batch No.: 9320
- Date of receipt: 1990-01-22
- Storage condition of test material: Room temperature

Administration /exposure

Route of administration: oral: unspecified

Vehicle: other: distilled water

Details on oral exposure: MAXIMUM DOSE VOLUME ADMINISTERED: 10 mL/kg bw

Rationale for the selection of doses: Dose-levels for the main study were selected based on the results of preliminary range-finding study.

Doses:

- Preliminary study: 20, 200, 2000 and 5000 mg/kg bw
- Main study: 4000 and 5000 mg/kg bw

No. of animals per sex per dose:

- Preliminary study: 2/sex/dose
- Main study: 5/sex/dose

Control animals: no

Details on study design:

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing:

Clinical observations: Animals were inspected four times on the day of dosing and twice daily thereafter (once daily at weekends and holidays). Body weights were recorded on the day before dosing (Day 1), Days 1, 8 and before necropsy.

- Necropsy of survivors performed: Yes; On Day 15, all animals were killed by carbon dioxide

inhalation and were subjected to a macroscopic examination.

Statistics: None

Results and discussion

Preliminary study: One male and one female died at 5000 mg/kg bw. No mortality was observed in lower dose groups.

Effect levels:

Sex: male/female

Dose descriptor: LD50

Effect level: > 5 000 mg/kg bw

Based on: test mat.

Mortality, clinical signs, body weight and gross pathology

Mortality:

- Four animals (two males and two females) were died at 5000 mg/kg bw.
- One female was died at 4000 mg/kg bw.
- Deaths occurred 6-9 days after dosing.

Clinical signs:

- Prone posture, decreased motor activity, ataxia, hunched posture, eyelids partially closed, piloerection, bradypnoea, pigmented orbital secretion, thinness, urogenital staining and failure to groom. The swollen ears seen in some animals were not considered to be related to treatment.
- Treatment related signs had resolved 10 days after dosing.

Body weight:

- Several survivors had lost weight when weighed a week after dosing, but normal body weight gains were achieved at the second week following administration of test material.

Gross pathology:

- No abnormality related to treatment was observed in animals sacrificed at termination.
- In animals dying during the observation period changes considered related to treatment with Potassium bromide included: externally, emaciation, muzzle, urogenital and periorbital staining. Internally, in the stomach, duodenum, jejunum, ileum, caecum, colon and rectum, haemorrhagic contents were noted. On the gastric glandular mucosa, congestion, haemorrhage, dark foci and ulcerations were noted; on the non-glandular mucosa dark foci and wall thickening were observed. Other lesions included meningeal vascular congestion, darkening of the kidneys, mucosal congestion of the urinary bladder and blood stained or haemorrhagic contents.

Other findings: None

Table 7.2.1/1: Results of mortality

Dose level (mg/kg bw)	Mortality		
	Male	Female	Combined
Preliminary study			
20	0/2	0/2	0/4
200	0/2	0/2	0/4
2000	0/2	0/2	0/4
5000	1/2	1/2	2/4
Main study			
4000	0/5	1/5	1/10
5000	2/5	2/5	4/10

Registrant's summary and conclusion

Conclusions:

Under the test conditions, the oral LD50 for Potassium bromide is higher than 5000 mg/kg bw in rats therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

Executive summary:

In an acute oral toxicity study performed according to EPA OPP 81 -1 guideline and in compliance with GLP, groups (5/sex//dose) of CD strain of Sprague Dawley rats were given a single oral dose of Potassium bromide at 4000 and 5000 mg/kg bw. Animals were then observed for mortality, clinical signs and bodyweights for 14 days and were all sacrificed on Day 15 for macroscopic examination. Before the main study, a preliminary range-finding study was conducted with the dose-levels of 20, 200, 2000 and 5000 mg/kg bw.

Four animals (two males and two females) and one female were died at 5000 and 4000 mg/kg bw, respectively and deaths occurred 6-9 days after dosing. Clinical signs observed were prone posture, decreased motor activity, ataxia, hunched posture, eyelids partially closed, piloerection, bradypnoea, pigmented orbital secretion, thinness, urogenital staining and failure to groom. Treatment related signs had resolved 10 days after dosing. Several survivors had lost weight when weighed a week after dosing, but normal body weight gains were achieved at the second week following administration of test material. No abnormality related to treatment was observed in animals sacrificed at termination. In animals dying during the observation period changes considered related to treatment with Potassium bromide included: externally, emaciation, muzzle, urogenital and periorbital staining. Internally, in the stomach, duodenum, jejunum, ileum, caecum, colon and rectum, haemorrhagic contents were noted. On the gastric glandular mucosa, congestion, haemorrhage, dark foci and ulcerations were noted; on the non-glandular mucosa dark foci and wall thickening were observed. Other lesions included meningeal vascular congestion, darkening of the kidneys, mucosal congestion of the urinary bladder and blood stained or haemorrhagic contents. In this study, the combined oral LD50 of Potassium bromide was considered to be higher than 5000 mg/kg bw in rats.

Under the test conditions, the oral LD50 for Potassium bromide is higher than 5000 mg/kg bw in rats

therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

3.8.1.5 [Study 10] Acute oral toxicity study of potassium bromide

Reference Study report, 1994

Guideline OECD Guideline 401 (Acute Oral Toxicity)
Deviations: body weight of one female of the preliminary study was outside the range on Day 1
GLP compliance

Reliability 2 (reliable with restrictions)

Species / strain Species: rat
Strain: Rat Ico : OFA.SD. (IOPS Caw)
Sex: male/female

Details on test animals and environmental conditions:
TEST ANIMALS
- Source: Iffa-Credo, L' Arbresle Cedex, France.
- Age at study initiation: 5-7 weeks
- Weight at study initiation: Preliminary study (males: 161-169 g; females: 125-128 g); Main study (males: 167 g; females: 138 g)
- Fasting period before study: Animals were fasted for an overnight period. Food was given back approximately 4 hours after administration of the test item.
- Housing: Animals were housed in groups at 5 (main study) or 2 (preliminary study) of the same sex and dose group in polycarbonate cages type FI (305 x 180 x 184 mm) for the preliminary study and type MI (365 x 225 x 180 mm) for the main study.
- Diet: Pelleted complete diet, ad libitum
- Water: Filtered (0.2 µm) mains drinking water, ad libitum
- Acclimation period: 5 days

ENVIRONMENTAL CONDITIONS
- Temperature: 19-25 °C
- Humidity: 30-70 %
- Air changes: Minimum 8 air changes per hour
- Photoperiod: 12 hours dark / 12 hours light

Test material Reference substance name: Potassium bromide
EC Number: 231-830-3/ Cas Number: 7758-02-3
Molecular formula: BrK
IUPAC Name: potassium bromide
Test material form: solid: particulate/powder
Remarks: migrated information: powder
Details on test material:
- Name of test material (as cited in study report): Bromure de Potassium
- Physical state: White powder
- Analytical purity: Assumed to be 100 %
- Lot/batch No.: 3044038
- Date of receipt: 1994-09-02

- Storage condition of test material: Room temperature

Study design

ADMINISTRATION/EXPOSURE

Route of administration: oral: gavage

Vehicle: other: water for injection

Details on oral exposure:

- Concentration in vehicle: Test article as 2.5, 5.0 and 10.0 % (W/V) solution in the vehicle (preliminary study); test article as 10.0 % (W/V) solution in the vehicle (main study).

- Lot/batch no.: LD 4011

Maximum dose volume administered: 20 mL/kg bw

Doses:

- Preliminary study: 500, 1000 and 2000 mg/kg bw

- Main study: 2000 mg/kg bw

No. of animals per sex per dose:

- Preliminary study: 2/sex/dose

- Main study: 5/sex/dose

Control animals: no

DETAILS ON STUDY DESIGN

Duration of observation period following administration: 14 days

Clinical observations: Examinations for mortality and abnormal clinical signs were performed 15 minutes after administration, then at 1, 2 and 4 hours, and then daily for the 14 day study period.

Body weight: All the animals were weighed the day before treatment (Day 1), immediately before administration of the test article (Day 1), on Days 8 and 15.

Necropsy of survivors performed: Yes; On Day 15, all animals were killed by carbon dioxide inhalation and were subjected to a macroscopic examination.

Statistics:

- Statistical analysis were performed where appropriate using the following accepted methods:

- Body weights: Analysis of variance and Student t test (LD50),

- Mortality rate was calculated (as a percentage) to determine the innocuity or degree of toxicity of the test article,

- Calculation of the LD50 expressed in mg of the test article per kg of body weight with a 95 % confidence limit interval evaluated according to Bliss and Litchfield and Wilcoxon's methods.

Results and discussion

Preliminary study: No mortality was observed in any of the dose groups.

Effect levels

Sex: male/female

Dose descriptor: LD50

Effect level: > 2 000 mg/kg bw

Based on: test mat.

Clinical signs, bodyweight, mortality and gross pathology

Mortality: No mortality was observed.

Clinical signs: All animals showed subdued behaviour from 15 minutes after administration of the test article to Day 2 as well as infrequent stools on Day 2. All animals were normal from Day 3 onwards.

Body weight: Body weight was not affected by treatment.

Gross pathology: No macroscopic abnormalities related to treatment were observed.

Other findings: None

Registrant's summary and conclusion

Conclusions

Under the test conditions, the oral LD50 for Bromure de Potassium is higher than 2000 mg/kg bw in rats therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

Executive summary:

In an acute oral toxicity study (limit test) performed according to OECD Guideline 401 and in compliance with GLP, group (5/sex/dose) of Ico : OFA.SD. (IOPS Caw) rats were given a single oral (gavage) dose of Bromure de Potassium at 2000 mg/kg bw. Animals were then observed for mortality, clinical signs and bodyweights for 14 days and were all sacrificed for macroscopic examination. Before the main study, a preliminary study was conducted with the dose-levels of 500, 1000 and 2000 mg/kg bw.

No mortality was observed. All animals showed subdued behaviour from 15 minutes after administration of the test article to Day 2 as well as infrequent stools on Day 2. All animals were normal from Day 3 onwards. Body weight was not affected by treatment. No macroscopic abnormalities were observed at study termination on Day 15. In this study, the combined oral LD50 of Bromure de Potassium was considered to be higher than 2000 mg/kg bw in rats.

Under the test conditions, the oral LD50 for Bromure de Potassium is higher than 2000 mg/kg bw in rats therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

3.8.1.6 [Study 11] Acute oral toxicity study of sodium bromide

Reference

A6.1.1/02, Doc. No. 521-002

Study report, 1988a

Guideline

According to guideline: EPA FIFRA 81-1

Equivalent or similar to guideline: OECD Guideline 401 (Acute Oral Toxicity)

Deviations: no

GLP: Yes

Reliability

1 (reliable without restriction)

Species / strain

Species: rat

Strain: CD [Crl: CD(SD)BR]

Sex: male/female

Details on test animals and environmental conditions:

- Source: Charles River U. K. Limited, Margate, Kent, England

- Age at study initiation: six weeks

- Weight at study initiation: 112 to 150 g

Test material

Reference substance name: sodium bromide

EC Number:231-599-9 / Cas Number: 7647-15-6

Molecular formula: NaBr

IUPAC Name: Active bromine generated from sodium bromide and sodium hypochlorite
Details on test material:
- Name of test material (as cited in study report): Active ingredient ´Sodium bromide, technical grade (99,23 %)
- Description: White powder
- Analytical purity: 99.23 %
- Lot/batch No.: Batch No.: 7320
- Stability under test conditions: not determined

Study design

ADMINISTRATION/EXPOSURE
Route of administration: oral: gavage
Vehicle: other: 1 % aqueous methylcellulose
Details on oral exposure:
- Concentration in vehicle: Sodium bromide was prepared at various concentrations in 1% aqueous methylcellulose and administered at a volume of 20 ml/kg.
- Amount of vehicle (if gavage): 20 ml/kg bodyweigh
MAXIMUM DOSE VOLUME APPLIED: 20 ml/kg bodyweight

Doses: Preliminary study: 2 g/kg bw and 5 g/kg bw
Main Study: 3.2 g/kg bw, 4 g/kg bw and 5 g/kg bw
No. of animals per sex per dose: 5/sex/group
Control animals: no

DETAILS ON STUDY DESIGN
- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: Animals were observed for clinical signs and mortality soon after dosing and at frequent intervals for the remainder of day 1. On subsequent days the animals were observed twice daily for 5 (preliminary study) and 14 days (main study) respectively.
Individual bodyweights of rats were taken on Days 1 (day of dosing), 8 and 15 and at death.
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight; A pre-test was carried out to establish a dosing regimen using groups of two male and two female rats at two dose levels of 2 and 5 g/kg bodyweight. Animals were observed for mortality and clinical signs for 5 days.

Results and discussion

Preliminary study: The results indicated that the acute median lethal oral dose of sodium bromide, technical grade, was greater than 5 g/kg bodyweight for male rats and between 2 and 5 g/kg bodyweight for female rats.

Effect levels

Sex: male/female

Dose descriptor: LD50

Effect level: 4.2 other: g/kg bw

Clinical signs/mortality/gross pathology

Mortality: Two female rats dosed at 5 g/kg bodyweight died on Day 4 of preliminary study. All other animals survived during the study period of five days. During the main study there were deaths amongst rats of both sexes dosed at 4 and 5 g/kg. Deaths occurred from Day 3 to Day 7. One male was sacrificed after being found moribund on Day 5.

Clinical signs: Signs of reaction to treatment observed were recorded for animals in the main study only. All treated animals showed piloerection within 5 minutes of dosing and abnormal body carriage (hunched posture), abnormal gate (waddling), lethargy, decreased respir

Gross pathology: Autopsy of rats that died commonly revealed slight congestion of the glandular region of the stomach; red coloured fluid was observed in the urinary bladder of a single male (4 g/kg); isolated cases of congestion or vascular congestion of other zones of the gastrointestinal tract. Terminal autopsy findings of rats killed at the end of the study, were normal.

Table A6.1.1/02-1 Summary of Acute Oral Toxicity

Dose g/kg	Number of dead / number of investigated		Time of death (range)	Observations
	male	female		
3.2	0/5	0/5	-	Piloerection, hunched posture, waddling, lethargy, decreased respiratory rate, ptosis, pallor of extremities, prostrate
4	1/5	3/5	Day 5-7	Piloerection, hunched posture, waddling, lethargy, decreased respiratory rate, ptosis, pallor of extremities, ataxia, prostrate
5	4/5	5/5	Day 3-6	Piloerection, hunched posture, waddling, lethargy, decreased respiratory rate, ptosis, pallor of extremities, ataxia, prostrate, moribund (apathy, prostrate, cyanosis, decreased respiration)
LD_{50} value: 4.5 g/kg bw (males), 3.9 g/kg bw (females), 4.2 g/kg bw (combined)				

Registrant's Conclusion

The acute oral LD50 was determined to be 4.5 g/kg bw (males), 3.9 g/kg bw (females) and 4.2 g/kg bw (both sexes combined). In accordance with CLP Regulation (EC) No 1272/2008, sodium bromide does not have to be classified and labelled with respect to acute oral toxicity.

Registrant's executive summary

Materials and methods

The study was designed to assess the toxicity following a single oral dose of sodium bromide. Groups of 5 fasted CD rats received a single oral dose of the test substance formulated in 1 % aqueous methylcellulose and administered at a volume of 20 ml/kg and dose levels of 3.2; 4 and 5 g/kg bw. Animals were observed for mortality and clinical signs soon after dosing, at frequent intervals for the

remainder of day 1 and twice daily on the subsequent days. Body weights were taken on Day 1, 8 and 15 and at death. All animals were subject to necropsy and LD50 was determined.

Results and discussion

Signs of reaction observed were piloerection, abnormal body carriage, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities, ataxia and prostration. There were death amongst rats of both sexes dosed at 4 and 5 g/kg. Autopsy of these rats revealed slight congestion of the glandular region of the stomach; red coloured fluid was observed in the urinary bladder of a single male (4 g/kg) and isolated cases of congestion or vascular congestion of other zones of the gastrointestinal tract were observed. Low bodyweight gains were recorded for surviving rats at all dose levels. Body weight losses were recorded for all rats that died

3.8.1.7 [Study 12] Acute oral toxicity study of ammonium bromide

Reference	A6.1.1/01, Doc. No. 521-001 Study report, 1986a
Guideline	OPP 81-1 which is comparable to OPPTS 870.1100 and OECD guideline 401 GLP Yes Deviations No
Reliability	Reliability 1 (reliable without restriction)
Species / strain	Species Rat Strain CD strain (remote Sprague-Dawley origin)
Test material	NH4Br (Ammonium Bromide) White crystalline powder Purity n.a. Stability: Material was kept at ambient temperature in the original container and was considered to be stable under these storage conditions.
Study design	**Test Animals** Sex: Male and female Age/weight at study initiation: At the time of administration of the test substance, males were within the bodyweight range 112-143 g and females 110-129 g. The animals were about 5 weeks old at this time. Number of animals per group: 5/sex/group Control animals No **Administration/Exposure** Oral gavage Postexposure period: 14 days Concentration: 2000/2714/3684/5000 mg/kg bw Vehicle: Distilled water Concentration in vehicle

The test material was prepared at appropriate concentrations in distilled water to permit administration at a constant volume-dosage of 20 ml/kg bodyweight.
Total volume applied: 20 ml/kg bw

Examinations

Post-treatment observations at least twice daily during the first 7 days after dosage and daily observations until day 15;
body weight was taken the day before and the day of dosage and then once per week; necropsy at the end of the post-observation period

Method of determination of LD50: Probit analysis by the method of Finney (1952)

Findings

RESULTS AND DISCUSSION

Clinical signs: The most frequent observations were lethargy, decreased motor activity, prone or hunched posture, ataxia and breathing irregularities.
Unconsciousness and tonic convulsions were also observed in a smaller number of animals.
All survivors receiving 2000 mg/kg had recovered fully by the morning of Day 3. All four male survivors receiving 2714 mg/kg had recovered by the morning of Day 4 and the single female of that dose group had recovered by Day 6.
Mortalities: There were no deaths among rats treated at 2000 mg/kg ammonium bromide. One male and four female rats treated at 2714 mg/kg died within one hour after dosing. All animals receiving 3684 mg/kg and 5000 mg/kg died between 30 minutes and one day after dosing.
Pathology: The necropsy of animals dying following administration of ammonium bromide revealed fur staining, abnormal gastro-intestinal contents, dark areas on the lungs and occasional thymic petechiae. Animals killed on day 15 showed enlarged cervical lymph nodes in four males and a dark submandibular salivary gland in one male. None of these lesions were considered to be an effect of the test material.
Other: All surviving animals achieved anticipated bodyweight gains over the 14-day study period.
LD50 2868 mg/kg (male), 2566 mg/kg (female), 2714 mg/kg (combined)

Table A6.1.1/01-1 Summary of Acute Oral Toxicity

Dose [mg/kg]	Number of dead / number of investigated		Time of death (range)	Observations
	Male	Female		
2000	0/5	0/5		Lethargic, unconscious, decreased motor activity, hunched, ataxia, prone, musculature tremor, bradypnoea, hyperpnoea, salivation, pigment of snout (duration of signs: 15 minutes – 2 days)
2714	1/5	4/5	½ - 1 hour	Lethargic, unconscious, decreased motor activity, hunched, ataxia, prone, bradypnoea, hyperpnoea, piloerection, ungroomed (duration of signs: 15 minutes – 5 days)
3684	5/5	5/5	½ - 48 hours	Lethargic, unconscious, decreased motor activity, hunched, ataxia, prone, tonic convulsions, bradypnoea, hyperpnoea, piloerection
5000	5/5	5/5	¼ - 1 hour	Prone, gasping
LD_{50} value	2868 mg/kg bw (male); 2566 mg/kg bw (female), 2714 mg/kg bw (combined)			

Conclusions REGISTRANT'S SUMMARY AND CONCLUSION

Materials and methods

The study was designed to investigate the acute oral toxicity of ammonium bromide in rats.

The test material was administered to groups of five male and five female rats as a single oral dose of 2000, 2714, 3684 and 5000 mg/kg at a constant volume of 20 ml/kg in distilled water.

Mortality, signs of reaction to treatment and body weight gain were recorded during a subsequent 14-day observation period after which LD50 was determined. Decendents and animals killed on day 15 were subjected to necropsy.

Results and discussion

The principal signs of reaction comprised lethargy, decreased motor activity, prone and hunched posture, ataxia and breathing difficulties.

Unconsciousness and tonic convulsions were also observed in a smaller number of animals.

Necropsy findings included fur staining, abnormal gastro-intestinal contents, dark areas on the lungs and occasional thymic petechiae. All survivors achieved anticipated bodyweight gains and necropsy findings on day 15 were unremarkable.

Conclusion The LD50 in this study was determined to be 2868 mg/kg bw (males), 2566 mg/kg bw (females) and 2714 mg/kg bw for both sexes combined.

3.8.2 Human data

3.8.2.1 [Study 1]

Study reference:

Gosselin R. E. (1976). Clinical Toxicology of Commercial Products. 4th ed. Baltimore: Williams and Wilkins, p. II-77.

3.8.2.2 [Study 2]

Study reference:

Grant W. M. (1974). Toxicology of the Eye. 2nd ed. Springfield, Illinois: Charles C. Thomas, p. 199, 200.

3.8.3 Other data

3.9 Specific target organ toxicity – repeated exposure

3.9.1 Animal data

3.9.1.1 [Study 1] Sub-chronic repeated dose toxicity: oral in dog

Reference	A6.10/17, Doc. No. 592-032
	March, P.A. Podell, M., Sams, R.A. (2002). Pharmacokinetics and toxicity of bromide following high-dose oral potassium bromide administration in healthy beagles. J. Vet. Pharmacol. Therap. 25, 425-432
Guideline	Not applicable, no guideline available for this particular investigation; study was performed according to good experimental practice.
	GLP: No
	Deviations: Not applicable, no guideline available for this particular investigation.
Reliability	Reliability 2
	Deficiencies Not applicable, no guideline available for this particular investigation
Species / strain	Species Dog
	Strain Beagle
	Sex male and female
Test material	Potassium bromide
Study design	3.2 Test Animals
	3.2.5 Age/weight at study initiation: Mean bodyweight was 11.3 kg (range 9.6-13.7 kg)
	3.2.6 Number of animals per group 3/sex
	3.2.7 Control animals No
	3.3 Administration/
	Exposure: Oral
	3.3.1 Duration of treatment 115 days
	3.3.2 Frequency of exposure: Daily
	3.3.3 Postexposure period: Not indicated
	3.3.4 Type in food
	3.3.5 Concentration 30 mg/kg bw/day
	3.3.6 Vehicle Distilled water
	3.3.7 Concentration in vehicle: 200 mg/mL distilled water, mixed with canned food

3.3.8 Total volume applied Not indicated

3.3.9 Controls No control animals included

3.4 Examinations

3.4.1 Observations

3.4.1.1 Clinical signs Yes; once per week

3.4.1.2 Mortality Not examined

3.4.2 Body weight Yes; once per week

3.4.3 Food consumption Yes

3.4.4 Water consumption: Not examined

3.4.5 Ophthalmoscopic examination: Not examined

3.4.6 Haematology Yes; complete blood count was performed during the acclimatisation period only (prior to start of the experiment).

3.4.7 Clinical Chemisty Yes; bromide concentration was determined periodically in order to determine attainment of a steady-state level. After the steady-state was attained, venous blood samples were collected every two hours over a 12-hour dosing interval in all dogs to determine if serum bromide concentrations underwent fluctuations between doses.

3.4.8 Urinalysis Yes; 24-hour collection every three days

3.5 Sacrifice and pathology

3.5.1 Organ Weights Not examined

3.5.2 Gross and histopathology

Muscle and nerve biopsies were collected at the termination of the study.

Quadriceps, cranial tibial and triceps muscle and sciatic nerve were submitted for histochemical evaluation. No further gross and histopathological examinations were performed.

3.5.3 Other examinations

Bromide concentrations in cerebrospinal fluid were determined. Samples were collected on Days 0, 9, 45 and 115. A complete neurologic examination was performed weekly and the degree of ataxia and/or paresis was scored. Electrodiagnostic testing was performed on Days 0, 9, 45 and 115. Blood samples were always collected just before dosing in the morning. Following serum separation, 0.5 ml aliquots were frozen (-20°C) until analysis. Cerebrospinal fluid (CSF) was collected under anesthesia from the cerebellomedullary cistern under aseptic conditions. Samples were centrifuged (2100 g, 5 min) and 0.5 ml aliquots were frozen. CSF cytology was performed on all samples to ensure that no blood contamination had occurred. Serum and CSF bromide concentrations in thawed samples were measured using a modified gold chloride method. For each assay standard curves were obtained and regression analysis were performed to calculate the concentrations. Urine aliquots from 24- hour samples of 1.5 ml were centrifuged (2100 g, 5 min) and samples were stored at -20°C until analysis. Urine bromide was measured using a bromide ion selective electrode which was calibrated for each assay using known standards. All serum, CSF and urine samples were run in duplicate or triplicate.

For EEG recordings, subjects were administered meperidine (5 mg/kg, i.m.) followed in 20 minutes by acepromazine (0.05-0.1 mg/kg i.v.). Platinum subdermal needle electrodes were placed over the scalp using a modified 10-20 electrode placement. Recording conditions included input range ±600 mV, high frequency filter 70 Hz and electrode impedance less than 15k□. EEG signals were quantified on-line using quantitative EEG software. For subsequent testing, dogs were induced with 2 % thiopental sodium intravenously, intubated and maintained on isoflurane in a semiclosed system. EMG, SSEPs, and BAERs were recorded using previously established methods. MNCV and RNS of the sciatic/tibial and ulnar nerves were performed using standardized protocols. A Nihon Kohden EMG/evoked potential unit was used for all EMG and evoked potential recordings.

3.5.4 Statistics One- compartment model for all sample results assuming a constant rate of administration of bromide. PK Analyst software was used to perform nonlinear, least-squares regression analysis.

Findings

4.1 Observations

4.1.1 Clinical signs There were no adverse neurologic side-effects in any of the dogs at an oral dose of 30 mg/kg bw/day. Following dose adjustment, two of the dogs exhibited grade III-IV caudal paresis and ataxia characterized by a wide-based and/or crouched pelvic limb stance, difficulty rising from a sitting position and decreased hemistanding and flexor withdrawal reflexes in the pelvic limbs. Neither the magnitude of nor the relative change in the serum bromide concentration was related to the development of neurologic deficits. Two of the dogs were agitated to hyperexcitable following the dose adjustment phase but neither showed

signs of weakness or ataxia.

4.1.2 Mortality No mortalities reported in this investigation

4.2 Body weight/body weight gain: Mean bodyweight throughout the study initiation was 11.3 kg, with a range of 9.6-13.7 kg.

4.3 Food consumption and compound intake: Food consumption and compound intake were observed by eye only. For that reason no further specification is available.

4.4 Water Consumption: No analysis of water consumption performed in this investigation.

4.5 Ophthalmoscopic examination: No ophthalmoscopic examination performed in this investigation.

4.6 Blood analysis

4.6.1 Haematology No haematological analysis performed after bromide administration.

4.6.2 Clinical chemistry The mean steady-state serum bromide concentration was 245 mg/dL. Steady-state bromide concentrations were widely variable between dogs, ranging from 178 to 269 mg/dL. From 60 days until the final sampling time during maintenance dosing (115 days), serum bromide concentrations increased very slowly. The subsequent dose adjustment successfully raised the serum bromide concentration of 400 mg/dL. The median serum bromide concentration post-dose adjustment was 397 mg/dL.

The mean median t1/2 using a one-compartment model was 15.2 days. A majority of dogs reached a serum bromide concentration that was 75% of the apparent steady-state concentration by 30 days and at least 90% of the apparent steady-state concentration by 60 days.

4.6.3 Urinalysis Median renal clearance was 8.2 mL/kg bw/day . Renal clearance varied between dogs (range 6.03-12.6 mL/kg bw/day) and was not correlated with total body clearance.

4.7 Sacrifice and pathology

4.7.1 Organ weights No organ weights were taken in this investigation.

4.7.2 Gross and histopathology: No gross and histopathology was performed in this investigation.

4.8 Other Electrodiagnostic tests revealed subtle changes over time. Spectrally analyzed EEG frequencies either remained unchanged or showed insignificant reductions in power at all frequencies with increasing bromide concentrations. Examination of individual trends revealed central latency shifts that either progressively increased over time or else coincided with the appearance of adverse side-effects. Cortical somatosensory potentials remained constant in latency and amplitude with no significant changes over time. The electromyography, motor nerve conduction velocity, repetitive nerve stimulation and characteristics of the compound muscle action potential after peripheral nerve stimulation were all normal at all time points and did not show significant differences over time. Muscle and nerve biopsies from all dogs were normal. Brain stem auditory evoked response latencies were prolonged with increasing serum bromide concentrations, then appeared to stabilize or even decrease slightly. After a dose increase, latencies again increased.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The purpose of the study was to develop a multidose method of bromide administration that targets serum bromide concentrations in the range of 200-300 mg/dL and to examine the dynamics of bromide pharmacokinetics during the accumulation phase and at steady-state.

Pharmacokinetic parameters from other studies have served as the basis for currently recommended KBr maintainance doses in the dog.

Recommended doses of 30-40 mg/kg bw/day of potassium bromide (20-27 mg Bromide/kg bw) will hypothetically produce a steady-state bromide concentration of 100-200 mg/dL. Therefore, the selection of the initial maintenance dose was based on calculation of a dose required to maintain a steady-state serum concentration of 275 mg/dL. Previously reported bromide pharmacokinetic values were used to calculate the maintainance dose.

Prior to administration of 30 mg/kg bw/day of potassium bromide (mixed with canned food over 12 hours) for a period of 115 days, baseline serum, urine and cerebrospinal fluid bromide concentrations were measured.

Urine was collected over a 24-hour period and total volume was recorded.. The daily dose was divided in order to reduce the likelihood of gastrointestinal side-effects.

After steady-state was attained, blood samples were collected every 2 hours over a 12-hour dosing interval in all dogs in order to determine if serum concentrations of bromide underwent significant fluctuations between doses. Cerebrospinal fluid samples were taken on Days 0, 9, 45 and 155. On the same days electrodiagnostic testing was performed. A complete neurologic examination was performed once per week and the degree of ataxia and/or paresis were scored. Bodyweights were determined weekly. Consumption of food was observed by eye to ensure entire uptake of the test substance.

Subsequent to Day 115, an adjusted loading dose of potassium bromide was administered as divided doses over a period of 5 days to rapidly increase and maintain bromide serum concentrations at 400 mg/dL. Dose adjustment was designed to increase serum bromide to a new steady-state concentration rapidly in the event of inadequate seizure control but may also increase the risk of bromide toxicity. The loading dose required to achieve the new stead-state concentration was calculated using the following formula:

Volume distribution at steady-state x (target serum concentration – observed serum concentration) with 400 mg/dL being the target serum concentration. After this 5-day-period, serum, urine and cerebrospinal fluid were collected and neurodiagnostic testing was performed. Following the supplemental loading dose, all dogs were administered a maintenance dose calculated to maintain a 400 mg/dL bromide serum concentration.

Muscle and nerve biopsies were collected at termination of the study (quadriceps, cranial tibial, and triceps muscle and sciatic nerve were submitted for histochemical evaluation).

Electrodiagnostic testing (electroencephalography (EEG), electromyography (EMG), motor nerve conduction velocity (MNCV), repetitive nerve stimulation (RNS), brain stem auditory evoked responses (BAERs), and cortical somatosensory evoked potentials (SSEPS)) was performed at Days 0, 9, 45 and 115.

5.2 Results and discussion

The results of all pharmacological parameters determined are summarized in table A6.10/17-1. Steady-state bromide concentrations were variable (range 178-268 mg/dL) reflecting differences in clearance and/or bioavailability between dogs. The mean steady-state serum bromide concentration was 245 mg/dL. From 60 days until the final sampling time during maintenance dosing (115 days), serum bromide concentrations increased very slowly. The subsequent dose adjustment successfully raised the serum bromide concentration of 400 mg/dL. The median serum bromide concentration postdose adjustment was 397 mg/dL. The mean median t1/2 using a one-compartment model was 15.2 days. Median apparent total body clearance was 16.4 mL/kg/day and median apparent volume of distribution was 0.4 L/kg. Median renal clearance was 8.2 ml/kg/day, varied between dogs and was not correlated with total body clearance. The CSF to serum bromide concentration ratio was 0.63 at 9 days after initial dosing (see also table A6.10/17-2). After a significant increase in the ratio between 9 and 45 days, there was no significant change in this ratio between Day 45 and 115. The ratio increased significantly from days 115 to 121, coincident with the supplemental dose that was administered. The median CSF bromide concentration postdose adjustment was 353.3 mg/dL and the median CSF to serum ratio was 0.86.

There were no adverse neurologic side-effects from Day 0 to 115 in any of the dogs. Following dose adjustment, two dogs exhibited caudal paresis and ataxia characterized by a wide-based and/or crouched pelvic limb stance, difficulty rising from a sitting position, and decreased hemistanding and

flexor withdrawal reflexes in the pelvic limbs. Neither the magnitude of nor the relative change in the serum bromide concentration was related to the development of neurologic deficits.

Electrodiagnostic tests revealed subtle changes over time. EEG frequencies and SSEPs either remained unchanged or showed insignificant reductions in power with increasing bromide concentrations. EMG, MNCV, RNS and characteristics of the compound muscle action potential after peripheral nerve stimulation were. Muscle and nerve biopsies from all dogs were normal. BAER latencies were prolonged with increasing serum bromide concentrations, than appeared to stabilize or even decrease slightly. After a dose increase, latencies again increased. These findings suggest that conduction along peripheral and central sensory pathways may be delayed when serum and CSF bromide concentrations are elevated. Slowed neuronal conduction may be related to the hyperpolarizing effect of bromide on neuronal membrane potential.

Conclusions

5.3 Conclusion There were no adverse neurologic side-effects in any of the dogs treated with 30 mg/kg/day of sodium bromide. Neither the magnitude of nor the relative change in the serum bromide concentration was related to the development of neurologic deficits. The mean steady-state serum bromide concentration was 245 mg/dL and the mean median t1/2 was 15.2 days.

Only BAER latencies were prolonged with increasing serum bromide concentrations. These findings suggest that conduction along peripheral and central sensory pathways may be delayed when serum and CSF bromide concentrations are elevated. The BAER may be useful in documenting and assessing the severity of clinical bromism.

5.3.1 LO(A)EL 400 mg bromide/dL (serum concentration)

5.3.2 NO(A)EL 269 mg bromide/dL (serum concentration)

3.9.1.2 [Study 2] Repeat Dose (gavage) 90-Day Toxicity Study of sodium bromide in Rats, Including Recovery Assessments

Reference

Study report, 2016b

Guideline

OECD Guideline 408, 1998. Repeated Dose 90-day Oral Toxicity Study in Rodents. With the relevant sections relating to oestrous cycles, sperm evaluation and histopathology OECD Guideline 416, 2001. Two-Generation Reproductive Toxicity Study

EPA Health Effects Test Guideline OPPTS 870.3100: 90-Day Oral Toxicity in Rodents

Reliability

Reliability: 1

Species / strain

Rat, Crl:CD(SD)

Test material

Sodium Bromide
Batch (Lot) No.: 710120333
CAS Number: 7647-15-6
Physical Description: White crystalline solid
Purity: 100.0%

Study design

The study design was as follows:

Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Males No. of Rats	Females
1. R.O. deionized water	0	5	10a + 10b	10a + 10b
2. Sodium Chloride	284	5	10a + 10b	10a + 10b
3. Sodium Bromide	60	5	10a	10a
4. Sodium Bromide	175	5	10a	10a

5. Sodium Bromide	500	5	10a + 10b	10a + 10b

a. The first 10 rats were euthanized following completion of the dosing period (Day 91 of study).

b. The remaining 10 rats were euthanized following completion of the 8 week recovery period (Day 148 of study).

The test or control substances were administered to the appropriate rats by once daily oral gavage from Days 1 to 90 of Study (DSs 1 to 90). The sodium chloride group was included to determine the effect of a sodium dose of equivalent osmolarity to that of the high dose sodium bromide group, and hence to distinguish effects of the sodium and bromide components of the test material.

Findings

Sodium Chloride Comparator Group

Administration of 284 mg/kg/day sodium chloride did not result in mortality, adverse clinical observations, or changes in body weight, food or water consumption. There was no effect on ophthalmic observations, hematology (including coagulation values), urine or clinical chemistry values. T3, T4 and TSH hormone levels were not affected although mean thyroid weights were higher than in any other group. There was no effect on organ weights, histopathology, or reproductive parameters (including estrous cycles, ovarian follicle counts or sperm count,

motility and morphology).

GENERAL TOXICITY:

MORTALITY, CLINICAL CONDITION (ROUTINE AND DETAILED CLINICAL OBSERVATIONS)

There were no deaths in the control group but one male (1558) showed adverse clinical signs and lung changes at histopathology which were considered likely due to aspiration of control substance into the lungs . Other control males and females showed common, transient signs (e.g. scab, misaligned incisors, swollen ear, ungroomed coat) during the treatment and recovery periods in one or two animals/sex only.

All males and females treated at 175 mg/kg/day survived to scheduled euthanasia. In males, treatment related signs of decreased motor activity postdose were observed in 6/10 males in week 2 but all animals recovered before the end of the working day. In females, these signs were only observed on DS 11-13, in 8/10 animals. Other clinical signs, including chromodacryorrhea, mild dehydration, swollen ear and/or periorbital area and hunched posture were generally infrequent and transient in both sexes.

At 500 mg/kg/day, multiple clinical signs, including numerous episodes of ataxia and decreased motor activity, prostration or breathing abnormalities (tachnypnea/dyspnea /hyperpnea), limb abnormalities (limited or no use and/or swollen/lacerated/purple color, no grip reflex in fore or hindlimbs) and signs indicative of poor condition (dehydration, ungroomed coat, chromodacryorrhea, chromorhinorrhea, fur staining), were considered evidence of marked toxicity in all males. In 4 of these animals, these signs were so severe as to require euthanasia on DS 52, 55, 86 or 107: all of these males had shown marked reductions in body weight, and 2/4 had decreased food intake. At necropsy, 2/4 had tan, firm lung lobes with masses: one also had tan material in the trachea and the other adhesions to the pericardium. Histopathology confirmed the presence of bacterial infections in these animals, possibly related to mis-intubation or aspiration of the dosing solution in animals which may have still been affected by the dose from the previous day.

In surviving males clinical signs, consistent with the known sedative effects of sodium bromide, were apparent from 2 hours after dosing on the first day of treatment in 5 males and increased in incidence, duration and severity over the treatment period, with all animals showing decreased motor activity by week 3 and all males showing ataxia/prostration which persisted beyond the end of the working day by week 11. Significant increases ($p \leq 0.01$, compared to controls) were observed in the incidence of decreased motor activity, dehydration (mild and moderate), ataxia, ungroomed coat, urine-stained abdominal fur, hunched posture, chromodacryorrhea, ptosis, low carriage and limited use of

limb(s)/paw(s). Impaired righting reflex , first observed in week 8 in 2 males, affected 3 males by the end of the treatment period. After approximately 6 weeks of dosing, one or more of these observations persisted throughout the working day, and was still evident at predose the next day in 2 or more males for the remainder of the dosing period. The number of males in poor clinical condition (defined by limbs/paws red or purple and/or swollen, scabbed, chromorhinorrhea, reduced feces, thin, piloerection, head tilt, hypernea, lost grip reflex, abrasion and/or mass present in the eye, limbs, paws or inguinal area) was also increased compared to the control group and, although not statistically significant, was considered treatment-related as many effects persisted throughout the dose period, then became less evident in the recovery period. After treatment ceased, 8 males ($p \leq 0.01$) continued to exhibit decreased motor activity, ungroomed coat, urine stained abdominal fur and dehydration for up to 30 days of the recovery period. The severity and incidence of these signs generally decreased with time off- dose but one male still showed signs of lack of grooming (urine-stained abdominal fur) until DS 120.

Females showed similar clinical signs, with significant increases ($p \leq 0.01$) in ataxia, decreased motor activity, hunched posture, ptosis, low carriage and limited use of limb(s)/paw(s), chromodacryorrhea, ungroomed coat, urine-stained abdominal fur and dehydration (mild and moderate) but they appeared later in the treatment period, did not persist until the following day and recovery was faster, suggesting a higher tolerance than males. Decreased motor activity was first observed in 5 females from 1 hour after dosing on DS 10, increasing to 16 females during the second week of treatment and generally affecting all females by week 3. Ataxia/prostration began after 2 weeks of dosing, generally between 1 to 2 hours after dosing and persisting until the end of the normal working day, initially in 9 females and increasing to 18 during week 8.

Impaired righting reflex was also observed in week 8. During the recovery period, ataxia/decreased motor activity was not apparent from the first day off-dose but 3 females continued to exhibit ungroomed coat, urine-stained abdominal fur and dehydration, for up to 9 days after treatment ceased.

Detailed clinical observations recorded once weekly generally confirmed the daily clinical observations reported above.

FUNCTIONAL OBSERVATION BATTERY & MOTOR ACTIVITY

Functional observations and motor activity generally reflected the clinical observations.

There was no effect of treatment at 60 mg/kg/day on functional observations or motor activity at any of the scheduled evaluations.

Functional observations were not affected by treatment at 175 mg/kg/day. Effects on motor activity were limited to significantly increased ($p \leq 0.05$) ambulations in week 4 (prior to dosing), only at the first 10 minute interval and only in males.

At 500 mg/kg/day, when assessed predose during week 4 of treatment, males showed a decreased incidence of rears, and two animals exhibited ataxia and abnormal gait. These signs were not apparent in females but there was evidence of lack of righting reflex in 5 females (4 landing on their side, one on the back) and a reduction in hindlimb grip strength. Increased motor activity ($p \leq 0.05$) was observed in males (ambulation and fine movements) and females (ambulations only).

When assessed postdose in week 9, ataxia and abnormal gait were observed during the Functional Observation Battery in all males and in 6/10 females, respectively. Five females also showed unusual posture. Increased ambulations ($p \leq 0.05$) were recorded for both sexes at the first 10 minute interval of motor activity monitoring only, and the incidence of unkempt appearance/stained fur was 4-6 in males and 3-5 in females.

By week 13 (post-dosing), although the number of males showing ataxia was slightly lower (7), 5 animals showed unusual posture and 8 showed lack of air righting response (landing on their side). Signs indicating lack of grooming (unkempt, urine/fecal staining) were more frequent and 9/10 males urinated in the open field. In females, ataxia was observed in 8 females which was a slightly higher incidence than week 9 (6 females), abnormal gait and unusual posture had increased slightly and 8/10 females were sleeping in the home cage prior to examination.

Decreased fine movement was observed in males but there was no effect on motor activity in females.

By the end of the recovery period, functional effects were limited to the males. There was an increased incidence of urination in the open field and there was an increase in forelimb grip strength (mean and maximum).

BODY WEIGHT

There was no significant effect of treatment at 60 or 175 mg/kg/day on body weight or body weight gain in males and females, although values at 175 mg/kg/day were very slightly lower than controls. At the end of the treatment period mean body weights of males were 97.2% and 93.0% of controls and for females 108.7% and 95.2%, in the 60 and 175 mg/kg/day groups, respectively. Body weight gain was similar, with values for males of 97.3% and 90% of controls and for females 118.1% and 92.1% of controls, in the 60 and 175 mg/kg/day groups, respectively.

At 500 mg/kg/day there was a significant reduction in body weight gain in males for most weekly intervals after week 2, and at the end of the dosing period body weight and body weight gain were significantly lower than control (81.2 % and 68.8% of control, respectively, $p \leq 0.01$). Body weight remained significantly lower than controls for the first two weeks post-dosing but increased to 90.4% of control values at the end of the recovery period, owing to weight gain markedly higher than control (134.7%). Female body weight and weight gain were unaffected during the treatment period but gain at the end of the recovery period was substantially lower (66.5% of control values).

FOOD INTAKE

Food intake generally paralleled changes in body weight. There was no adverse effect in males or females at 60 mg/kg/day and at 175 mg/kg/day significant reductions over the treatment period were limited to males with absolute intake of 91.4% of controls ($p \leq 0.05$).

At 500 mg/kg/day, average and relative food consumption in males were significantly reduced ($p \leq 0.01$) over the dosing period (DS 1 to 90) and for each weekly interval after week 1. During the recovery period, average absolute food consumption values were significantly reduced ($p \leq 0.01$) in first 2 weeks, remained lower than controls for the next 2 weeks but were similar thereafter. Relative food consumption values in males were significantly lower ($p \leq 0.05$) at the start of the recovery period (Days 90 to 92), comparable to controls for the next two weeks and significantly increased ($p \leq 0.01$) for each weekly interval for the remainder of the recovery period, resulting in an overall significant increase (DS 90 to 147, $p \leq 0.01$). In females treated at 500 mg/kg/day, within the dosing period there were transient decreases in absolute (DS 43 to 50, $p \leq 0.01$) and in relative food consumption (DS 8 to 15, $p \leq 0.01$ and DS 43 to 50, $p \leq 0.05$) values for the entire period (DS 1-90) were comparable to controls. Over the entire recovery period (DS 90-147) average absolute and relative food consumption were similar to control values as reductions ($p \leq 0.01$) observed in the first 2 weeks were followed by increases ($p \leq 0.05$ to $p \leq 0.01$) in the last 2/3 weeks.

WATER INTAKE

There was no adverse effect on water intake in any group during the first period measured (DS 1-7). In the second period (DS 64-70), significant reductions were observed in both sexes in all groups treated with sodium bromide. A dosage-related trend was apparent for males, with values of 87.4%, 81.7% and 78.8% in the low, intermediate and high dose groups, respectively, but not for females (82.5%, 77.3% and 83.9% of controls, respectively). However, in the low dose group significant reductions in water intake were confined to DS 66 to 67 in males (when a similar reduction was seen the sodium chloride treatment group males) and DS 66 to 67 and 67 to 68 in females.

CLINICAL CHEMISTRY AND HEMATOLOGY

There was no treatment- related effect on any hematology, coagulation or clinical chemistry value in male or female rats from any treated group when compared to concurrent and/or historical control values.

THYROID HORMONE ANALYSIS

Thyroid hormone analysis in serum was conducted on a single occasion in Week 4. Interpretation of thyroid hormone data was constrained by the limited amount of historical control data (2 studies), the consequent variation of some control values from this study in comparison to these values and the high number of values below the LLOQ.

There was however, an apparent reduction ($p \leq 0.01$) in T3 (males only) and in T4 (males and females) at 500 mg/kg/day. At 175 mg/kg/day, differences from control in T3 ($p \leq 0.05$, males) and T4 ($p \leq 0.01$, males and females) were less marked and values were comparable to historical control values. Single animals in these groups also showed depletion of colloid in the thyroid at histopathology (2 males and 2 females in each group) but there was generally no correlation between this finding, hormone levels or thyroid weight in individuals.

In the absence of effects on thyroid weight and histopathology at 60 mg/kg/day, the slight decreases in T3 and T4, to values which were within the range of historical control data, are considered to be of questionable toxicological significance and may fall into the normal adaptive response range of the thyroid. There was no effect on TSH in males or females at 60 mg/kg/day. Although not statistically significant (probably due to wide variation in individual results) mean TSH levels were 36% and 74% higher than controls in the 175 and 500 mg/kg/day male groups, respectively. All mean values, including controls were, however, markedly higher (~2 to 6 fold) than the historical control range. There was no significant effect on TSH in females.

URINALYSIS

Effects on urine were limited to the 500 mg/kg/day group. Males and females showed increases in leucocytes and males also showed increased incidences of yellow coloration and hemolysed blood in the urine, possibly indicating infection.

OPHTHALMOLOGY

Ophthalmology revealed no significant changes in any treated group.

GROSS PATHOLOGY

One male in each of the 60 and 175 mg/kg/day groups and 1 male and 2 females in the 500 mg/kg/day group were found to have a mass on the limbs at gross necropsy. Histopathology confirmed these findings as abscesses associated with bacterial infection and not directly related to treatment.

ORGAN WEIGHTS

There were no statistically significant differences in absolute thyroid weight and increases relative to body weight were only significant ($p \leq 0.05$) in females treated at 500 mg/kg/day. This did not, however, generally correlate with hormone levels or the microscopic finding of colloid depletion in individual animals. No other significant organ weight changes were observed. The effects on the reproductive organs are discussed in the Reproductive Toxicology section below.

HISTOPATHOLOGY

Mild/moderate depletion of colloid (noted when the majority of follicles in the thyroid contained no identifiable colloid) was observed in 2 males and 2 females in the groups treated at 175 and 500 mg/kg/day. It is, however, noteworthy that there was no correlation between these findings and decreased T3 and T4 levels in individual animals. These changes were not apparent at 60 mg/kg/day.

Microvesicular vacuolation in the periportal hepatocytes was observed in 2, 5, 4 males and 2, 3, 4

females in the 60, 175 and 500 mg/kg/day groups, respectively. This is a common incidental finding in rats, but can also result from nutritional disturbances.1

Tension lipidosis, often related to increased body weights, was observed at a higher incidence in females at 500 mg/kg/day, in which terminal body weights were lower than control females. This is a common incidental finding in rodents.2 At terminal euthanasia 1/19, 1/20 and 3/19 animals in the 60, 175 and 500 mg/kg/day groups multiple abscesses in the skin/subcutis containing bacteria with associated formation of Splendore-Hoppeli material which usually forms secondary to local antigen-antibody reaction. Skin abscess with evidence of bacteria and neutrophilic infiltrates were also contributory to the moribund condition of 2 males in the 500 mg/k/day group killed before scheduled termination. As these findings rarely occur spontaneously in animals at this facility they were considered treatment-related. As the skin abscesses were all located on the limbs/paws, however, these infections may be secondary to damage sustained during periods of test-substance related immobility or abnormal movement and/or poor clinical condition and lack of grooming.3 At 175 and 500 mg/kg/ day these findings tended to occur in animals also showing lung changes of accumulation of macrophages (within alveoli) and mixed cell infiltrates scattered in the pulmonary parenchyma. Other microscopic findings observed at the end of the treatment period were considered incidental. At the end of the recovery period, there was no evidence of previous signs other than macrophage aggregates in the lung. This is a common incidental finding in rats.4 As this was observed in 3/17 animals treated at 500 mg/kg/day sodium bromide/day, a comparable incidence to the control and sodium chloride treated groups (2/20 and 3/20 animals, respectively), it was considered not to indicate any irreversible effect of treatment.

Text Table 19
Summary of Microscopic Findings - Terminal Euthanasia (Day 91)

	Males					Females				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Sodium Chloride Dose (mg/kg/day)	**0**	**284**	**0**	**0**	**0**	**0**	**284**	**0**	**0**	**0**
Sodium Bromide Dose (mg/kg/day)	**0**	**0**	**60**	**175**	**500**	**0**	**0**	**60**	**175**	**500**
No. Rats Examined	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**9**	**10**	**10**
Gland, thyroid (No. Examined)	**10**	**10**	**10**	**10**	**8**	**10**	**10**	**9**	**10**	**10**
Depletion, colloid	(0)[a]	(0)	(0)	(2)	(2)	(0)	(0)	(0)	(2)	(2)
Minimal[b]	0	0	0	1	2	0	0	0	2	2
Mild	0	0	0	1	0	0	0	0	0	0
Liver (No. Examined)	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**9**	**10**	**10**
Vacuolation, periportal	(0)	(0)	(2)	(5)	(4)	(0)	(0)	(2)	(3)	(4)
Minimal	0	0	2	4	3	0	0	2	2	2
Mild	0	0	0	1	1	0	0	0	1	2
Tension lipidosis	(1)	(0)	(0)	(0)	(1)	(0)	(0)	(2)	(1)	(5)
Minimal	1	0	0	0	1	0	0	1	1	3
Mild	0	0	0	0	0	0	0	1	0	2
Lung (No. Examined)	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**9**	**10**	**10**
Macrophage aggregation	(1)	(0)	(2)	(1)	(4)	(2)	(0)	(0)	(1)	(8)
Minimal	1	0	2	1	4	2	0	0	1	6
Mild	0	0	0	0	0	0	0	0	0	2
Lung (No. Examined)	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**9**	**10**	**10**
Infiltration, mixed cell	(0)	(0)	(0)	(5)	(5)	(0)	(0)	(1)	(6)	(2)
Minimal	0	0	0	2	3	0	0	0	4	2
Mild	0	0	0	2	1	0	0	1	2	0
Moderate	0	0	0	1	1	0	0	0	0	0
Ovary (No. Examined)	**-**	**-**	**-**	**-**	**-**	**10**	**10**	**9**	**10**	**10**
Depletion, corpus luteum						(0)	(0)	(0)	(0)	(3)
Mild						0	0	0	0	2
Moderate						0	0	0	0	1
Skin (No. Examined)	**10**	**10**	**0**	**1**	**9**	**10**	**10**	**1**	**0**	**10**
Abscess	(0)	(0)	-	(1)	(1)	(0)	(0)	(1)	-	(2)
Mild	0	0		0	0	0	0	1		1
Moderate	0	0		1	1	0	0	0		1
Testes (No. Examined)	**10**	**10**	**0**	**10**	**9**					
Retained Spermatids/spermatid heads	(0)	(0)	-	(2)	(9)					

[a] Numbers in parentheses represent the number of rats with the finding.
[b] There were five severity grades available for assigning histopathology changes. Minimal, mild and moderate were considered unlikely to have clinical significance but have increasing amount of the tissue involved with the change, marked and severe changes are likely to have clinical significance as these are used when a large portion of the section (greater than ~50%) is affected.

REPRODUCTIVE TOXICITY:

Females

Estrous cycles

There was no adverse effect of treatment with sodium chloride or sodium bromide on estrous cycles.

Ovary weight

There were no statistically significant differences in ovary weight (absolute or relative to brain or body weight) in any treatment groups compared with controls.

Female reproductive organ histopathology and ovarian follicle counts

Histopathology revealed no effects on the uterus, oviduct, vagina or mammary gland at any dose level. Depletion of corpora lutea was observed in 3 females in the 500 mg/kg/day group, which were all in estrus at termination. Further sections taken from these females, examined in the Ovarian Follicle Quantification investigation, also had no corpora lutea present, although no effect on follicle count was apparent. At recovery euthanasia, all females in the 500 mg/kg/day group had corpora lutea but one control female was also found to have no corpora lutea.

As sectioning of the ovaries for follicle counts involved some deviations from protocol in positioning of the sections within the ovary, allocation of left and right ovaries and some incomplete sections (including the females with no corpora lutea) and as there were no other effects on female reproductive organ weight or histopathology, it is considered that no conclusions can be drawn from the above observations and further investigation would be necessary to investigate any potential effect on female reproductive function. The absence of corpora lutea did not correlate with depletion of colloid in the thyroid or with thyroid hormone levels in individual animals.

Males

Organ weights

There was no adverse effect on reproductive organ weights in males treated at 60, 175 or 500 mg/kg/day.

Some statistically significant differences in absolute weights were considered not biologically significant, but a consequence of the lower body weights, as values relative to body weight were comparable to controls or significantly higher.

Histopathology

Microscopic examination of testes, prostate gland, epididymides, cauda epididymis, seminal vesicles and coagulating glands indicated no effect of treatment at any dose level with the exception of sodium bromide -related findings that were found in 2/10 in the 175 and in 9/9 500 mg/kg terminal euthanasia animals. All 4 early deaths (two of which were recovery group animals that died either during the dosing period or shortly thereafter) in the NaBr 500 mg/kg dose groups had NaBr-related findings. NaBr-related findings consisted of minimal to moderate retained spermatids at the lumen of the seminiferous tubule epithelium-primarily at Stages X-XII and minimal to moderate retained spermatid heads in the Sertoli cell near the basement membrane-primarily at Stages XI-XII. A secondary change in the epididymis, originating from the corresponding testis, was increased cellular debris in the lumen.

Seminology

There was no effect on sperm count, motility or morphology at 60 mg/kg/day or 175 mg/kg/day.

At 175 mg/kg/day, the number of sperm with detached/no head was higher than the concurrent

control but considered unlikely to have been an effect of treatment as it was closer to the expected (historical control mean) value than was the unusually low control value.

At 500 mg/kg/day, there was a reduction (88.6% of control, p≤ 0.01) in the number of normal sperm, a reduction in the percent motile sperm from the vas deferens (75.3% of control, p≤ 0.05) and increases in mean non-motile sperm (110.4% of control, p≤ 0.01), percent abnormal sperm (11.9%, p≤ 0.01) and mean number of sperm with detached head (20.6%, p≤ 0.01) or no head (3.2%, p≤ 0.01) compared to the control group values. Mean epididymal sperm counts were also below the historical control range, although not significantly different (68.5%) from the concurrent control.

Testicular sperm counts (heads of homogenized sperm) were, however, comparable among the groups. The lack of effect on this parameter, or on testis weight or histopathology suggests that the changes in epididymal sperm count and vas deferens sperm parameters, may be indicative of an effect of sodium bromide on the sperm occurring only after they have left the testes. The changes observed may therefore have been a direct effect on the sperm or may have been mediated by a change in the osmolarity of the fluids in the epididymal lumen or vas deferens, possibly associated with altered electrolyte balance and/or the hydration state of the male.

Text Table 21
Selected Sperm Evaluation Parameters - Male Rats

		Dose Level (mg/kg/day)					HCD[a]
	N	0	284	60	175	500	Mean Min - Max
Vas Deferens							
Number Motile	10	386.5	345.1	379.7	375.3	265.8 (9)	481.0 308.4-821.1
%	10	91.2	88.4	91.7	87.1	68.7* (9)	95.6 80.3- 437.8
Static Count	10	41.0	40.6	37.3	52.7	110.4* (9)	50.5 15.6- 101.8
Total Count	10	427	385	417	428	376 (9)	531.1 348.4- 852.6
Caudal Epididymal							
Sperm Count	10	270.8	241.0	259.9	219.6	185.6 (9)	299.4 230.0-410.5
Sperm Concentration[b]	10	1015	922.3	987.9	872.2	912.0 (9)	1150.6 599.6-1713.08
Testicular							
Spermatid Concentration[c]	10	85.0	103.4	76.4	69.3	85.0 (9)	66.8 28.4-100.6
Morphology (epididymal)							
Normal	10	198.9	195.9	198.9	194.1	176.2** (9)	190.4 168.8-202.1
% Abnormal	10	0.6 [0-1.5]	2.0 [0-6.5]	0.6 [0-1.5]	3.0** [0-7.5]	11.9** (9) [6-21]	4.9 0.5-15.8
Detached Head	10	0.8 [0-3]	3.2 [0-9]	1.0 [0-2]	5.0* [0-15]	20.6** (9) [7-40]	6.0 1.0-19.4
No Head	10	0.3 [0-1]	0.9 [0-4]	0.1 [0-1]	0.9 [0-3]	3.2** (9) [1-8]	3.2 0.1-12.3

( ) = number of values averaged
a. Historical Control Data for the vas deferens, caudal epididymal, and testicular generated from 203 studies from 1998 to 2012. Historical Control Data for morphology generated from 54 studies from 1996 to 2012.
b. million sperm/mL.
c. million spermatid/mL.

Conclusion

CONCLUSION

Administration of 284 mg/kg/day of sodium chloride at an equivalent osmololarity as 500 mg/kg/day of sodium bromide produced no toxicity, indicating that the toxicity observed in the 500 mg/kg/day

dose group was due to exposure to bromide. The 500 mg/kg/day dose of sodium bromide was also demonstrated to be too toxic for use as a high dose in any evaluation of the effect of bromide on reproduction or other functional parameters as the general toxicity would preclude an appropriate evaluation of these functions.

General Toxicity

In conclusion, administration of 500 mg/kg/day of sodium bromide for 90 days produced severe toxicity, characterized by adverse clinical observations and reductions in body weight gain, food intake and water consumption, with effects generally more severe in males than females.

Histopathology indicated adverse effects in the liver, lungs and skin, with indicators of an increased susceptibility for bacterial infection , especially in the limbs, probably associated with the treatment-related decreased mobility and lack of grooming.

Administration of 175 mg/kg/day of sodium bromide produced similar but less severe toxicity including adverse clinical observations and reductions in food and water consumption, occurring more frequently in males than females. Effects on thyroid hormones, brain and lung weights and thyroid, liver, lung and skin histopathology were observed in occasional males only and cannot unequivocally be attributed to the test substance.

Effects observed in the 60 mg/kg group were comparable to the sodium chloride or historical controls and/or were findings common in this strain of rat, which cannot be unequivocally related to treatment and are regarded as not adverse. This dose level is therefore considered a NOAEL.

Reproductive Toxicity

In the presence of severe toxicity in males treated at 500 mg/kg/day sodium bromide, there were effects on sperm motility, morphology and sperm count but no adverse effects on reproductive organ weights, or testicular spermatid counts. Retained spermatids at the luminal surface or in basal Sertoli cell cytoplasm occurred in 2/10 in the 175 and in 9/9 500 mg/kg terminal euthanasia animals.

Three of 10 females in the 500 mg/kg/day dose group had no corpora lutea noted in the ovary, but overall follicle counts were not affected and there were no other adverse effects on reproductive organ weight, histopathology or estrous cycles.

The 500 mg/kg/day dose of sodium bromide was demonstrated to be too toxic for use as a high dose in any further evaluation of the effect of bromide on reproduction or other functional parameters as the general toxicity would preclude an appropriate evaluation of these functions. There were minimal toxic effects on reproductive parameters at 175 mg/kg/day and no significant adverse effects on the reproductive parameters evaluated at 60 mg/kg/day sodium bromide. The NOAEL for reproductive effects was therefore established as 60 mg/kg/day.

3.9.1.1 [Study 3] Three-generation reproductive toxicity study, sodium bromide

A6.8.2/02, Doc. No. 592-002

Van Leeuwen, F. X. R. et al. (1983) Toxicity of Sodium Bromide in Rats – Effects on endocrine system and Reproduction. Fil. Che. Toxic; Vol 21, No. 4, 383-399

See study details under reproductive toxicity

3.9.1.1 [Study 4] No guideline study: 28-day oral repeated dose toxicity study

Reference	A6.4.1/03, Doc. No. 592-007 van Logten, M. J. et al. 1973. Short-term Toxicity Study on Sodium Bromide in Rats. Toxicology 1, 321-327
Guideline	no guideline available GLP compliance:no
Reliability	Reliability: 2 (reliable with restrictions) Rationale for reliability incl. deficiencies:other: study was performed at a time when no official testing guidelines were available and is considered to have been conducted according to good experimental practice
Species / strain	Species: rat Strain:Wistar Sex: female
Test material	Name of test material (as cited in study report): Sodium bromide Analytical purity: 99.5 %
Study design	ADMINISTRATION / EXPOSURE Route of administration:oral: feed Vehicle:not specified Analytical verification of doses or concentrations: not specified Duration of treatment / exposure: 4 weeks Frequency of treatment:daily Doses / concentrations Remarks:Doses / Concentrations: 300, 1200, 4800 or 19200 ppm Basis: nominal in diet No. of animals per sex per dose:4/group Control animals:yes, plain diet CAGE SIDE OBSERVATIONS: No data DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: daily BODY WEIGHT: Yes

- Time schedule for examinations: once per week

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: No; food consumption taken once per week and expressed as mean per group per week

FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): Yes
- Time schedule for examinations: once per week

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

CLINICAL CHEMISTRY: Yes; but bromide and total halide concentrations were determined only; performed before the beginning of the experiment and from the start on at weekly intervals.

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No
GROSS PATHOLOGY: Yes ; brain, liver and kidneys were examined only.
HISTOPATHOLOGY: Yes ; brain, liver and kidneys were examined only.
Other examinations: Bromide and total halide content was determined in brain, liver and kidney.

Statistics: Student´s Test

Findings

RESULTS
CLINICAL SIGNS AND MORTALITY
No animal died in the course of the experiment
After a few days the animals fed 19200 ppm sodium bromide in their diet looked very dirty; they did not clean themselves sufficiently. Furthermore these animals showed signs of motor incoordination of their hind legs.

BODY WEIGHT AND WEIGHT GAIN
In the third week growth was retarded in the highest dosage group.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
Animals fed 4800 and 19200 ppm sodium bromide showed in comparison with the control group, a somewhat higher food intake.

CLINICAL CHEMISTRY
During the first week of the experiment the plasma concentration of bromide increased rapidly, thereafter a plateau was reached by the third week in all experimental groups. A relationship between the bromide level in the diets and the bromide concentration in plasma was observed. Only at the beginning of the experiment did the total halide concentration in the blood show a tendency to increase, thereafter there was no longer any influence detectable.
No other clinical chemical investigation was performed.

ORGAN WEIGHTS
Sodium bromide did not have any influence on the relative weight of the brain. Addition of 19200 ppm sodium bromide to the diet caused an increase in the weight of the kidneys. The relative weight of the liver showed a tendency to a dose-dependent increase.

GROSS AND HISTOPATHOLOGY
Microscopic examination of brain, liver and kidneys of the animals given 19200 ppm sodium bromide revealed no evidence of histopathological changes related to the treatment.

OTHER FINDINGS
There was a relationship between the bromide dosage in the diet and the concentration in kidney, brain and liver. Average bromide concentration in brain and liver was lower than in kidney and plasma. In the highest dosage group (19200 ppm) about 50% of the chloride in plasma, brain, kidneys and liver had been replaced by bromide. In the other dosage-groups there was a dose-related replacement of chloride by bromide.

NOAEL:4800 ppm Sex:female 4800 ppm (in the diet) corresponding to 480 mg/kg bw/day
LOAEL:19200 ppm Sex:female 19200 ppm (in the diet) corresponding to 1920 mg/kg bw/day based on reduced body weight gain, changes in absolute kidney weight and relative liver weight as well as on clinical signs at this dose level.

Table 6.4.1/03-1: Growth, Food and Water Intake of Rats Fed Diets containing Sodium Bromide

Dose [ppm]	Growth during week [g]				Food consumption during week [g]				Water consumption during week [ml]			
	1	2	3	4	1	2	3	4	1	2	3	4
0	21	17	14	13	12	11	12	12	18	18	18	17
300	22	15	17	10	11	11	13	12	19	19	18	16
1200	26	18	18	13	13	12	14	13	21	21	20	20
4000	27	22	11	20	13	14	16	14	23	23	22	21
19200	19	22	0*	20	13	18	17	16	23	24	21	24

* $0.01 \leq P < 0.05$

Table A6.4.1/03-2: Total Halide concentration in Plasma after Feeding of Sodium Bromide

Dose [ppm]	Total Halide Concentration [mEq/L]			
	1 week	2 weeks	3 weeks	4 weeks
0	103 ± 2	101 ± 2	102 ± 5	106 ± 2
300	106 ± 1	99 ± 2	105 ± 2	104 ± 2
1200	111 ± 5	99 ± 2	106 ± 3	101 ± 1
4800	120 ± 13	98 ± 3	109 ± 4	100 ± 1
19200	126 ± 8	103 ± 3	116 ± 1	101 ± 2

Table A6.4.1/03-3: Bromide Concentration in Brain, Liver, Kidneys and Plasma of Rats Fed Diets Containing Sodium Bromide for 4 Weeks

Dose [ppm]	Bromide Concentration [mEq/L]			
	Brain [mEq/kg]	Kidneys [mEq/kg]	Liver [mEq/kg]	Plasma [mEq/L]
0	0.4 ± 0.1	0.7 ±0.1	0.1 ± 0.1	0.2 ± 0.1
300	0.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.3	2.0 ± 0.1
1200	1.8 ± 0.1	5.1 ± 0.3	3.1 ± 0.3	8.1 ± 0.3
4800	7 ± 1	16 ± 1	9 ± 2	25 ± 2
19200	20 ± 1	34 ± 6	21 ± 2	56 ± 1

Table A6.4.1/03-4: Relative Organ Weights of Rats Fed Diets Containing Sodium Bromide

Dose [ppm]	Relative Weight [%]		
	Brain	Kidneys	Liver
0	0.923 ± 0.030	0.842 ± 0.044	4.231 ± 0.359
300	0.974 ± 0.090	0.845 ± 0.022	4.320 ± 0.281
1200	0.896 ± 0.090	0.841 ± 0.071	4.480 ± 0.241
4800	0.883 ± 0.064	0.843 ± 0.080	4.614 ± 0.381
19200	0.912 ± 0.096	1.021 ± 0.037	4.903 ± 0.437

EXECUTIVE SUMMARY:

MATERIALS AND METHODS
A short-term toxicity study of sodium bromide in rats is reported. Each treatment group consisted of four female rats receiving either a standard diet (control) or were given 300-19200 ppm sodium bromide in the diet (corresponding to 30-1920 mg/kg bw/day) respectively. Body weight, food intake and water intake were recorded weekly. Animals were investigated for clinical signs and mortality daily. No haematological or clinical-chemical examinations were performed. Special attention was paid on the accumulation of bromide in the blood and several other tissues. Halide content of the blood and other tissues was also determined. After 4 weeks all animals were killed and examined macroscopically. Liver, kidneys and brain were weighed and studied histopathologically in the control and highest dosage group.

RESULTS AND DISCUSSION
There were no dead animals in neither treatment group observed in the course of the experiment.

After a few days the animals fed 19200 ppm sodium bromide in their diet looked very dirty; they did not clean themselves sufficiently. Furthermore these animals showed signs of motor incoordination of their hind legs. These symptoms are an indication of neurotoxicity at high levels of sodium bromide affecting the behaviour and the nervous system of the animals.

In the third week of the experiment growth was retarded in the 19200 ppm group, but at the end of the test period there was so significant difference between the total weight gain of the treated groups and the control group. Animals fed 4800 and 19200 ppm sodium bromide showed in comparison with the control group, a somewhat higher food and water intake which in view of unchanged body weight is not considered to be a toxicologically relevant effect.

During the first week of the experiment the plasma concentration of bromide increased rapidly, thereafter a plateau was reached by the third week in all experimental groups. A relationship between the bromide level in the diets and the bromide concentration in plasma was observed. Only at the beginning of the experiment did the total halide concentration in the blood show a tendency to increase, thereafter there was no longer any influence detectable.

The relationship between the bromide dosage in the diet and the concentrations in the tissues examined is obvious. The average bromide concentration in the brain and liver was lower than in the kidneys and in plasma. In the highest dosage group about 50 % of the chloride in plasma, brain, kidneys and liver had been replaced by bromide. In the other treatment groups there was also a dose-related replacement of chloride by bromide.

An addition of 19200 ppm sodium bromide to the diet caused an increase in the weight of the kidneys. The relative weight of the liver showed a tendency to a dose-dependent increase. There was no influence on the relative weight of the brain.
Microscopic examination of brain, liver and kidneys of animals treated with 19200 ppm of sodium bromide revealed no evidence of histopathological changes that could be ascribed to the treatment. There was no indication of nephrotoxicity resulting from the administration of the high osmotic load.

Conclusion

CONCLUSIONS
After feeding of 19200 ppm sodium bromide for four weeks the chloride/bromide ratio in the brain is essentially the same as in liver, kidneys and plasma.

3.9.1.2 [Study 5] No guideline study: 90-day oral repeated dose toxicity study of sodium bromide study in rat

Reference

A6.4.1/04, Doc. No. 592-005

van Logten, M. J., et al., 1974. Semichronic Toxicity Study of Sodium Bromide in Rats. Toxicology 2, 257-267

Guideline

No guideline available

Study is a publication; no official testing guidelines were available at the time of study conduct

GLP compliance:no

Reliability

Species / strain

Species:rat

Strain:Wistar

Sex:male/female

Test material

Name of test material (as cited in study report): Sodium bromide

Analytical purity: 99.5 %

Study design

ADMINISTRATION/EXPOSURE
Route of administration:oral: feed
Vehicle:other: test substance was mixed with food
Analytical verification of doses or concentrations:not specified
Duration of treatment / exposure:90 days
Frequency of treatment:daily
Doses / concentrations: 75, 300, 1200, 4800 or 19200 ppm
Basis: nominal in diet
No. of animals per sex per dose:10/sex/group
Control animals:yes, plain diet

CAGE SIDE OBSERVATIONS: No data

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: weekly

BODY WEIGHT: Yes
- Time schedule for examinations: once per week

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: in Weeks 1, 2, 5, 9 and 12

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: 10 and 11 weeks after treatment
- Anaesthetic used for blood collection: No data
- Animals fasted: No data
- Parameters checked in table [No.A6.4.1/04-2] were examined.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: 12 and 13 weeks after treatment had started
- Animals fasted: No data
- Parameters checked in table [No. A6.4.1/04-3] were examined.

URINALYSIS: Yes
- Time schedule for collection of urine: 10 weeks after treatment had started
- Metabolism cages used for collection of urine: No data
- Animals fasted: No data
- Parameters: urine pH, glucose, ketones, bilirubin, and protein were determined

NEUROBEHAVIOURAL EXAMINATION: No

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
The following organs were examined by routine histopathological techniques: heart, brain, lungs, liver, kidneys, spleen, thymus, adrenals, thyroid, pituitary gland, uterus, ovaries, prostate, testes, mesenteric lymph nodes, pancreas, stomach, duodenum, ileum, jejunum, caecum, colon, rectum, urinary bladder, spinal cord, sciatic nerve and musculature.Other examinations:weights of heart, brain, lungs, liver, kidneys, spleen, thymus, adrenals, thyroid, pituitary gland, uterus, ovaries, prostate and testes were taken.

Statistics: Student´s t test

Findings

RESULTS AND DISCUSSION

Results of examinations
Clinical signs:no effects observed
Mortality: no mortality observed
Body weight and weight changes:not specified
Food consumption and compound intake (if feeding study):effects observed, treatment-related
Food efficiency: not examined
Water consumption and compound intake (if drinking water study): not examined
Ophthalmological findings:not examined
Haematological findings:effects observed, treatment-related
Clinical biochemistry findings: effects observed, treatment-related
Urinalysis findings: effects observed, treatment-related
Behaviour (functional findings): not examined
Organ weight findings including organ / body weight ratios: effects observed, treatment-related
Gross pathological findings:effects observed, treatment-related
Histopathological findings: non-neoplastic:effects observed, treatment-related
Histopathological findings: neoplastic: not specified

CLINICAL SIGNS AND MORTALITY
After 4 weeks of treatment a female rat from the 19200 ppm group had her tail eaten by her cage mate and had to be killed. Otherwise there was no mortality.
The animals on the highest dosage level (19200 ppm) did not groom themselves sufficiently and exhibited signs of motor incoordination. The male animals in this group showed significant growth retardation.

BODY WEIGHT AND WEIGHT GAIN
No analysis of body weight gain was performed in this investigation. Bodyweights were only determined to express the organ weights in relation to bodyweight. But neither individual nor mean bodyweights are indicated.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
Food intake in the 19200 ppm group was significantly increased in the females from the second week onwards. Food conversion was decreased in both sexes during the first few weeks.

HAEMATOLOGY
In the highest dosage group PCV and erythrocyte counts were somewhat decreased, the latter significantly only in the male animals. The percentage of neutrophil granulocytes was doubled from 6 to 12 in both sexes in this treatment group without distinct changes in total leucocyte counts.

CLINICAL CHEMISTRY
Biochemical changes were not seen except a tendency toward raised alkaline phosphatase values in both sexes in the highest dosage group. There were no effects on the microsomal aromatic hydroxylating enzyme activities. In male rats there was a tendency to a dose-related decrease of microsomal aminopyrine demethylating enzyme activity towards levels in females. There was a decrease in APDM activity in male rat.

Plasma bromide levels increased steeply and reached a plateau in the third week. The molar

concentration of total halogens in plasma was 105 ± 4 mmol/L and remained constant within narrow limits in constant to the transient rise in the 19200 ppm group during the previously performed short-term study.

URINALYSIS
Except for traces of haemoglobin in isolated instances no clinical pathological changes were seen in the urine.

ORGAN WEIGHTS
The relative thyroid weight was increased of females treated with 1200, 4800 and 19200 ppm sodium bromide and in males of the highest dosage group (19200 ppm). In addition, the relative thymus weight was reduced in the female animals of the 19200 ppm group, whereas the relative weight of spleen and adrenals was increased at this dose level. In animals of the 4800 and 19200 ppm groups relative prostate weight was reduced.

GROSS AND HISTOPATHOLOGY
Pituitary glands of male animals of the 19200 ppm group contained cysts which occur normally only to some extent in females. There was an activation of the thyroid in the highest dosage group in both sexes and in the 4800 ppm group in females only. In the adrenals in all dosage groups there was strikingly less vacuolisation of the zona fasciculate, compared with the controls.
In the highest dosage group there was a tendency to a decrease of spermatogenesis and less secretory activity of the prostate. This latter tendency was also seen in the 4800 ppm group. In other organs and tissues no marked or consistent changes in the histological picture were found.

OTHER
Bromide concentrations in brain and kidneys were, like the plasma values, directly proportional to the bromide concentrations in the corresponding diets, except in the highest dosage group. The ratio of brain bromide to plasma bromide and of kidney bromide to plasma bromide was 0.2 and 0.6 respectively, independent of bromide dosage.
Total halogen levels in kidneys and brain in the highest dosage group did not differ from those in the control group. For that reason no further total halogen determinations in the lower dosage groups were carried out.

NOAEL Effect level: 1200 ppm
Sex:male/female
Basis for effect level:other: 1200 ppm (corresponding to 120 mg bromide/kg bw/day for young rats and 60 mg/kg bw/day for older rats)
LOAEL Effect level:4800 ppm
Sex:male/female

Table A6.4.1/04-4: Median Bromide Content of Plasma, Kidneys and Brain of rats after 12 weeks of Sodium Bromide Treatment

NaBr [ppm]	Plasma [mmol/L]	Kidneys [mmol/kg]	Brain [mmol/kg]
0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
75	0.6 ± 0.1	0.3 ± 0.1	0.2 ± 0.2
300	2.1 ± 0.1	1.1 ± 0.1	0.3 ± 0.1
1200	7.7 ± 1.1	4.1 ± 0.4	1.1 ± 0.1
4800	25 ± 2	14 ± 2	4.3 ± 0.4
19200	51 ± 3	30 ± 2	11 ± 1

1 mmol/L or 1mmol/kg corresponds to 80 ppm bromide or 103 ppm NaBr.

Table A6.4.1/04-5: Selected Organ Weights Expressed in Relation to Bodyweight (%) from rats treated with Sodium Bromide for 12 weeks

Organs	Controls	NaBr [ppm]				
		75	300	1200	4800	19200
Female animals						
Spleen	0.248	0.234	0.237	0.219*	0.237	0.271
Thymus	0.155	0.15	0.156	0.135	0.139	0.118*

Adrenals	0.0245	0.256	0.0237	0.0251	0.0238	0.0269
Thyroid	0.0071	0.0087	0.0078	0.0094**	0.0087**	0.0136***
Pituitary Gland	0.0063	0.0065	0.0056	0.0057	0.0052	0.0052
Uterus	0.183	0.175	0.175	0.19	0.163	0.16
Ovaries	0.0337	0.0263**	0.033	0.0327	0.0337	0.0284
Male animals						
Spleen	0.208	0.196	0.199	0.205	0.222	0.26**
Thymus	0.125	0.128	0.12	0.13	0.132	0.129
Adrenals	0.016	0.0157	0.0149	0.0162	0.0163	0.021***
Thyroid	0.0065	0.0069	0.0069	0.0065	0.0068	0.0102***
Pituitary Gland	0.0033	0.0032	0.0031	0.0032	0.0031	0.0037
Prostate	0.153	0.155	0.153	0.166	0.103**	0.076***
Testes	0.857	0.8	0.83	0.839	0.767	0.826

* $0.01 \leq P < 0.05$
** $0.001 \leq P < 0.01$
*** $P < 0.001$

EXECUTIVE SUMMARY:

Materials and Methods

The investigation was performed to extend the existing short-term toxicity study with sodium bromide in the rat. Animals were administered 75, 300, 1200, 4800 or 19200 ppm of sodium bromide (corresponding to about 5.63, 22.5, 90, 360 and 1440 mg/kg bw/day based on a default conversion of 1 ppm = 0.075 mg/kg bw/day (mean of 0.05 mg/kg bw/day and 0.1 mg/kg bw/day for older and young rats, respectively)) with the diet or left untreated as a control. For each treatment group 10 animals of each sex were used. The rats received the test material for 90 days and were killed at the end of the treatment period. Animals were observed for clinical signs, haematological examinations, urinalysis and clinical chemistry were performed at different time points throughout the investigation. After sacrifice rats were subject to gross and histopathology and weights of several organs were taken and expressed in relation to bodyweight.

Results and Discussion

Grooming was depressed in the rats of the highest treatment dosage group (19200 ppm) and as a consequence their fur was brownish yellow and caky. They also showed motor incoordination of the hind legs. Both symptoms were also seen in the short-term study performed before. The symptoms remained at more or less constant intensity during the entire study, but seemed more intensive in the first week. One female rat of the 19200 ppm group had to be killed because it had here tail eaten by her cage mate. There were no other mortalities during the study. Effects on growth were seen only during the first 6 weeks. Food intake in the 19200 ppm group was significantly increased in the females from the second week onwards. Food conversion was decreased in both sexes during the first few weeks. Except for traces of haemoglobin in isolated instances no pathological changes were seen in the urine. In the highest dosage group PCV and erythrocyte counts were somewhat decreased, the latter significantly only in the male animals. The percentage of neutrophil granulocytes was doubled from 6 to 12 in both sexes in this treatment group without distinct changes in total leucocyte counts. Biochemical changes were not seen except a tendency toward raised alkaline phosphatase values in both sexes in the highest dosage group. There were no effects on the microsomal aromatic hydroxylating enzyme activities. In male rats there was a tendency to a dose-related decrease of microsomal aminopyrine demethylating enzyme activity towards levels in females. There was a decrease in APDM activity in male rats which might be linked to endocrine changes. APDM activity is sex-dependent, whereas AH activity is not. The observed changes might be caused by an altered pituitary function leading to increased output of the adrenocorticotropic and thyroid-stimulating hormones and decreased output of the luteotropic and follicle-stimulating hormones. Plasma bromide levels increased steeply and reached a plateau in the third week. Applying accumulation kinetics to the observed data, one may estimate the biological half-life of bromide under the conditions of this investigation to be of the order of 3-5 days. Except for the highest dosage group these plateaus were directly proportional to the bromide concentrations in the corresponding diets. The molar concentration of total halogens in plasma was 105 ± 4 mmol/L and remained constant within narrow limits in constant to the transient rise in the 19200 ppm group during the previously performed short-term study. Bromide concentrations in brain and kidneys were, like the plasma values, directly proportional to the bromide concentrations in the corresponding diets, except in the highest dosage

group. The ratio of brain bromide to plasma bromide and of kidney bromide to plasma bromide were 0.2 and 0.6 respectively, independent of bromide dosage. As total halogen levels in kidneys and brain in the highest dosage group did not differ from those in the control group, no further total halogen determinations in the lower dosage groups were carried out.
The relative thyroid weight was increased of females treated with 1200, 4800 and 19200 ppm sodium bromide and in males of the highest dosage group (19200 ppm). In addition the relative thymus weight was reduced in the female animals of the 19200 ppm group, whereas the relative weight of spleen and adrenals was increased at this dose level. In animals of the 4800 and 19200 ppm groups relative prostate weight was reduced. A complex of presumably related changes was found in the endocrine system. Pituitary glands of male animals of the 19200 ppm group contained cysts which occur normally only to some extent in females. Related to the changes in organ weights there was an activation of the thyroid in the highest dosage group in both sexes and in the 4800 ppm group in females only. In the adrenals in all dosage groups there was strikingly less vacuolisation of the zona fasciculate, compared with the controls. These changes in the adrenals point to an increased corticosteroid production and output. In the highest dosage group there was a tendency to a decrease of spermatogenesis and less secretory activity of the prostate. This latter tendency was also seen in the 4800 ppm group. The effect on ovaries, testes and prostate suggests a diminished production of gonadotropic hormones. In other organs and tissues no marked or consistent changes in the histological picture were found.

Conclusion After treatment with 4800 and 19200 ppm of sodium bromide (corresponding to about 360 and 1440 mg/kg bw/day) via the diet for a period of 90 days, several effects on the endocrine system were observed as demonstrated by a decrease in APDM activity in males as well by the histological findings made in the pituitary, adrenals and gonads. Sodium bromide also reduced the relative thyroid weight in animals dosed at 1200 ppm or higher.

3.9.1.1 [Study 6] No guideline study: 90-day oral repeated dose toxicity study of sodium bromide study in rat on a low chloride diet

Reference

A6.4.1/05, Doc. No. 592-006

Van Logten M.J. et al., 1976

Publication

Semichronic Toxicity Studies of Sodium Bromide in Rats on a Normal Diet and a Low Chloride Diet

Author: Van Logten M.J., Rauws A.G., Kroes R., Den Tonkelaar E.M. and van Esch G.J. 1976. Med. Fac. Landbouww Rijksuniv. Gent 41 (2), 1499-1507

Guideline

No guideline

GLP compliance: no

Reliability

Reliability: 2 (reliable with restrictions) Rationale for reliability incl. deficiencies:other: study was performed at a time when no official testing guidelines were available and is considered to have been conducted according to good experimental practice

Species / strain

Species: rat

Strain: Wistar

Sex: male/female

Test material

Name of test material (as cited in study report): Sodium bromide

No further information on test substance is given in the report.

Study design

ADMINISTRATION / EXPOSURE
Route of administration:oral: feed
Vehicle:other: No vehicle used; The test substance was mixed with food
Analytical verification of doses or concentrations:not specified
Duration of treatment / exposure: 90 days
Frequency of treatment: daily

Doses / Concentrations:
75, 300, 1200, 4800 and 19200 ppm (normal chloride diet) Basis: nominal in diet
8, 31, 125, 500 and 2000 ppm (low chloride diet) Basis: nominal in diet

No. of animals per sex per dose:10/sex/group
Control animals:yes, plain diet

CAGE SIDE OBSERVATIONS: No data
DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: weekly
BODY WEIGHT: Yes
- Time schedule for examinations: regularly (the exact time points are not further specified within the report)

FOOD CONSUMPTION AND COMPOUND INTAKE:
- Time schedule: regularly (the exact time points are not further specified within the report)
FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No data

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood:at termination only.
- How many animals: all animals examined
- Parameters checked in table [No.A6.4.1/05-2] were examined.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: at termination only.
- How many animals: all animals examined
- Parameters examined: Bromide level, total halogenide content, serum alkaline phosphatase and glutamate-pyruvate transaminase activity

URINALYSIS: Yes
- Parameters examined: urine pH, glucose, ketones, bilirubin and protein

NEUROBEHAVIOURAL EXAMINATION: No
Sacrifice and pathology:GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
the following organs were examined by routine histopathological techniques: heart, brain, lungs, liver, kidney, spleen, thymus, adrenals, thyroid, pituitary gland, uterus, ovaries, prostate, testes, mesenteric lymph node, pancreas, stomach, duodenum, ileum, jejunum, caecum, colon, rectum, urinary bladder, spinal cord, sciatic nerve and musculature.
Other examinations: Bromide level and total halides were determined in plasma and several organs

Statistics: Not specified

Findings

RESULTS
CLINICAL SIGNS AND MORTALITY
In the animals on a normal chloride diet no mortality was observed, whereas in the study with a low chloride diet 3 males and 3 females of the highest dosage group (2000 ppm of sodium bromide) died during the experiment.

In both experiments the animals showed motor incoordination of their hind legs

BODY WEIGHT AND WEIGHT GAIN
The growth in the 2000 ppm group of the rats on a low chloride diet was retarded, whereas the bodyweight gain of the 19200 ppm group was also somewhat lower.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
Results for food consumption are given for the normal chloride diet receiving rats only. Food intake in the 19200 ppm group of rats on a normal chloride diet was significantly increased in the females from the second week onwards. Food conversion was decreased in both sexes during the first few weeks.

HAEMATOLOGY
There was an increase in the percentage and total number of neutrophil granulocytes at the highest dosage group of both experiments. Moreover, the total leucocyte count was increased in the 2000 ppm sodium bromide group of the rats on a low chloride diet.

CLINICAL CHEMISTRY
Bromide concentration in plasma of the rats on a normal chloride diet rose to a plateau within 3 weeks. In the experiment with a low chloride diet it took about 8 weeks to reach a plateau. Except for the highest dosage group of both experiments, these plateaus were directly proportional to the bromide concentration in the diet. However, in animals on a low chloride diet the accumulation level was about 10 times higher than in animals on a normal chloride diet.

URINALYSIS
Urinalysis was performed in the normal chloride diet receiving group only. Except for traces of haemoglobin in isolated instances no clinical pathological changes were seen in the urine.

ORGAN WEIGHTS
In both experiments, the relative weights of the adrenals and thyroid were increased in the highest sodium bromide groups, whereas the weights of the pituitary gland, ovaries and testes were decreased. The thyroid of the female animals of the 1200 and 4800 ppm groups of male rats on a normal chloride diet were also increased.

HISTOPATHOLOGY: NON-NEOPLASTIC
Results of the histopathological examinations are given in Table A6.4.1/05-5.

OTHER
Bromide concentrations in brain and kidneys of both experiments were directly proportional to the bromide concentrations in the diets. In the low chloride experiment one tenth of the bromide dose was sufficient to reach the same bromide concentrations compared with the normal chloride diet.
The average bromide concentration in the brain was lower than in the kidneys. At the highest dose group of the normal chloride diet about 25 % of the chloride in the brain and 50 % in the kidney had been replaced by bromide.

NOAEL normal chloride diet**:** 1200 ppm **Sex:** male/female
NOAEL low chloride diet**:** 125 ppm **Sex:** male/female
LOAEL normal chloride diet**:** 4 800 ppm **Sex:** male/female other: based on depressed grooming and changes within the thyroid gland
LOAEL low chloride diet**:** 500 ppm **Sex:** male/female other: based on depressed grooming and changes within the thyroid gland

Table A6.4.1/05-1: Mean Bodyweight gain of Rats fed Sodium Bromide withChloride or Low Chloride Diet for 12 weeks

NaBr [ppm]	Weight Gain [g]	
	females	males
Normal Chloride Intake		
0 (control)	140	265
75	131	285
300	138	273
1200	139	278

4800	138	256
19200	130	204*
Low Chloride Intake + 1 % K_2SO_4		
0 (control)	151	265
8	139	266
31	161	274
125	143	271
500	143	283
2000	98**	183**

*0.001 < P < 0.01
**P < 0.001

Table A6.4.1/05-2: Result of Total Leucocytes and Differential Count at the end of the Studies

Treatment	Cell Type counted					
	Leucos [10^7/L]	Eos [%]	Baso [%]	Neutro [%]	Lympho [%]	Mono [%]
Normal Chloride Intake						
Females						
0 (control)	1040	1.2	0.1	7.1	85.8	5.8
19200 ppm NaBr	1049	0.9	0.0	12.3*	81.8	5.0
Males						
0 (control)	1635	0.9	0.2	5.8	87.6	3.3
19200 ppm NaBr	1667	1.4	0.1	12.2*	81.3	5.0
Low Chloride Intake						
Females						
0 (control)	1018	1.6	0.1	7.2	87.1	4.0
19200 ppm NaBr	1283	1.4	0.0	15.7	79.5	3.4
Males						
0 (control)	1130	0.9	0.2	9.2	84.9	4.8
19200 ppm NaBr	1336	0.6	0.1	16.7	79.6	3.0*

* 0.01 < P < 0.05
Leucos Leucocytes
Eos Eosinophil Granulocytes
Baso Basophil Granulocytes
Neuto Neutrophil Granulocytes
Lympho Lymphocytes
Mono Monocytes

Table A6.4.1/05-3: Bromide Content of Kidneys and Brain of Rats fed Sodium Bromide withChloride or Low Chloride Diet for 12 weeks

NaBr [ppm]	Kidneys [mmol bromide/kg]	Brain [mmol bromide/kg]
Normal Chloride Intake		
0 (control)	0.1 ± 0.1	0.1 ± 0.1
75	0.3 ± 0.1	0.2 ± 0.2
300	1.1 ±0.1	0.3 ± 0.1
1200	4.2 ± 0.4	1.1 ± 0.1
4800	14 ± 2	4.3 ± 0.4
19200	30 ± 2	11 ± 1
Low Chloride Intake + 1 % K_2SO_4		
0 (control)	0.1 ± 0.1	0.1 ± 0.1
8	0.5 ± 0.1	0.2 ± 0.1
31	0.8 ± 0.1	0.3 ± 0.1
125	3.3 ± 0.3	1.3 ± 0.1
500	12 ± 1.3	6.2 ± 0.4
2000	25 ± 2	13 ± 0.7

Table A6.4.1/05-4: Selected Organ weights, expressed in relation to heart weight of rats after 12 weeks of Sodium Bromide either in Chloride or Low Chloride Diet

Treatment	Organ weight expressed in relation to heart weight [%]			
	adrenals	thyroid	pituitary gland	ovaries/testes
Normal Chloride Intake				
Females				
0 (control)	0.069	0.02	0.018	0.095
19200 ppm NaBr	0.074	0.037***	0.014*	0.078*
Males				
0 (control)	0.055	0.022	0.011	2.944
19200 ppm NaBr	0.067**	0.032***	0.012	2.588
Low Chloride Intake				
Females				
0 (control)	0.07	0.025	0.018	0.096
19200 ppm NaBr	0.065	0.024	0.013***	0.079
Males				
0 (control)	0.049	0.024	0.011	2.992
19200 ppm NaBr	0.063*	0.03	0.011	2.79

* $0.01 < P < 0.05$
** $0.001 < P < 0.01$
*** $P < 0.001$

Table A6.4.1/05-5: Synopsis of histopathological investigations on rats treated with Sodium Bromide for 12 weeks in Chloride or Low Chloride Diet respectively

Organ	Normal Chloride Intake (19200 ppm NaBr)	Low Chloride Intake (2000 ppm NaBr)
Thyroid	Activation*	Activation**
Adrenals	less vacuolization in zona fasciculata	less vacuolization in zona fasciculate**
Ovaries	less corpora lutea	less corpora lutea
Testes	spermatogenesis inhibited	spermatogenesis inhibited
Pituitary gland	cysts (males)	-
Prostate	less secretory activity	-
Thymus	involution	-
Brain	-	hyperaemic
Heart	-	degeneration in myocard
Lungs	-	granulocytes along bloodvessels
Salivary gland	-	less secretory activity
Uterus	-	retardation in maturation
Pancreas	-	less zymogen granulae**

* also in 4800 ppm NaBr group
** also in 500 ppm NaBr group

EXECUTIVE SUMMARY

MATERIALS AND METHODS

In a preliminary study it was confirmed that bromide caused a dose-related replacement of chloride in the plasma and several organs. The influence of chloride in the drinking water on the excretion rate of bromide was investigated previously. It was shown that the bromide half-life is strongly influenced by the chloride intake. This investigation compares the results of two 90 day studies with sodium bromide in rats on a normal chloride diet and a low chloride diet. To the latter one, 1 % K2SO4 was added. Animals on a normal chloride diet received 75, 300, 1200, 4800 and 19200 ppm of sodium bromide (corresponding to 5.6, 22.5, 90, 360 and 1440 mg/kg bw/day for a medium of young and old rats) or were fed the diet without test substance for control. The animals on the low chloride diet were treated with 0, 8, 31, 125, 500 and 2000 ppm of sodium bromide (corresponding to 0.6, 2.3, 9.4, 37.5 and 150 mg/kg bw/day for a medium of young and old rats). Each group consisted of 10 male and 10 female animals. During the studies weight gain, food intake, bromide and total halide concentration in plasma were determined regularly. At the end of the experiments clinical-

pathology determinations were carried out in blood, urine and liver. Bromide and total halide were determined in plasma and several organs. The organs were weighed and examined histopathologically.

RESULTS AND DISCUSSION
Grooming was depressed in the animals of the highest dosage groups in both experiments and as a consequence their fur was brownish yellow and caky. The animals also showed motor incoordination of their hind legs. In the animals on a normal chloride diet no mortality was observed, whereas in the study with a low chloride diet 3 males and 3 females of the highest dosage group (2000 ppm of sodium bromide) died during the experiment. Especially the growth of the rats of the 2000 ppm group was retarded, whereas the bodyweight gain of the 19200 ppm group was also somewhat lower. Bromide concentration in plasma of the rats on a normal chloride diet rose to a plateau within 3 weeks. In the experiment with a low chloride diet it took about 8 weeks to reach a plateau. Except for the highest dosage group of both experiments, these plateaus were directly proportional to the bromide concentration in the diet. However, in animals on a low chloride diet the accumulation level was about 10 times higher than in animals on a normal chloride diet. The haematological investigations revealed a striking increase in the percentage and total number of neutrophil granulocytes at the highest dosage group of both experiments. Moreover, the total leucocyte count was increased in the 2000 ppm sodium bromide group. Bromide concentrations in brain and kidneys of both experiments were directly proportional to the bromide concentrations in the diets. In the low chloride diet experiment one tenth of the bromide dose was sufficient to reach the same bromide concentrations compared with the normal chloride diet. The average bromide concentration in the brain was lower than in the kidneys. At the highest dose group of the normal chloride diet about 25 % of the chloride in the brain and 50 % in the kidney had been replaced by bromide. In the low chloride diet experiments the replacement percentages are much higher, due to the lower total halide concentration in the organs. As an overall tendency, in both experiments the relative weights of the adrenals and thyroid were increased in the highest sodium bromide groups, whereas the weights of the pituitary gland, ovaries and testes were decreased. The thyroid of the female animals of the 1200 and 4800 ppm groups of the males were also increased. These alterations in adrenals and thyroid and the increased percentage of neutral granulocytes point to an increased output of the adrenocorticitropic and thyroid-stimulating hormones by the pituitary gland of which the relative weight was also decreased. The effect on ovaries and testes suggest a diminished secretory activity of the pituitary gland. There is a clear tendency that 2000 ppm sodium bromide in a low chloride diet is more toxic than 19200 ppm in a normal diet. In the 2000 ppm group 6 out of 20 rats died, whereas only one rat had to be killed in the 19200 ppm group because it was attacked by its cage mate. The influence on bodyweight gain is also more pronounced in the 2000 ppm group.

Conclusion

CONCLUSIONS
The toxicity of sodium bromide in rats on a low chloride diet is about 10 times higher in comparison with the toxicity for rats on a normal diet due to the competition for uptake between bromide and chloride ions. A main result of the investigation is that bromide had marked effects on the endocrine system, with activation of the thyroid, histological changes in ovaries and testes (less vacuolisation in zona fasciulata and less corpora lutea respectively) and less secretory activity of the prostate.

3.9.1.1 [Study 7] Sub-chronic repeated dose toxicity: oral in dog

Reference A6.4.1/07. Doc. No. 592-027

Rosenblum I (1958). Bromide Intoxication: I. Production of Experimental Intoxication in Dogs. J. Pharmacol Exp. Ther. 122 (3), 379-385

Guideline no guideline available

GLP compliance: no

Reliability 3 (not reliable)

Rationale for reliability incl. deficiencies:other: there were no official guidelines in place at the time of study conduct

Species / strain

Dog

Strain:other: Mongrel

Sex:male/female

Test material

Name of test material (as cited in study report): Sodium bromide

There are no further details given on test material within the study report

Study design

Administration / exposure

Route of administration:oral: feed

Vehicle: other: gelatine capsule

Details on oral exposure: The dose of sodium bromide was put into gelatine capsules and these were fed wrapped in a small ball of dog food.

Analytical verification of doses or concentrations: not specified

Duration of treatment / exposure: Animals were treated with increasing doses of sodium bromide at intervals of six weeks until death resulted (between 44 and 185 days).

Frequency of treatment: daily

Doses / concentrations:

Group 1: 100 mg/kg bw, Group 2 and 3: initial doses of 100 (group 2) and 200 mg/kg bw (group 3), increments of 100 or 200 mg/kg/day in group 3 at intervals of 6 weeks until death resulted, Group 4: 400 mg/kg bw

Basis: actual ingested

No. of animals per sex per dose: 4 dogs per treatment group

Control animals:yes, plain diet

Examinations

Observations and examinations performed and frequency:

CAGE SIDE OBSERVATIONS: No data

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule: daily

BODY WEIGHT: Yes

- Time schedule for examinations: time points are not stated in the publication

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

- test substance was administered via food, for that reason dogs were observed for food consumption after test substance in food had been offered.

FOOD EFFICIENCY:

- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No data

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): No

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

CLINICAL CHEMISTRY: Yes; blood bromide levels (method of Brodie and Friedman) and total halide

levels (method of Sendroy) were investigated. No other clinical chemistry parameters were examined.

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No data

GROSS PATHOLOGY: No

HISTOPATHOLOGY: No

Findings

Clinical signs: effects observed, treatment-related

Mortality:mortality observed, treatment-related

Body weight and weight changes:effects observed, treatment-related

Food consumption and compound intake (if feeding study):no effects observed

Food efficiency:not examined

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

Ophthalmological findings:not examined

Haematological findings:not examined

Clinical biochemistry findings: effects observed, treatment-related

Urinalysis findings: not examined

Behaviour (functional findings) :not examined

Organ weight findings including organ / body weight ratios:not examined

Gross pathological findings:not examined

Histopathological findings, non-neoplastic: not examined

Histopathological findings, neoplastic: not examined

Details on results:

CLINICAL SIGNS AND MORTALITY

Three of the four dogs from group 4 were dead within 44 days, 2 of these within 27 days. Five dogs on dosage groups 2 and 3 receiving the same dose of bromide (400 mg/kg/day) survived for at least 42 days and three survived for at least 87 days on even higher doses of bromide. The animals on dosage group 1 were maintained for 140 days without any fatalities.

Some form of neurotoxicity was seen in all dogs that received 200 mg/kg/day or more of sodium bromide. The usual progression as the blood bromide level rose was as follows: slight ataxia, stupor, severe ataxia (unable to stand) and coma. The shivering which was seen in some dogs may also have been a result of the irritant effect of bromide. Slightly ataxic dogs could continue to eat and drink and often showed no other signs of intoxication. Stuporous dogs ate and drank but they did this mechanically without any apparent interest. Severely ataxic dogs and dogs that became comatose were unable either to eat or drink and these usually died within two or three days after first becoming comatose.

Signs of gastrointestinal toxicity appeared almost as frequently as did those of neurotoxicity. Some were transient and often did not recur in the same animal; these were diarrhea, bloody stool and vomiting. They propably resulted from the irritant effect of bromide on the gastrointestinal tract and in only one dog on dosage group 4 repeated vomiting was observed which made it necessary to withdraw the animal from the experiment.

Skin lesions were observed in some of the animals. These were mainly nonsuppurative white macules overlaid by scales and varying in size from about 1 to 3 cm in diameter. They appeared on the hind limbs, head and sternum. One dog developed furunculosis and the organism cultured from its wounds was identified as S. fecalis.

BODY WEIGHT AND WEIGHT GAIN

There was emaciation and weight loss on undiminished food intake noticed which occurred in most dogs even at doses of sodium bromide which caused no other signs of toxicity. This effect appears

characteristic of bromide intoxication. In order to rule out the possibility that this observation was partially due to the diet and environment, two groups, each of four dogs, were placed on a similar diet and in a similar environment. After a control period, one group was treated with increasing doses of sodium bromide beginning with 100 mg/kg/day, the other was given a comparable amount of sodium chloride. The group which was fed sodium bromide lost weight progressively (a loss of 15.4 % from the mean control bodyweight in week 10) and gave the appearance of marked emaciation while the other group lost little weight and appeared healthy throughout.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)

Consumption of 40 g/kg of canned dog food was confirmed by eye. It was confirmed that this amount was eaten within 1-3 hours.

CLINICAL CHEMISTRY

Dogs appear able to tolerate very large doses of bromide and blood bromide levels. The highest mean blood bromide level of approximately 50 mEq /L was produced by a daily dose of 400 mg/kg/day of sodium bromide. A comparison of the data from groups 2 and 3 with that of group 4 indicates that rapid elevation of the blood bromide level increases the lethality of bromide.

OTHER FINDINGS

Since one of the factors determining the blood bromide level that results from ingestion of a dose of bromide is the ratio of Br-/Cl-, in addition to measure the blood bromide level, the total blood halide was measured by the method of Sendroy. The dog food used in this study had a sodium chloride content of 200 mg/kg/day, so that by holding the amount of food constant, the amount of chloride ingested was in effect also held constant. The ratio of Br-/Cl- ingested was known for each dose of bromide. Under such conditions it is suggested that when the blood bromide level reaches a relatively steady state, the ratio

(Br- blood/Halide blood) / (Br- ingested/Halide ingested)

is constant (K).

From this one could predict the blood bromide level resulting from administrating a defined amount of bromide. In the 6th week of administration K values for treatment groups 1-3 were obtained by using the mean blood bromide levels. The total blood halide remained within the normal range in animals at this point. For calculation an average of 85 mEq/l was used (corresponding to approximate mean concentration in animals from treatment group 2). Maximum bromide blood levels did nor exceed an approximate mean value of 50 mEq/l. This may be explained by the fact that fecal elimination of bromide becomes significant when large doses of bromide are given.

Body temperature was examined in a group of four animals. The mean pre-treatment body temperature for this group was 101.5°F (= 38.6°C). A slight rise to 102.3°F (= 39.1°C) occurred on the second week of administration of 200 mg/kg/day of sodium bromide. No further significant increases were seen during the remainder of the experimental period. It appears unlikely that the elevated temperature is associated with bromide intoxication.

Table A6.4.1/07-1: Results of clinical signs of intoxication, frequency and range of blood bromide levels (of 20 dogs examined)

Signs	Frequency	Range Blood Bromide [mEQ/L]
Ataxia	17	39-50
Emaciation	10	26-45
Stupor	11	50-57
Coma	11	35-59
Skin lesions	5	33-48
Shivering	2	49, 53
Salivation	1	53

Diarrhea	1	50
Vomiting	2	33, 40
Bloody stool	1	23

Table A6.4.1/07-2: Mean Blood bromide levels in dogs on various dosage schedules of Sodium Bromide

Treatment Group 1 (100 mg/kg/day)			Treatment Group 2 (increasing dosage, initial dose of 100 mg/kg/day)			Treatment Group 3 (increasing dosage, initial dose of 200 mg/kg/day)			Treatment Group 4 (400 mg/kg/day)		
Day	Dose NaBr [mg/kg bw/day]	Blood Br- [mEq/L]	Day	Dose NaBr [mg/kg bw/day]	Blood Br- [mEq/L]	Day	Dose NaBr [mg/kg bw/day]	Blood Br- [mEq/L]	Day	Dose NaBr [mg/kg bw/day]	Blood Br- [mEq/L]
14	100	19.8	14	100	13.2	7	200	22.4	7	400	27.4
28	100	22.6	28	100	27.2	14	200	25.9	14	400	40.6
42	100	32.0	42	100	32.4	21	200	31.2	21	400	43.1
56	100	33.8	56	200	34.7	28	200	33	23	*	-
70	100	30	70	200	38.9	29	*	-	27	*	-
84	100	30.6	82	*	-	35	200	34.7	28	400	44.7
96	100	30	84	200	36.6	42	200	36.2	35	500+	41
112	100	30.2	98	400	50	45	*		42	500++	49.9
126	100	28.6	99	*	-	49	300	48.6	44	*	-
140	100	29.4	105	*	-	54	*	-	-	-	-
-	-	-	122	400	51.1	56	300	43.3	-	-	-
-	-	-	133	400	49.3	63	300	47.3	-	-	-
-	-	-	134	*	-	-	77	300	48	-	-
-	-	-	-	-	-	84	300	42.3	-	-	-
-	-	-	-	-	-	91	400	54.5	-	-	-
-	-	-	-	-	-	98	400	53	-	-	-
-	-	-	-	-	-	101	*		-		-
-	-	-	-	-	-	105	400	52.5	-	-	-
-	-	-	-	-	-	110	*		-	-	-
-	-	-	-	-	-	112	400	48.6	-		-
-	-	-	-	-	-	119	400	52.5	-	-	-
-	-	-	-		-	126	400	51	-	-	-
-	-	-	-		-	131	*	-	-	-	-
-	-	-	-	-	-	133	500	48.8	-	-	-
-	-	-	-	-	-	140	500	51.7	-	-	-
-	-	-	-	-	-	147	500	54.4	-	-	-

-	-	-	-	-	-	154	500	54.9	-	-	-
-	-	-	-	-	-	156	*	-	-	-	-
-	-	-	-	-	-	161	500	55.1	-	-	-
-	-	-	-	-	-	168	500	49.6	-	-	-
-	-	-	-	-	-	175	500	60.4	-	-	-
-	-	-	-		-	181	*		-	-	-
-	-	-	-	-	-	182	500	57.4	-	-	-
-	-	-	-	-	-	185	*	-	-	-	-

* death of animal/s

\+ dog began vomiting

++ discontinued because dog continued to vomit

Table A6.4.1/07-3: Relationship between dose and blood concentration of bromide

Dose NaBr [mg/kg/day]	100	200	300	400	500
Blood bromide concentration [mEq/L], mean ± SD	32.2±3.8	36.3±4.5	45.9±9.1	51.0±6.2	49.6±1.9

Conclusion The maximum bromide blood levels reached in this study did not exceed an approximate mean value of 50 mEq/L. This may be explained by the fact that fecal elimination of bromide becomes significant when large doses of bromide are given.

Although the relationship between dose and blood bromide level is at least partially predictable in these experiments, appearance of signs of intoxication are not. Effects on nervous system, gastrointestinal tract and skin appeared over a moderate range of blood levels.

Emaciation and loss of body weight was observed during administration of sodium bromide. This occurred on undiminished ingestion of food and could not be attributed to vomiting. This suggests the possibility that bromide may interfere with the absorption or utilization of neutrients from the gastrointestinal tract.

EXECUTIVE SUMMARY:

Materials and Methods

The study was investigated to receive detailed information on the course of bromide intoxication in dogs. Groups of four dogs each were treated with either constant or increasing doses of sodium bromide for 44-185 days. Sodium bromide was put into gelatine capsules and mixed with the diet. Group 1 was treated with 100 mg/kg/day. To group 4, 400 mg/kg/day were administered to create rapid intoxication. In two additional groups animals were treated with increasing concentrations of sodium bromide, starting with 100 mg/kg bw/day (group 2) and 200 mg/kg bw/day (group 3) respectively. Doses were increased at 6 week intervals and increments were either 100 mg/day or 200 mg/day. Dogs were observed for body weight gain, signs of toxicity and viability and blood bromide concentrations were measured. In order to exclude that the observed effects were due to diet or environment a control group was treated with similar diet in similar environment. After a control period, 4 animals of that group were treated with increasing doses of sodium bromide, the other four dogs were treated with comparable amounts of sodium chloride.

Results and Discussion

Some form of neurotoxicity was seen in all dogs that received 200 mg/kg bw/day or more of sodium bromide (please refer to table 6.4.1/07-1). The usual progression as the blood bromide level rose was slight ataxia, stupor, severe ataxia (unable to stand) and coma. Signs of gastrointestinal toxicity appeared almost as frequently as did those of neurotoxicity. Transient diarrhea, bloody stool and vomiting were observed which was probably due to the irritant effect of bromide on the gastrointestinal tract. Skin lesions were observed on the hind limbs, head and sternum in some of the animal and were characterised mainly by nonsuppurative white macules overlad by scales and varying in size. As this often is the case in human bromide intoxication, there was no relationship between blood bromide levels and the appearance of skin changes. However, animals receiving 100 mg/kg bw/day of sodium bromide did not develop these changes. Comparison of the data from dosage groups 2 and 3 (increasing dosages) with that of dosage group 4 (400 mg/kg bw/day) indicates that rapid elevation of the blood bromide level increases the lethality of bromide (please refer to table 6.4.1/07-2). Three of the four dogs from group 4 were dead within 44 days, 2 of these within 27 days. Five dogs on dosage groups 2 and 3 receiving the same dose of bromide (400 mg/kg/day) survived for at least 42 days and three survived for at least 87 days on even higher doses of bromide. The animals on dosage group 1 were maintained for 140 days without any fatalities and only minimal signs of toxicity were observed; the mean bromide blood level was 30 mEq/L. Treatment with 400 mg/kg/day resulted in a blood bromide level of 50 mEq/L. The most interesting sign of gastrointestinal toxicity was the appearance of emaciation and loss of body weight. This occurred on undiminished ingestion of food and could not be attributed to vomiting as it was observed in most dogs even at doses of sodium bromide which caused no other signs of toxicity. Maximum bromide blood levels did not exceed an approximate mean value of 50 mEq/L. This may be explained by the fact that fecal elimination of bromide becomes significant when large doses of bromide are given. Although the relationship between dose and blood bromide level is at least partially predictable in these experiments, appearance of signs of intoxication are not (please refer to tables 6.4.1/07-1 and 6.4.1/07-3). Effects on nervous system, gastrointestinal tract and skin appeared over a moderate range of blood levels. The severity of neurotoxicity appears to increase as the blood bromide level rises, but in many cases appearance of severe intoxication occurred suddenly at blood levels which more typically caused mild neurotoxicity (see table 6.4.1/07-2).

3.9.1.1 [Study 8] Short-term repeated dose toxicity of ammonium bromide: Dose-range finding study, oral in rat

Reference	A6.3.1/01, Doc. No. 532-001 Study report, 1999
Guideline	Guideline:other: 4 week dose range-finding study Principles of method if other than guideline: The methods and procedures applied were in accordance with OECD guideline 407 (subacute oral toxicity study in rodents). GLP compliance: yes
Reliability	Reliability:1 (reliable without restriction) Rationale for reliability incl. deficiencies:other: GLP Dose range-finding study
Species / strain	Species: rat Strain: Sprague-Dawley Sex: male/female
Test material	Details on test material:- Name of test material (as cited in study report): Ammonium bromide - Physical state: White crystalline solid - Analytical purity: 99.94%

- Lot/batch No.: 980060

- Stability under test conditions: Not determined; considered to be stable under the storage conditions

- Storage condition of test material: The material was stored in the dark at ambient temperature

Study design

ADMINISTRATION / EXPOSURE
Route of administration:oral: feed
Vehicle:unchanged (no vehicle)
Details on oral exposure:
PREPARATION OF DOSING SOLUTIONS:
DIET PREPARATION
First a premix was prepared consisting of test material which was sieved through a 250 µm sieve and manually mixed with approximately the same weight of sieved diet (250 mm) prior to addition to the bulk premix. This was then mixed on a Hobart mixer for 2 hours before being transferred to a labelled premix container and booked in as a Test Material. Formulated diets were prepared from the premix. Fresh diets were prepared weekly.

Analytical verification of doses or concentrations:yes
Details on analytical verification of doses or concentrations:Trial formulations of the lowest and highest concentrations were investigated for stability, concentration and homogeneity. Triplicate samples of diet were taken from each formulation (including control) immediately after preparation during Weeks 1 and 4 of the study.
Analysis of formulated diets carried out during Weeks 1 and 4 were acceptable in terms of concentration and homogeneity with the percentage differences from the nominal value being within the specified range of ± 10%.
Duration of treatment / exposure:4 weeks (28 days)
Frequency of treatment: Daily

Doses / Concentrations: 100, 500, 1000 mg/kg bw/day Basis: nominal in diet
No. of animals per sex per dose: 5/sex/dose
Control animals:yes, plain diet
Dose selection rationale: Range finding study for dose determination in 90-day study

DETAILED CLINICAL OBSERVATIONS: Yes
All animals were examined for reaction to treatment during the day. Once a week they received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.
Mortality: All animals were checked for viability twice each day.

BODY WEIGHT: Yes
- Time schedule for examinations: Body weights were recorded twice each week commencing one week pre-trial up until the end of the dosing period.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes
The quantity of food consumed by each animal was measured and recorded twice each week commencing one week pretrial up until the end of the dosing period.

FOOD EFFICIENCY: No

WATER CONSUMPTION: Yes
- Time schedule for examinations: Water consumption was monitored by visual inspection of the water bottles on a weekly basis throughout the study

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

CLINICAL CHEMISTRY: No

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No
GROSS PATHOLOGY: Yes
Epididymis, heart, implant, kidneys, liver, lung, ovaries, spleen and testis were taken from all animals, weighed and placed in 10% neutral buffered formalin (fixation).
Organ weights: Epididymis, heart, implant, kidneys, liver, lung, ovaries, spleen, testis
HISTOPATHOLOGY: No
Other examinations:Not applicable
Statistics: Body weight data was analysed for homogeneity of variance using the "F-max" test. If the group variance appeared homogeneous, a parametric ANOVA was used and individual between group comparisons were made using Fischer´s F-protected LSD method via student´s t-test. If the variances were heterogenous, then a non-parametric test such as a Kruskal-Wallis ANOVA was used. Individual between group comparisons were made using Fisher´s F-protected LSD method.
Organ weights were also analysed conditional on body weigh ie analysis of covariance (ANCOVA).

Findings

CLINICAL SIGNS AND MORTALITY
In the Intermediate and high dose group of both sexes the clinical signs most frequently observed included agitated, nervous and hyperactive behaviour, subdued behaviour, rolling gait, hunched posture, piloerection, and eyes partially closed indicative for signs of neurotoxicity. Other signs appeared to a lesser extent and included unkempt coat and irregular breathing. There were no notable signs in the low dose or control groups. All other signs were in the usual range of signs commonly seen in rats of this age and strain in the testing facility.
There were no premature deaths.

BODY WEIGHT AND WEIGHT GAIN
Mean body weight was decreased in high dose males and females reaching significance in the males from Day 10 of treatment onwards when compared to the Control group. Mean body weight gain was significantly decreased in high dose males (-49%) and high dose females (-31%) when compared to the respective control groups. Body weight and body weight gain were only slightly reduced in males of the mid dose group, but remained unaffected by treatment in females of this dosage group. Animals from the low dose group (both males and females) remained unaffected by treatment.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
Food consumption was decreased in high dose males on Day 10 of treatment (-24%) and more markedly decreased in this dose group from Day 21 onward (about -31%) On Day 28 of treatment, food consumption was reduced by about 29%. Food consumption in females was considered to have been similar throughout all the dose groups. Food intake rates remained unaffected by treatment in the low and mid dose groups.
Achieved dosages were satisfactory for most dose groups, with the mean dosage within ± 10% of target. This was due to the low food consumption of this dose group.

ORGAN WEIGHTS
High dose males showed marked decreases in mean epididymides, heart, liver, kidneys, lungs and testes absolute weights when compared to the control group. Intermediate dose males also showed significantly relevant decreases in mean epididymides, kidneys and testes absolute weights when compared to control group. However, due to the decreased mean body weight of the intermediate and high dose males (statistically significant in the high dose group) these apparent effects disappeared after covariance analysis and so were not considered to be attributable to the administration of ammonium bromide in the diet. After covariance analysis high dose females showed a significant increase in mean liver weight. This effect was not noted in the absolute organ weight analysis.

GROSS PATHOLOGY
At necropsy there were no significant findings and, with the exception of staining being recorded for some rats, most animals of the treatment groups did not show any treatment-related macroscopic

findings.

NOEL:100 mg/kg bw/day (nominal) Sex:male/female

LOAEL:500 mg/kg bw/day (nominal) Sex:male/female

Table 1: Incidence of selected clinical observations

Observation/Finding	**Group/Dose Level (mg/kg/day)**							
	1 (0)		**2 (100)**		**3 (500)**		**4 (1000)**	
Sex	m	f	m	f	m	f	m	f
Number of animals per group	5	5	5	5	5	5	5	5
Subdued behaviour	0	0	0	0	5	0	5	4
Agitated behaviour	0	0	0	0	1	4	0	5
Rolling gait	0	0	0	0	5	5	5	5
Hunched posture	0	0	0	0	1	2	3	5
Nervous/hyperactive behaviour	0	0	0	0	0	5	0	4
Eyes partially closed	0	0	0	0	2	2	4	5
Piloerection	0	0	0	0	3	3	2	5
Irregular breathing	0	0	0	0	0	0	0	1
Unkempt	0	0	0	0	1	2	2	3

m: male; f: female

Table 2: Mean food consumption, Bodyweights, absolute organ weights and achieved doses

	Control (0 mg/kg/day)		**Low dose (100 mg/kg/day)**		**Medium dose (500 mg/kg/day)**		**High dose (1000 mg/kg/day)**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals examined	5	5	5	5	5	5	5	5
	Food consumption (g/animal/day)							
Days 1-8	31.5	20.9	33.5	20.9	32	20	25.8	19.6
Day 8-14	31	22.7	31.1	19.8	29.3	21.4	25	21
Days 15-21	33.6	24	34.7	20.8	29.3	23.2	22.5**	20.3
Days 22-28	33.6	24.1	32	22.8	32	23.2	23.9**	21.5
	Body weights (g)							
Day 0	198	141	197	136	193	136	196	138
Day 7	258	164	264	163	260	169	234	159
Day 14	313	190	321	183	309	187	268	173
Day 21	363	204	369	203	345	201	286	185
Day 28	406	218	409	218	378	217	303	191
	Bodyweight gain (g)							
Day 0 - 28	208	77	212	82	185	81	107***	53*
	Mean absolute organ weights (g)							
Epididymides	0.89	-	0.84	-	0.79	-	0.75	-
Heart	1.47	0.89	1.44	0.95	1.34	0.86	1.16**	0.8
Kidneys	3.22	1.84	3.13	1.79	2.82 **	1.86	2.35 ***	1.74
Liver	18.38	9.28	19.19	9.56	17.56	10.39	13.19 **	10.21
Lung	1.7	1.19	1.56	1.31	1.56	1.28	1.38 **	1.02
Spleen	0.74	0.48	0.77	0.53	0.71	0.47	0.66	0.49
Testes	3.49	-	3.12 *	-	3.09 *	-	2.92 **	-
Ovaries	-	0.086	-	0.092	-	0.112	-	0.095
	Achieved Dosages (ppm)							
Week 1	-	-	108	105	524	484	884	986
Week 2	-	-	91	82	441	435	859	911
Week 3	-	-	105	93	462	517	888	917
Week 4	-	-	93	109	485	560	976	1083

Mean Week 1-4 (as % of target)	-	-	99%	97%	96%	100%	90%	97%

m male
f female
* statistically significantly different from controls with p< 0.05
** statistically significantly different from controls with p< 0.01
*** statistically significantly different from controls with p< 0.001

Conclusion

EXECUTIVE SUMMARY:
The objective of the study was to provide dose range finding information on the toxicity of Ammonium Bromide in the rat after administration by the diet continuously for 4 weeks. The test material was administered at dose levels of 0, 100, 500 and 1000 mg/kg/day. Dose levels were selected after evaluation of existing data and took into account the maximum tolerated dose in rats and other factors such as anticipated human exposure. The concentration of test material in the diet was adjusted regularly to achieve a constant dose level.

Formulated diets were prepared as follows: First a premix was prepared consisting of test material which was sieved through a 250 µm sieve and manually mixed with approximately the same weight of sieved diet (250 mm) prior to addition to the bulk premix. This was then mixed on a Hobart mixer for 2 hours before being transferred to a labelled premix container and booked in as a Test Material. Formulated diets were prepared from the premix. Fresh diets were prepared weekly. Prior to study commencement trial formulations of the lowest and highest concentrations were investigated for stability, concentration and homogeneity. This was carried out in the Product Chemistry laboratories of Inveresk Research under a separate protocol and contract (Inveresk Project No. 379673). Analysis of formulated diets was undertaken with regard to stability, concentration and homogeneity. Triplicate samples of diet were taken from each formulation (including control) immediately after preparation during Weeks 1 and 4 of the study.

Intermediate and high dose group animals of both sexes were noted as having agitated behaviour, a rolling gait, hunched posture and eyes partially closed indicative for signs of neurotoxicity. All other animals were considered normal throughout the study. Both body weight gain and food consumption were reduced in the high dose males.

High dose male showed marked decreased mean absolute weights in epididymides, heart, kidneys, lung, liver and testes when compared to control group. Intermediate dose males also showed decreases in mean epididymides, kidneys and testes absolute weights when compared the control group.

The lower body weights and reduced body weight gain noted in the high dose group males are undoubtly related and contributed to the more variable achieved dosage values for this dose group throughout the study. It should be noted when considering dose levels for future studies that these effects were presented at ca 90% of target dose i.e. ca 900 mg/kg/day as opposed to the target of 1000 mg/kg/day.

High dose females showed a significant increase in mean liver weight after covariance analysis. This effect is of unclear toxicological significance in the absence of any corroborative histological or clinical pathology evidence and, given the absence of any similar finding in males, this increase may not be related to the treatment with ammonium bromide.

There were no premature deaths.

There were no visual differences in water consumption between groups treated with ammonium bromide and the control group.

Analyses of diets were acceptable in terms of concentration andhomogeneity with the percentage difference from the nominal value being within the specified range of ± 10%.

In this study clinical signs were noted in male and female rats at a dose level of 500 mg/kg bw/day and above. The observed clinical signs consisted of agitated, nervous and hyperactive behaviour,

subdued behaviour, rolling gait, hunched posture, piloerection and eyes partially closed. To a lesser extent unkempt coat and irregular breathing were noted. Statistically significant reduced body weight gains (males:-49%; females:-31%), reduced food consumption (males) and increased rel. liver weight (females) were also noted in animals of the high dose group (1000 mg/kg bw/day).

The NOEL for male and female rats was determined at 100 mg/kg bw/day. The NOAEL for male and female rats was determined at 100 mg/kg bw/day (corresponding to 82 mg bromide/kg bw/day) on clinical signs indicating neurotoxicity noted in both sexes at the dose level of ≥500 mg/kg bw/day, reduced body weight gain noted in both sexes at the dose level of 1000 mg/kg bw/day and increased liver weight noted in females at 1000 mg/kg bw/day.

3.9.1.2 [Study 9] Repeated Dose 90-Day Oral Toxicity of ammonium bromide in Rats (OECD TG 408)

Reference

A6.4.1/01, Doc. No. 533-001

Study report, 2000a

Guideline

according to Guideline: OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)

Test guideline 2 : according to Guideline: EPA OPPTS 870.3100 (90-Day Oral Toxicity in Rodents)

GLP compliance:yes

Reliability

Reliability:1 (reliable without restriction)

Species / strain

Species:rat

Strain:Sprague-Dawley

Sex:male/female

Test material

Details on test material:- Name of test material (as cited in study report): Ammonium bromide

- Physical state: White crystalline solid

- Analytical purity: 99.91%

- Lot/batch No.: 980060

- Stability under test conditions: Not determined; considered to be stable under the storage conditions

- Storage condition of test material: The material was stored in the dark at ambient temperature

Study design

ADMINISTRATION/EXPOSURE

Route of administration:oral: feed

Vehicle:unchanged (no vehicle)

Details on oral exposure:

PREPARATION OF DOSING SOLUTIONS:

DIET PREPARATION

Formulated diets were prepared from premix (Groups 3 and 4) or from the Group 4 diet (group 2). Treated diets were formulated weekly throughout the treatment period. The exact concentration (ppm) of test material in the diet varied over the treatment period to allow for the varying bodyweight and food consumption data produced by the animals. This enabled as near a constant dose level (mg/kg/day) as possible to be achieved.

Analytical verification of doses or concentrations:yes

Details on analytical verification of doses or concentrations:Analysis of formulated diets was undertaken with regard to stability, concentration and homogeneity. Triplicate samples were taken

from each formulated diet (including control) immediately after preparation on Day 1 and during Weeks 7 and 13 of treatment.

Analysis of formulated diets indicated that actual dose levels administered were within ± 10 % of the nominal concentration, indicating acceptable accuracy of formulation. The coefficients of variation were all less than 10 %, indicating acceptable homogeneity.

Duration of treatment / exposure:13 weeks (90-days)

Post-exposure period: 4 weeks for the control and high dose group (10 animals/sex/group); no postexposure period for the low and intermediate dose group and the remaining animals of the control and high dose group

Frequency of treatment: Daily

Doses / Concentrations: 0, 100, 225, 500, 750 mg/kg bw/day Basis: nominal in diet

No. of animals per sex per dose:25/sex/group (control and high doses), 15/sex/group (low and intermediate dose)

Control animals:yes, plain diet

Dose selection rationale: 4 week dose range-finding study.

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule: All animals were examined for reaction to treatment each day. The onset, intensity and duration of any signs were recorded.

In addition, each week, all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Mortality: Each animal was checked twice per day for viability

BODY WEIGHT: Yes

- Time schedule for examinations: All animals were weighed once weekly during the pretrial period, up until the end of the recovery period. Animals showing weight loss or deterioration in condition were weighed more frequently as necessary.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

The quantity of food consumed by each animal was measured and recorded once during the week prior to the start of treatment and then weekly up until the end of the recovery period.

FOOD EFFICIENCY:

- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): Yes

- Time schedule for examinations: Water consumption was monitored by visual inspection on a weekly basis throughout the study, commencing during the pretrial period.

OPHTHALMOSCOPIC EXAMINATION: Yes

Ophthalmoscopic examination was carried out on all animals before treatment commenced and on control and high dose animals during Week 12 of treatment. The eyes were examined using an indirect ophthalmoscope after the application of 1 % Tropicamide (Mydriacyl). Anterior, lenticular and fundig areas were evaluated.

HAEMATOLOGY: Yes

- Time schedule for collection of blood: Time points: during Week 12, and additionally for control and high dose rats during Week 17 (end of recovery)

- Anaesthetic used for blood collection: Yes (identity) / No / No data

- Animals fasted: Yes / No / No data

- How many animals: All animals

- Parameters examined:

CLINICAL CHEMISTRY: Yes

- Time schedule for collection of blood: Time points: during Week 12, and additionally for control and high dose rats during Week 17 (end of recovery)

- Animals fasted: Yes / No / No data

- How many animals: All animals

- Parameters examined:

URINALYSIS: Yes

- Time schedule for collection of urine: Time points: during Week 12, and additionally for control and high dose rats during Week 17 (end of recovery);

- Metabolism cages used for collection of urine: Yes / No / No data

- Animals fasted: Yes

- Parameters examined:

NEUROBEHAVIOURAL EXAMINATION: Yes

Detailed functional observations were performed during pretrial and in Weeks 4, 8, 13 and 17. Observations included, but were not limited to: posture/condition, ease of removal from the cage, body temperature, condition of eyes and coat, presence of salivation, overall ease of handling, latency, level of mobility, rearing, grooming, urination/defecation, arousal (level of alertness), tremor/convulsions, vocalisation, piloerection. Palpebral closure, gait abnormalities, stereotypy and/or unusual behaviours, reaction to sudden sound, reaction to touch on the rump, grip strength, pain perception, landing foot splay, motor activity.

GROSS PATHOLOGY: Yes

On completion of 13 weeks of treatment 10 males and 10 females per group were killed and underwent detailed necropsy. All control and high dose animals also underwent a full histological examination. A further 5 males and 5 females per group were killed by perfusion fixation (injection of 1 % sodium nitrite and 1 % heparin via the tail vain, perfusion with phosphate buffered saline to remove most of the blood, fixation by perfusion with paraformaldehyde/glutaraldehyde-mixture) for neuropathological examination, with nervous tissues and organs from all perfused animals being examined histologically.

The control and high dose animals that were retained for 4 weeks after treatment received a detailed necropsy, but no histological examination was conducted.

Organ weights: adrenal, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary, prostate, spleen, submaxillary salivary gland, testis, thymus, thyroid, parathyroid, uterus

HISTOPATHOLOGY: Yes

adrenal, aortic arch, brain, epididymis, eye, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, heart, kidney, larynx, liver, lung, mesenteric lymph node, nasal cavity, oesophagus, optic nerve, ovary, pancreas, pharynx, pituitary, prostate, sciatic nerve, seminal vesicles, skin and mammary gland, spinal cord, sleen, sternum, submandibular lymph node, submaxillary salivary gland, testis, tigh muscle, thymus, thyroid, parathyroid, tongue, trachea, urinary bladder, uterus, vagina

Other examinations: None

Statistics: Bodyweight, food consumption, haematology, clinical chemistry and urinalysis data were subjected to analysis of variance or the Kruskal-Wallis non-parametric analysis, as appropriate.

Organ weight data were subjected to analysis of variance and of covariance using the terminal bodyweight as the single covariate.

Histological incidence data were analysed using Fisher´s Exact Probability test where considered appropriate.

Findings

RESULTS

CLINICAL SIGNS AND MORTALITY

The principal signs were rolling gait, intermittent staggering, subdued behaviour, nasal bleeding, unkempt coat, hunched posture, discharge from the eyes and splayed hind limbs. Some or all of these signs were observed for all animals at 500 mg/kg/day (males) and 750 mg/kg/day (females); among males 225 mg/kg/day, 11/15 showed subdued behaviour, sometimes accompanied by other signs. These signs generally became apparent after approximately 8 weeks of treatment, and persisted until necropsy (main study animals) or until at least the third week of the recovery period.

Additionally, the claws of many male rats at 225 and 500 mg/kg/day and females at 750 mg/kg/day were longer than normal. The presence of occasional females at 100 and 225 mg/kg/day with longer claws could not be positively associated with treatment.

Irregular respiration was noted for 7 males at 500 mg/kg/day, although 2 animals did not show this finding until the recovery period.

There were 3 premature descendents, all among males at 500 mg/kg/day. One animal was killed in the 6th week of treatment, with signs including laboured respiration; necropsy and histology indicated a widespread purulent pneumonia. Another animal was killed in the 13th week of treatment, with limping/swelling affecting the right hind foot; necropsy and histology indicated marked pododermatitis. The third animal was killed in the 9th week of treatment due to limping. None of the reasons for the premature terminations appeared to be associated with treatment.

BODY WEIGHT AND WEIGHT GAIN

Males:

At 500 mg/kg/day there was a slight increase in bodyweight gain to Day 6; from Day 6-13, gain was similar to control, but by Day 28 mean weights at this level were lower than Control, with differences from Day 35 attaining statistical significance (-23% on Day 91 of treatment). During the 4th week recovery period, there was an increase in weight gain, compared with control, although absolute weights remained significantly lower. At 225 mg/kg/day, there was a slight increase in bodyweight gain to Day 13, with the difference at Day 13 attaining statistical significance. From Day 13, gain was slightly lower than control, although absolute weights were slightly greater to Day 28. By Day 49, however, weights at this level were lower than control, attaining statistical significance from Day

63 (-10% on Day 91 of treatment). At 100 mg/kg/day, bodyweight performance was similar to control.

Females:

At 750 mg/kg/day, there was a slight increase in bodyweight gain to Day 6, with the difference at Day 6 attaining statistical significance, but by Day 21 mean weights at this level were lower than control, with the differences from Day 35 attaining statistical significance (-22% on Day 91 of treatment). During the 4th week recovery period, there was a slight increase in weight gain, compared with control, although absolute weights were significantly lower in most weeks. At 225 mg/kg/day, mean weight gain and absolute weights were greater than Control in the early part of the dosing period, with many differences being statistically significant. During the latter part of the dosing period, bodyweight gain was slightly lower than control, with absolute weights at this level being similar to control. At 100 mg/kg/day, bodyweight performance was similar to control.

FOOD CONSUMPTION AND COMPOUND INTAKE

Because the animals were receiving powdered diet and were housed in solid bottomed cages containing bedding material, it was difficult to measure scattered diet, and this impacted upon the accuracy of the food consumption calculations. However, it was considered that the inherent errors in assessment of scatter would apply equally to animals in all groups of the study, and therefore the validity of the study was not affected.

Males: At 500 mg/kg/day, mean food consumption during the first 3 weeks of treatment was significantly greater than control. During Weeks 4-13, consumption was lower than control, with most differences statistically significant. During the first week of recovery, consumption was slightly greater than control, in the other weeks of recovery, consumption was similar to control. At 225 mg/kg/day, mean food consumption during the first 3 weeks of treatment was also significantly greater than control, but thereafter consumption was slightly lower; some differences were statistically significant. At 100 mg/kg/day, there was no consistent effect on food consumption. Slight differences from the food consumption of the controls were recorded, a small number of which achieved statistical significance, but these were considered to be of no toxicological significance.

Females: At 750 mg/kg/day, food consumption in the first and fourth week of treatment was significantly greater than control; in later weeks, consumption was generally lower than control, with occasional differences attaining statistical significance. During the first week of recovery, consumption was significantly greater than control; in the other weeks of recovery, consumption was similar to control. At 225 mg/kg/day, mean consumption was generally greater than control, with some differences attaining statistical significance. At 100 mg/kg/day, there was a tendency towards slightly increased food consumption, although sporadic values were lower than the corresponding control values.

HAEMATOLOGY

Week 12:

For males treated at 500 mg/kg/day, mean values for haemoglobin and haematocrit were higher than those of the concurrent controls. Although the statistical significance of the increase may have been exaggerated by the concurrent controls being at the lower end of the background control range, both of the values for treated males were above the background control range. Females treated at 750 mg/kg/day also showed minimal increases in haemoglobin and haematocrit, but the differences from the concurrent control values did not achieve statistical significance. For males at 100 and 225 mg/kg/day mean haemoglobin level was slightly increased compared with the concurrent controls, the difference achieving statistical significance, but no dose-relationship was apparent and there was no corresponding increase in haematocrit. The increase in these groups was considered to be a consequence of the low concurrent control value, as the haemoglobin level of both treated groups was similar to the background control mean, and, therefore, was of no toxicological significance. Values for females in the low and intermediate treatment groups were similar to those of the controls.

For males and females in the high dose group (500 and 750 mg/kg/day respectively), the mean total white blood cell count was slightly increased, although the differences from the controls were not statistically significant. In the case of males, the increase was largely due to two animals. For both

sexes the overall increase reflected increases in the majority of white cell types, with the values for neutrophils and monocytes in females achieving statistical significance. At 225 mg/kg/day for females, minimal increases were seen in the same parameters but males were unaffected, as were both sexes at 100 mg/kg/day.

There were no obvious treatment related differences in platelet count and prothrombin time of males and females at any treatment level.

Week 17:

At the end of the 4-week recovery period, mean haematocrit and haemoglobin values of males and females previously treated with 500 and 750 mg/kg/day respectively were similar to control values. For males, mean cellular haemoglobin (MCH) was statistically significantly higher than that of the concurrent controls. However, the increase was considered to be of doubtful toxicological significance, as the concurrent control value was lower than the Week 12 control value and was at the lower end of the Week 12 background control range, whereas the value for the previously treated males was similar to both the Week 12 control value and background control mean value. MCH for previously treated females was similar to that of the controls.

In contrast to Week 12 findings, mean values for total and differential white cell counts of previously treated males and females were generally lower than those of the corresponding concurrent controls, with the differences for total white cell count and lymphocytes (both sexes), monocytes (males) and large unclassified cells (females) achieving statistical significance.

For previously treated females, although mean prothrombin time appeared to be significantly increased, the magnitude of the increase was minimal and it was considered not to represent any toxicological response to treatment.

CLINICAL CHEMISTRY

Week 12:

In both sexes, mean assayed chloride levels were apparently greater in all groups, with values for males at 225 and 500 mg/kg/day and females at all levels attaining statistical significance. However, to some extent these increases may have reflected increased plasma bromide levels, because the bromide ion has been found to interfere with the chloride ion in the assay.

Mean cholesterol levels in males at 225 and 500 mg/kg/day and females at all levels were significantly lower than control.

The mean total bilirubin level in females at 750 mg/kg/day was significantly reduced.

Mean phosphate levels of males at 225 and 500 mg/kg/day were significantly higher than control; a similar effect was not observed for females or for males in the low treatment group. The apparent increase in calcium levels among females at 100 and 225 mg/kg/day was influenced by a control group in which only 2 animals had the calcium level determined.

The mean glucose level among males at 100 mg/kg/day was significantly lower than control; this observation was not seen at higher levels.

Week 17:

After 4 weeks of recovery, mean assayed chloride levels of both sexes of treated animals were very slightly, but significantly greater than control, although the levels were slightly lower than the control values at Week 12.

Mean calcium levels of both sexes were significantly lower than control, but the actual differences were small and considered to be of doubtful toxicological significance.

Mean cholesterol among females at 750 mg/kg/day was significantly lower than control.

Among females at 750 mg/kg/day, mean glucose level was significantly greater than control, but this observation was not supported by a similar finding in males. Alkaline phosphatise at this level was significantly increased, but this increase was influenced by a low control value. Albumin at this dose

level was decreased, but was within the background range; the control value was at the upper and of that range: the slight differences in globulin and A-G ratio were considered to be secondary observations. The differences in glucose, alkaline phosphatise and albumin were considered not to be of toxicological significance.

URINALYSIS

At Week 12, the urine pH of both sexes at the high dose levels was lower than control, with the value for females attaining statistical significance; this finding was not seen at Week 17.

At Week 17, slight differences in specific gravity were considered to be incidental, despite their statistical significance, in view of the low actual differences in the values and because males showed a decrease whilst females showed an increase. The increased urine volume of males at Week 17 was also considered to be incidental.

NEUROBEHAVIOUR

Males: At 500 mg/kg/day many males were limp when handled (Weeks 4, 8 and 13) and/or showed stumble/rolling gait (Weeks 8 and 13). These signs were observed for occasional animals at 225 mg/kg/day, and limpness was also recorded for a few animals at 100 mg/kg/day of ammonium bromide. Occasional males had reduced alertness (225 and 500 mg/kg/day) and unkempt coat (500 mg/kg/day). All of the signs at 500 mg/kg/day had disappeared by the end of the recovery period.

Females: At 750 mg/kg/day, many females were limp in the hand (Weeks 8 and 13) and/or showed stumble/rolling gait (Weeks 4, 8 and 13). Limpness was also recorded for 4 animals at 225 mg/kg/day in Week 8. All of these signs at 750 mg/kg/day had disappeared by the end of the recovery period. Occasional females at 750 mg/kg/day had unkempt coat, which persisted into the recovery period.

Some inter-group variations in motor activity parameters were recorded for males and females at each of the test intervals, a small number of which achieved statistical significance compared with the concurrent controls. There were, however, no consistent findings that were indicative of a dose or treatment related response to treatment regarding motor activity.

Detailed functional observations:

Males: Among males, landing foot splay at 225 and 500 mg/kg/day was increased during Week 4, with differences for trial 2 attaining statistical significance. At other times, values for foot splay showed no statistically significant inter-group differences. At 500 mg/kg/day, hind limb grip strength was significantly lower at Weeks 4, 8 and 13; fore limb grip strength was significantly lower at Weeks 8 and 13. By the end of the 4th week recovery period, there were no significant differences in these parameters. At 225 mg/kg/day, hind limb strength at Week 4 was significantly lower than control, although the fore limb grip strength was slightly increased. At Weeks 8 and 13, hind limb grip strength values were also lower than concurrent control values although statistical significance was not attained; forelimb grip strength was unaffected. At 500 mg/kg/day the tail flick response was slightly slower than control, with the difference for the trial 3 value at Week 13 attaining statistical significance. However, the control value for trial 2 was low compared with the trial 1 value, which tended to exaggerate the group 4 result and by the end of the 4th week recovery period, there was no difference in response time. At 225 mg/kg/day, values for tail flick were similar to Control. At 500 mg/kg/day, the number of sectors traversed was significantly increased in Week 8; this observation was not repeated at other times. At 100 mg/kg/day, there were no obvious differences in the detailed functional observations for male rats.

Females: Among females, landing foot splay at 225 and 750 mg/kg/day was increased during Week 4, with differences for trial 1 at 750 mg/kg/day attaining statistical significance. At other times, foot splay values were similar to control. At 750 mg/kg/day fore and hind limb grip strength were reduced at Weeks 4, 8 and 13, with the differences at Weeks 8 and 13 attaining statistical significance. At the end of the 4th week recovery period, hind limb strength remained lower than control, although fore limb strength was similar to control. There were no other obvious differences among females in the detailed functional observations.

ORGAN WEIGHTS

Week 13:

Among males, most absolute organ weights at 500 mg/kg/day, together with weights for brain, epididymides, heart, liver and testes at 225 mg/kg/day, were significantly lower than control, reflecting the lower mean bodyweights at these levels. Following covariance analysis to adjust for bodyweight, none of the differences were statistically significant, although the validity of this statistical method was questionable because of the marked treatment-related effect on bodyweight. At 100 mg/kg/day, brain and epididymides weights were significantly lower than control, although adjusted weights were not significantly decreased.

In females at 750 mg/kg/day, absolute brain, lung and thymus weights were significantly lower than control, although the differences were no longer statistically significant after covariance adjustment. Absolute salivary gland and uterus weights at this level were significantly reduced; for these organs in this study, the covariance adjustment indicated decreasing organ weight with increasing body weight, and therefore, the assessment was based on the absolute weights.

The thyroid weights of males at 100 and 225 mg/kg/day, both absolute and adjusted, were significantly lower than control, although weights at the high dose (500 mg/kg/day) were not significantly different. A similar but slighter effect was noted for females, although none of the differences were statistically significant. Thyroid weights were taken by two technicians. The weights from control group animals were separated between the two technicians and recorded weights from one technician were slightly lower than from the other. Because of this difference and because of the small actual weights and the absence of an effect at the high dose, the apparent decreases at 100 and 225 mg/kg/day were not attributed to treatment.

Among females at 100 and 225 mg/kg/day, there were no significant differences for any organ.

Week 17:

At 500 mg/kg/day, following the 4-week recovery period, epididymides weights (absolute and adjusted) were significantly lower than control. Absolute prostate and testes weights were significantly reduced, although the statistical significance had disappeared after covariance adjustment. Adjusted spleen weights were increased, although absolute weights were similar to control; this apparent increase appeared to have been due to an over-correction by the analysis of covariance.

At the end of the 4-week recover period, the thyroid weights (absolute and adjusted) of high dose males were significantly greater than control, although the high dose value was very similar to the control value at Week 13. A slight, but not significant increase was noted for thyroid weights of high dose females. The apparent slight increases were attributed to 4 animals with noticeably higher weights rather than to an increase across all animals, and the differences considered to be of no toxicological significance.

At 750 mg/kg/day, following the recovery period, absolute adrenal gland, brain and lung weights were significantly lower than control, although differences were no longer statistically significant after covariance adjustment.

GROSS PATHOLOGY

There were no necropsy findings attributed to administration of ammonium bromide. One female rat treated at 750 mg/kg bw/dayhad a subcutaneous mass on the head. The left testis and epididymis of another animal of the 500 mg/kg bw/day group were small and flaccid. All other findings recorded reflect the usual range of congenital or spontaneously arising background findings in rats of this strain, at this age, on this type of study at the performing laboratory.

HISTOPATHOLOGY:

Main study animals:

The subcutaneous mass on the head of one female rat was found to be a granuloma, containing areas of acute inflammation, bacterial colonies, and pigmented macrophages. The male animal with the abnormal finding had widespread left testicular tubular atrophy. Due to the isolated occurrence in individual animals only and since no dose-response relationship was evident, neither of these findings was attributed to the test material. All other findings recorded reflect the usual range of congenital or spontaneously arising background findings in rats of this strain, at this age, on this type of study at the performing laboratory.

Neuropathology study animals:

Ventricular dilatation was seen in animals from all groups. Occasional ballooned axons and digestion chambers, extending one internode length, were noted in animals from all groups. An epidermal cyst was seen in the white matter of the lumbar cord of one female at 225 mg/kg/day. Epidermal cysts are congenital lesions of the spinal cord. They are attributed to improper closure of the neural tube in the later stage of embryonal development. The finding of ventricular dilatation was considered to be artefactual, as perfusion fixation is known to cause minimal to mild ventricular distension. Occasional ballooned axons and digestion chambers were seen. Such small segmental demyelination lesions are recognised spontaneous lesions of peripheral nerves.

Recovery study animals: The thyroids from the control and high dose recovery study animals showed no changes that were attributed to treatment.

EXECUTIVE SUMMARY:

MATERIALS AND METHOD

The objective of this study was to assess the toxicity (including neurotoxicity) of the test material in the rat after oral administration with the diet for 13 weeks including as recovery period of 4 weeks for control and high dose animals. As part of the assessment of toxicity, it was also intended to identify the no observed effect level (NOEL) and any toxic effects.

The main study and neuropathology animals were treated continuously by the diet for at least 13 consecutive weeks. The recovery animals were fed treated diet for 13 weeks, and were then fed untreated diet for a period of at least 4 weeks. The concentration of the test material in the diet was adjusted at weekly intervals to achieve a constant dose level of test material per kg of bodyweight per day.

All animals were observed for viability and examined for reaction to treatment. In addition, each week, all animals received a detailed clinical examination. Detailed functional observations were performed during pretrial and at several time points throughout the study. Once per week bodyweight and food and water consumption were recorded. Ophthalmoscopic examination was performed during the pretrial and for control and high dose animals again during Week 12 of treatment.

At the end of the treatment and recovery periods organ weights were taken; several organs, including nerve tissue, were fixed and stained with haematoxylin and eosin for histological examination.

RESULTS AND DISCUSSION

Clinical signs of reaction at the highest treatment levels of ammonium bromide tested in this study (500 mg/kg/day for males and 750 mg/kg/day for females) included subdued behaviour and abnormalities of gait. Additional findings included hunched posture, unkempt coat and claws that were longer than normal. The detailed neurotoxicological findings included increased limpness, decreased alertness, increases in landing foot splay, and decreases in fore and hind limb grip strength, with hind limb strength remaining lower in females after the 4 week recovery period.

At the intermediate treatment level (225 mg/kg/day) subdued behaviour, abnormalities of gait, hunched posture and claws longer than normal were observed for males. Neurological findings at this level were limpness and increased landing foot splay in both sexes, and decreased hind limb grip strength in males. At the low level (100 mg/kg/day) the findings were limited to slight limpness in 3 males; of these, only one showed the finding on more than one occasion. Slight limpness was only noted during the detailed neurotoxicity examination, but was not recorded during routine daily clinical examination.

Bromide is known to have a sedative effect and the decreased alertness and reduced muscle tone (as

indicated by reduced grip strength, and increased limpness and landing foot splay) can be regarded as expected effects. The increased claw lengths were probably related to the decreased activity.

The pattern of effects on bodyweight was an initial increased gain, compared with control, followed by reduced gain, which was observed for males at 225 and 500 mg/kg/day and for females at 750 mg/kg/day. A lesser effect, manifest as a weight gain followed by a return to control values was observed for females at 225 mg/kg/day, During the recovery period, there was an increase in weight gain, although absolute weights remained lower than control. Food consumption essentially mirrored the body weight performance.

At the high treatment level, for both sexes after 12 weeks, there were increases in haemoglobin, haematocrit and total white cell count, the latter reflecting increases in the majority of white cell types. At the intermediate treatment level, in females only, minimal increases were recorded in total and differential white cell counts. After the 4-week recovery period, haemoglobin and haematocrit values of animals in the high treatment group were similar to those of the controls. In contrast, total and differential white cell counts of previously treated animals were significantly lower than those of the controls. Other inter-group variations in haematological parameters were considered to be of no toxicological significance.

Mean assayed chloride levels were greater in all test groups. To some extent these increases may have reflected increased bromide levels, because bromide ion interfered with the chloride ion assay. Only a slight increase was still present after the 4-week recovery period, and the values were similar to control values at Week 12. Because of the interference of the bromide ion it was not possible to comment on the actual chloride levels.

Mean cholesterol levels in males at 225 and 500 mg/kg/day and females at all levels were lower than control. For the females, the control value was slightly larger than the expected value and therefore the effect, particularly at 100 mg/kg/day may have been incidental.

Mean total bilirubin level in females at 750 mg/kg/day was reduced. Mean phosphate levels of males at 225 and 500 mg/kg/day were significantly increased. Urine pH was decreased at the high dose levels of both sexes.

Assessment of the effect of ammonium bromide on organ weights was complicated by the marked effect on bodyweight. Many weights were reduced, especially for males at 500 mg/kg/day, but it was not clear whether the reductions were true effects on the organ or were merely a consequence of the lower bodyweight. Taking all the data into consideration, it was considered that epididymis weight was reduced at the high dose group and that this reduction persisted into the recovery period. A lesser effect, never significant by analysis of covariance, was seen on the testes. Neither of these organs had any histological finding after Week 13 necropsy.

Apparent slight differences in thyroid weights of main study animals were attributed to variations between the recordings of two technicians, rather than to an effect of treatment. This opinion is reinforced by the absence of any treatment related finding at histopathological examination. Equally, the apparent slight increase in thyroid weights among recovery study animals were also considered to be of no toxicological significance in the absence of histopathological findings.

In assessing neurotoxicity, it was noted that effects (decreased alertness and grip strength, and increased limpness and landing foot splay) were seen in males at 225 and 500 mg/kg/day and in females at 750 mg/kg/day, together with slight limpness in 3 males at 100 mg/kg/day. All of the effects had reversed following the 4-week recovery period except for hind limb grip strength in females at the highest dosage group. Hind limb grip strength was the more sensitive marker in this study in that decreased hind limb strength occurred without the fore limb being affected, but decreased fore limb strength only occurred when the hind limb strength was also reduced. There was no indication of any histological effect on the nervous system following 13 weeks of treatment. It was considered that all neurotoxicological effects were probably reversible.

During the pretrial period (observation of animals one week prior to study initiation) one animal had an unacceptable ophthalmoscopic finding and another one showed poor performance during detailed functional observations. These animals were replaced by spare animals prior to study initiation.

Analysis of formulated diets indicated that all were within ± 10 % of the nominal concentration, indicating acceptable accuracy of formulation. The achieved intakes of the test material, expressed as mg ammonium bromide/kg/day, were close to the target dose levels.

LOAEL males: 100 mg/kg bw/day. The observed effect of slight limpness noted in males at 100

mg/kg bw/day during the detailed neurotoxicological observations is an expected effect and should be regarded as an adverse effect, although the sign had disappeared by the end of the recovery period. LOAEL: females: 225 mg/kg bw/day.

Clinical signs of subdued behaviour and neurotoxic effects were noted during the routine daily clinical examination (in males at ≥225 mg/kg bw/day; in females at 750 mg/kg bw/day). Additional findings included hunched posture, unkempt coat and claws that were longer than normal. Clinical signs of neurotoxicity were also noted during the detailed neurotoxicity examination (in males at ≥100 mg/kg bw/day; in females at ≥225 mg/kg bw/day). The findings noted during the detailed neurotoxicity examination (included viability, clinical signs, detailed functional observations) consisted of increased limpness, decreased alertness, increases in landing foot splay and decreases in fore and hind limb grip strength. At the low dose (100 mg/kg bw/day) the findings were limited to slight limpness in 3 males; of these, only one showed the finding on more than one occasion. Functional alterations were noted in both sexes at ≥225 mg/kg bw/day. All of these effects had reversed following the 4 week recovery period, except for hind limb grip strength in females at 750 mg/kg bw/day. It was considered that all neurotoxicological effects were probably reversible. Other effects noted in the study consisted of: reduced bodyweight gain (in males at ≥225 mg/kg bw/day; in females at 750 mg/kg bw/day), reduced food consumption (in males at 500 mg/kg bw/day), minor changes in haematology parameters (in males at 500 mg/kg bw/day; in females at 750 mg/kg bw/day), minor changes in clinical parameters (in males at ≥225 mg/kg bw/day; in females at ≥100 mg/kg bw/day), decreased urine pH (in females at 750 mg/kg bw/day) and reduced epididymides weight (in males at 500 mg/kg bw/day). No treatment related necropsy or histopathological findings were observed.

No NOEL was determined for male and female rats. The NOAEL for female rats was determined at 100 mg/kg bw/day based on clinical signs of neurotoxicity noted at ≥225 mg/kg bw/day, clinical signs of subdued behaviour noted at 750 mg/kg bw/day, reduced bodyweight gain noted at 750 mg/kg bw, changes in haematological parameters noted at ≥225 mg/kg bw/day, changes in biochemical parameters noted at 750 mg/kg bw/day and decreased urine pH noted at 750 mg/kg bw/day. No NOAEL was determined for male rats but the NOAEL was considered to be close to the dose level of 100 mg/kg bw/day.

Conclusion

CONCLUSIONS:

LOAEL males: 100 mg/kg bw/day. The observed effect of slight limpness noted in males at 100 mg/kg bw/day during the detailed neurotoxicological observations is an expected effect and should be regarded as an adverse effect, although the sign had disappeared by the end of the recovery period. LOAEL: females: 225 mg/kg bw/day.

Clinical signs of subdued behaviour and neurotoxic effects were noted during the routine daily clinical examination (in males at ≥225 mg/kg bw/day; in females at 750 mg/kg bw/day). Additional findings included hunched posture, unkempt coat and claws that were longer than normal. Clinical signs of neurotoxicity were also noted during the detailed neurotoxicity examination (in males at ≥100 mg/kg bw/day; in females at ≥225 mg/kg bw/day). The findings noted during the detailed neurotoxicity examination (included viability, clinical signs, detailed functional observations) consisted of increased limpness, decreased alertness, increases in landing foot splay and decreases in fore and hind limb grip strength. At the low dose (100 mg/kg bw/day) the findings were limited to slight limpness in 3 males; of these, only one showed the finding on more than one occasion. Functional alterations were noted in both sexes at ≥225 mg/kg bw/day. All of these effects had reversed following the 4 week recovery period, except for hind limb grip strength in females at 750 mg/kg bw/day. It was considered that all neurotoxicological effects were probably reversible. Other effects noted in the study consisted of: reduced bodyweight gain (in males at ≥225 mg/kg bw/day; in females at 750 mg/kg bw/day), reduced food consumption (in males at 500 mg/kg bw/day), minor changes in haematology parameters (in males at 500 mg/kg bw/day; in females at 750 mg/kg bw/day), minor changes in clinical parameters (in males at ≥225 mg/kg bw/day; in females at ≥100 mg/kg bw/day), decreased urine pH (in females at 750 mg/kg bw/day) and reduced epididymides weight (in males at 500 mg/kg bw/day). No treatment related necropsy or histopathological findings were observed.

No NOEL was determined for male and female rats. The NOAEL for female rats was determined at

100 mg/kg bw/day based on clinical signs of neurotoxicity noted at ≥225 mg/kg bw/day, clinical signs of subdued behaviour noted at 750 mg/kg bw/day, reduced bodyweight gain noted at 750 mg/kg bw, changes in haematological parameters noted at ≥225 mg/kg bw/day, changes in biochemical parameters noted at 750 mg/kg bw/day and decreased urine pH noted at 750 mg/kg bw/day. No NOAEL was determined for male rats but the NOAEL was considered to be close to the dose level of 100 mg/kg bw/day.

3.9.1.1 [Study 10] No guideline study: 4 week dose range-finding study of ammonium bromide in rat

Reference	A6.3.1/01, Doc. No. 532-001 Study report, 1999
Guideline	Not applicable,dose range finding-study; the methods and procedures applied were in accordance with OECD guideline 407 (subacute oral toxicity study in rodents). GLP compliance: yes
Reliability	1 (reliable without restriction)
Species / strain	Species: Rat Strain: Sprague-Dawley
Test material	- Name of test material (as cited in study report): Ammonium bromide - Physical state: White crystalline solid - Analytical purity: 99.94% - Lot/batch No.: 980060 - Stability under test conditions: Not determined; considered to be stable under the storage conditions - Storage condition of test material: The material was stored in the dark at ambient temperature
Study design	**Administration / exposure** Route of administration: oral: feed Vehicle: unchanged (no vehicle) Details on oral exposure: PREPARATION OF DOSING SOLUTIONS: DIET PREPARATION First a premix was prepared consisting of test material which was sieved through a 250 µm sieve and manually mixed with approximately the same weight of sieved diet (250 mm) prior to addition to the bulk premix. This was then mixed on a Hobart mixer for 2 hours before being transferred to a labelled premix container and booked in as a Test Material. Formulated diets were prepared from the premix. Fresh diets were prepared weekly. Analytical verification of doses or concentrations: yes Details on analytical verification of doses or concentrations: Trial formulations of the lowest and highest concentrations were investigated for stability, concentration and homogeneity. Triplicate samples of diet were taken from each formulation (including control) immediately after preparation during Weeks 1 and 4 of the study. Analysis of formulated diets carried out during Weeks 1 and 4 were acceptable in terms of concentration and homogeneity with the percentage differences from the nominal value being within the specified range of ± 10%. Duration of treatment / exposure: 4 weeks (28 days) Frequency of treatment: Daily Doses / concentrations: 100, 500, 1000 mg/kg bw/day No. of animals per sex per dose: 5/sex/dose Control animals: yes, plain diet Details on study design: Dose selection rationale: Range finding study for dose determination in 90-day study Positive control:Not applicable **Exanimations**:

DETAILED CLINICAL OBSERVATIONS: Yes

All animals were examined for reaction to treatment during the day. Once a week they received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Mortality: All animals were checked for viability twice each day.

BODY WEIGHT: Yes

-Time schedule for examinations: Body weights were recorded twice each week commencing one week pre-trial up until the end of the dosing period.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

The quantity of food consumed by each animal was measured and recorded twice each week commencing one week pretrial up until the end of the dosing period.

FOOD EFFICIENCY: No

WATER CONSUMPTION: Yes

- Time schedule for examinations: Water consumption was monitored by visual inspection of the water bottles on a weekly basis throughout the study

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

CLINICAL CHEMISTRY: No

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology:

GROSS PATHOLOGY: Yes

Epididymis, heart, implant, kidneys, liver, lung, ovaries, spleen and testis were taken from all animals, weighed and placed in 10% neutral buffered formalin (fixation).

Organ weights: Epididymis, heart, implant, kidneys, liver, lung, ovaries, spleen, testis

HISTOPATHOLOGY: No

Other examinations:

Not applicable

Statistics:

Body weight data was analysed for homogeneity of variance using the “F-max” test. If the group variance appeared homogeneous, a parametric ANOVA was used and individual between group comparisons were made using Fischer´s F-protected LSD method via student´s t-test. If the variances were heterogenous, then a non-parametric test such as a Kruskal-Wallis ANOVA was used. Individual between group comparisons were made using Fisher´s F-protected LSD method.

Organ weights were also analysed conditional on body weigh ie analysis of covariance (ANCOVA).

Results and discussion

Results of examinations

Clinical signs: effects observed, treatment-related (see details below)

Mortality: mortality observed, treatment-related (see details below)

Body weight and weight changes: effects observed, treatment-related (see details below)

Food consumption and compound intake (if feeding study): effects observed, treatment-related (see details below)

Food efficiency: not examined

Water consumption and compound intake (if drinking water study): no effects observed

Ophthalmological findings: not examined

Haematological findings: not examined

Clinical biochemistry findings: not examined

Urinalysis findings: not examined

Behaviour (functional findings): not examined

Organ weight findings including organ / body weight ratios: effects observed, treatment-related (see details below)

Gross pathological findings: effects observed, treatment-related (see details below)

Histopathological findings: non-neoplastic: not examined

Histopathological findings: neoplastic: not examined

Details on results:

CLINICAL SIGNS AND MORTALITY

In the Intermediate and high dose group of both sexes the clinical signs most frequently observed included agitated, nervous and hyperactive behaviour, subdued behaviour, rolling gait, hunched posture, piloerection, and eyes partially closed indicative for signs of neurotoxicity. Other signs appeared to a lesser extent and included unkempt coat and irregular breathing. There were no notable signs in the low dose or control groups. All other signs were in the usual range of signs commonly seen in rats of this age and strain in the testing facility.

There were no premature deaths.

BODY WEIGHT AND WEIGHT GAIN

Mean body weight was decreased in high dose males and females reaching significance in the males from Day 10 of treatment onwards when compared to the Control group. Mean body weight gain was significantly decreased in high dose males (-49%) and high dose females (-31%) when compared to the respective control groups. Body weight and body weight gain were only slightly reduced in males of the mid dose group, but remained unaffected by treatment in females of this dosage group. Animals from the low dose group (both males and females) remained unaffected by treatment.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)

Food consumption was decreased in high dose males on Day 10 of treatment (-24%) and more markedly decreased in this dose group from Day 21 onward (about -31%) On Day 28 of treatment, food consumption was reduced by about 29%. Food consumption in females was considered to have been similar throughout all the dose groups. Food intake rates remained unaffected by treatment in the low and mid dose groups.

Achieved dosages were satisfactory for most dose groups, with the mean dosage within ± 10% of

target. This was due to the low food consumption of this dose group.

ORGAN WEIGHTS

High dose males showed marked decreases in mean epididymides, heart, liver, kidneys, lungs and testes absolute weights when compared to the control group. Intermediate dose males also showed significantly relevant decreases in mean epididymides, kidneys and testes absolute weights when compared to control group. However, due to the decreased mean body weight of the intermediate and high dose males (statistically significant in the high dose group) these apparent effects disappeared after covariance analysis and so were not considered to be attributable to the administration of ammonium bromide in the diet. After covariance analysis high dose females showed a significant increase in mean liver weight. This effect was not noted in the absolute organ weight analysis.

GROSS PATHOLOGY

At necropsy there were no significant findings and, with the exception of staining being recorded for some rats, most animals of the treatment groups did not show any treatment-related macroscopic findings.

Table: Incidence of selected clinical observations

Observation/Finding	Group/Dose Level (mg/kg/day)							
	1 (0)		2 (100)		3 (500)		4 (1000)	
Sex	m	f	m	f	m	f	m	f
Number of animals per group	5	5	5	5	5	5	5	5
Subdued behaviour	0	0	0	0	5	0	5	4
Agitated behaviour	0	0	0	0	1	4	0	5
Rolling gait	0	0	0	0	5	5	5	5
Hunched posture	0	0	0	0	1	2	3	5
Nervous/hyperactive behaviour	0	0	0	0	0	5	0	4
Eyes partially closed	0	0	0	0	2	2	4	5
Piloerection	0	0	0	0	3	3	2	5
Irregular breathing	0	0	0	0	0	0	0	1
Unkempt	0	0	0	0	1	2	2	3

m: male; f: female

Table: Mean food consumption, Bodyweights, absolute organ weights and achieved doses

	Control (0 mg/kg/day)		Low dose (100 mg/kg/day)		Medium dose (500 mg/kg/day)		High dose (1000 mg/kg/day)	
	m	f	m	f	m	f	m	f
Number of animals	5	5	5	5	5	5	5	5

examined								
	Food consumption (g/animal/day)							
Days 1-8	31.5	20.9	33.5	20.9	32	20	25.8	19.6
Day 8-14	31	22.7	31.1	19.8	29.3	21.4	25	21
Days 15-21	33.6	24	34.7	20.8	29.3	23.2	22.5**	20.3
Days 22-28	33.6	24.1	32	22.8	32	23.2	23.9**	21.5
	Body weights (g)							
Day 0	198	141	197	136	193	136	196	138
Day 7	258	164	264	163	260	169	234	159
Day 14	313	190	321	183	309	187	268	173
Day 21	363	204	369	203	345	201	286	185
Day 28	406	218	409	218	378	217	303	191
	Bodyweight gain (g)							
Day 0 - 28	208	77	212	82	185	81	107***	53*
	Organ weights (g)							
Epididymides	0.89	-	0.84	-	0.79	-	0.75	-
Heart	1.47	0.89	1.44	0.95	1.34	0.86	1.16	0.8
Kidneys	3.22	1.84	3.13	1.79	2.82	1.86	2.35	1.74
Liver	18.38	9.28	19.19	9.56	17.56	10.39	13.19	10.21
Lung	1.7	1.19	1.56	1.31	1.56	1.28	1.38	1.02
Spleen	0.74	0.48	0.77	0.53	0.71	0.47	0.66	0.49
Testes	3.49	-	3.12	-	3.09	-	2.92	-
Ovaries	-	0.086	-	0.092	-	0.112	-	0.095
	Achieved Dosages (ppm)							
Week 1	-	-	108	105	524	484	884	986
Week 2	-	-	91	82	441	435	859	911
Week 3	-	-	105	93	462	517	888	917
Week 4	-	-	93	109	485	560	976	1083
Mean Week 1-4 (as % of target)	-	-	99%	97%	96%	100%	90%	97%

m male;

f female

* statistically significantly different from controls with $p < 0.05$

** statistically significantly different from controls with $p < 0.01$

*** statistically significantly different from controls with $p < 0.001$

Effect levels

Dose descriptor: NOEL
Effect level: 100 mg/kg bw/day (nominal)
Based on: test mat.
Sex: male/female

Dose descriptor: LOAEL
Effect level: 500 mg/kg bw/day (nominal)
Based on: test mat.
Sex: male/female

Applicant's summary and conclusion

The NOEL for male and female rats was determined at 100 mg/kg bw/day. The NOAEL for male and female rats was determined at 100 mg/kg bw/day (corresponding to 82 mg bromide/kg bw/day) on clinical signs indicating neurotoxicity noted in both sexes at the dose level of ≥500 mg/kg bw/day, reduced body weight gain noted in both sexes at the dose level of 1000 mg/kg bw/day and increased liver weight noted in females at 1000 mg/kg bw/day.

Executive summary:

The objective of the study was to provide dose range finding information on the toxicity of Ammonium Bromide in the rat after administration by the diet continuously for 4 weeks. The test material was administered at dose levels of 0, 100, 500 and 1000 mg/kg/day. Dose levels were selected after evaluation of existing data and took into account the maximum tolerated dose in rats and other factors such as anticipated human exposure. The concentration of test material in the diet was adjusted regularly to achieve a constant dose level.

Formulated diets were prepared as follows: First a premix was prepared consisting of test material which was sieved through a 250 µm sieve and manually mixed with approximately the same weight of sieved diet (250 mm) prior to addition to the bulk premix. This was then mixed on a Hobart mixer for 2 hours before being transferred to a labelled premix container and booked in as a Test Material. Formulated diets were prepared from the premix. Fresh diets were prepared weekly. Prior to study commencement trial formulations of the lowest and highest concentrations were investigated for stability, concentration and homogeneity. This was carried out in the Product Chemistry laboratories of Inveresk Research under a separate protocol and contract (Inveresk Project No. 379673). Analysis of formulated diets was undertaken with regard to stability, concentration and homogeneity. Triplicate samples of diet were taken from each formulation (including control) immediately after preparation during Weeks 1 and 4 of the study.

Intermediate and high dose group animals of both sexes were noted as having agitated behaviour, a rolling gait, hunched posture and eyes partially closed indicative for signs of neurotoxicity. All other animals were considered normal throughout the study. Both body weight gain and food consumption were reduced in the high dose males.

High dose male showed marked decreased mean absolute weights in epididymides, heart, kidneys, lung, liver and testes when compared to control group. Intermediate dose males also showed decreases in mean epididymides, kidneys and testes absolute weights when compared the control group.

The lower body weights and reduced body weight gain noted in the high dose group males are undoubtly related and contributed to the more variable achieved dosage values for this dose group throughout the study. It should be noted when considering dose levels for future studies that these effects were presented at ca 90% of target dose i.e. ca 900 mg/kg/day as opposed to the target of 1000 mg/kg/day.

High dose females showed a significant increase in mean liver weight after covariance analysis. This effect is of unclear toxicological significance in the absence of any corroborative histological or clinical pathology evidence and, given the absence of any similar finding in males, this increase may not be related to the treatment with ammonium bromide.

There were no premature deaths.

There were no visual differences in water consumption between groups treated with ammonium bromide and the control group.

Analyses of diets were acceptable in terms of concentration andhomogeneity with the percentage difference from the nominal value being within the specified range of ± 10%.

In this study clinical signs were noted in male and female rats at a dose level of 500 mg/kg bw/day and above. The observed clinical signs consisted of agitated, nervous and hyperactive behaviour, subdued behaviour, rolling gait, hunched posture, piloerection and eyes partially closed. To a lesser extent unkempt coat and irregular breathing were noted. Statistically significant reduced body weight gains (males:-49%; females:-31%), reduced food consumption (males) and increased rel. liver weight (females) were also noted in animals of the high dose group (1000 mg/kg bw/day).

The NOEL for male and female rats was determined at 100 mg/kg bw/day. The NOAEL for male and female rats was determined at 100 mg/kg bw/day (corresponding to 82 mg bromide/kg bw/day) on clinical signs indicating neurotoxicity noted in both sexes at the dose level of ≥500 mg/kg bw/day, reduced body weight gain noted in both sexes at the dose level of 1000 mg/kg bw/day and increased liver weight noted in females at 1000 mg/kg bw/day.

3.9.1.2 [Study 11] Pre-natal developmental toxicity study (OECD TG 414) of ammonium bromide in rat

A.6.8.1/04, Doc. No. 551-002

Study report, 2007

See study details under reproductive toxicity

3.9.1.3 [Study 12] Dose range-finder for OECD 416; 2-generation reproduction study

A6.8.2/01, Doc. No.553-001

Study report, 2001.

See study details under reproductive toxicity

3.9.2 Human data

3.9.2.1 [Study 1]

Study reference:

A6.10.05, Doc. No. 592-001

Van Gelderen C. E. M., Savelkoul T. J. F., Blom J. L., van Dokkum W. and Kroes R. (1993). The No-Effect Level of Sodium Bromide in Healthy Volunteers. Human and Experimental Toxicology, 12, 9-14.

3.9.2.2 [Study 2]

Study reference:

A6.10/03, Doc. No. 592-012

Sangster B., Krajnc E. I., Loeber J. G., Rauws A. G. and van Logten M. J. (1982). Study of Sodium Bromide in Human Volunteers, with Special Emphasis on the Endocrine System. Human Toxicol., 1, 393-402.

3.9.2.3 [Study 3]

Study reference:

A6.10/04, Doc. No. 592-013

Sangster B., Blom J. L., Sekhuis V. M., Loeber J. G., Rauws A. G. and Koedam J. C. (1983). The Influence of Sodium Bromide in Man: A Study in Human Volunteers with Special Emphasis on the Endocrine and the Central Nervous System. Fd. Chem. Toxic., Vol. 21, No. 4, 409-419.

3.9.2.4 [Study 4]

Study reference:

McDanal C. E., Owens D. and Bolman W. M. (1974). Bromide Abuse: A Continuing Probl. Am. J. Psychiatry 131:8, August 1974.

3.9.2.5 [Study 5]

Study reference:

Tsutaoka B.T., Anderson I.B., Luu L., Arora A. and Olson K.R. (2004). Severe Pseudohyperchloremia from an Intentional Ingestion of Sodium Bromide. J. Toxicol. Clin. Toxicol., 42(4), 549.

3.9.2.6 [Study 6]

Study reference:

Yu C.Y., Yip P.K., Chang Y.C. and Chiu M.J. (2004). Reversible dysphagia and dementia in a patient with bromide intoxication. Journal of Neurology, Vol. 251, No. 10, pp. 1282-1284.

3.9.2.7 [Study 7]

Study reference:

Gosselin R. E. (1976). Clinical Toxicology of Commercial Products. 4th ed. Baltimore: Williams and Wilkins, p. II-77.

3.9.3 Other data

3.9.3.1 [Study 1] 16 or 66 days repeated dose toxicity study of potassium bromide to investigate effects on the thyroid gland of the rat

Reference	A6.10/11; Doc. No. 592-037 Velický J., Titlbach M., Dusková J., Vobecký M., Strbák V. and Raska I. (1997a). Potassium Bromide and the Thyroid Gland of the Rat: Morphology and Immunohistochemistry, RIA and INAA Analysis. Annals of anatomy = Anatomischer Anzeiger: official organ of the Anatomische Gesellschaft, Vol. 179, No. 5, pp. 421-31.
Guideline	2.1 Guideline study No, there is no guideline available for this special investigation and study was performed according to good experimental practice. 2.2 GLP No. Study is a publication and was performed according to good experimental practice. 2.3 Deviations Not applicable; no guideline available for mechanistic studies
Reliability	2
Species / strain	3.2.1 Species Rat 3.2.2 Strain Wistar 3.2.4 Sex male
Test material	Potassium bromide
Study design	3.2 Test Animals 3.2.5 Age/weight at study initiation Animals were 41 days of age and within a weight range of 100-120 g.

3.2.6 Number of animals per group: 6/group

3.2.7 Control animals Yes

3.3 Administration/Exposure

3.3.1 Duration of treatment: 16 and 66 days

3.3.2 Frequency of exposure daily

3.3.3 Postexposure period: No postexposure period; animals were sacrificed at the end of the treatment period.

3.3.4 Oral

3.3.4.1 Type in drinking water

3.3.4.2 Concentration drinking water:10, 50 or 100 mg/L corresponding to 1.25, 6.25 and 12.5 mg/kg bw/day, drinking water consumption: 15 mL/animal, average animal weight: 120 g/animal

3.3.4.3 Vehicle water

3.3.4.4 Concentration in vehicle: Not applicable; KBr was administered with the drinking water at 10, 50 or 100 mg/L

3.3.4.5 Total volume applied: Approximately 15 mL/animal/day

3.3.4.6 Controls water

3.4 Examinations

3.4.1 Observations

3.4.1.1 Clinical signs Not examined

3.4.1.2 Mortality Yes

3.4.2 Body weight Not examined

3.4.3 Food consumption The diet was given ad libitum, but the consumption was approximately 20 g/animal/day.

3.4.4 Water consumption Yes

3.4.5 Ophthalmoscopic examination: Not examined

3.4.6 Haematology Not examined

3.4.7 Clinical Chemisty Free T4, total T4, total T3 and plasma TSH levels were determined at

termination of the investigation.

3.4.8 Urinalysis Not examined

3.5 Sacrifice and pathology

3.5.1 Organ Weights Weights of the thyroid gland were taken only.

3.5.2 Gross and histopathology: Yes; Thyroid gland was examined only.

3.5.3 Other examinations

Immunohistochemistry:

The assay of thyroglobulin was carried out by an indirect immunoperoxidase technique with 3,3´-diaminobenzidine tetrahydrochloride visualisation. Rabbitanti- human-thyroglobulin was used as primary, and swine-anti-rabbit as secondary antibody.

Morphometric analysis:

The morphometric and immunohistochemical evaluation was performed with a Nikon Microphot FXA microscope and the Lucia M image analysis program Lucia 3.5. The proportion of the colloid area relative to the remaining parenchyma, as well as the size distribution of colloid deposits in the follicular lumen and the circularity of the colloid deposits, were measured and evaluated.

Radioimmunoassay (RIA):

For the plasma concentration determination of thyroid hormones and TSH in all experimental animals, 2-3 mL of blood were collected at the end of the experiment by cardiac puncture under ether anaesthesia. Thyroid hormones in nonextracted plasma were determined using the RIA T3 and RIA T4 kits. The RIA of TSH in the rats was assayed with the following reagents: rat TSH-PR-3, AFP-5512B, TSH-antibodies anti-rat TSH-RIA-6, AFP-329 661 Rb.

Tissue and diet bromine and iodine levels:

Bromine and iodine levels in the thyroid gland dry weight of experimental animals were determined by instrumental neutron activation analysis (INAA) during a short-term irradiation regime in a nuclear reactor. The bromine level in the diet was determined by INAA during long-term irradiation regime using the 82Br radionuclide, and the iodine level by means of kinetic photometry.

3.5.4 Statistics The differences between the mean values were tested by variance analysis, followed by the F-test, the ANOVA test and the Kruskal-Wallis test.

3.6 Further remarks The mean content of bromine and iodine in the diet was 10.04 mg bromide/kg and 0.52 mg iodine/kg

Findings

4.1 Observations

4.1.1 Clinical signs There were no clinical signs reported in the investigation.

4.1.2 Mortality There were no premature decedents during the treatment period.

4.2 Body weight gain No body weight analysis was performed in this investigation.

4.3 Food consumption

and compound

intake

Approximately 20 g of diet were consumed per animal per day. The amount of drinking water containing potassium bromide was approximately 15mL/animal/day.

4.4 Ophthalmoscopic examination: No ophthalmoscopic examination was performed in this investigation.

4.5 Blood analysis

4.5.1 Haematology No haematological analysis was performed during this investigation.

4.5.2 Clinical chemistry The following results were obtained by radioimmunoassay. The plasma thyroxin concentration was significantly decreased in animals exposed to all bromide concentrations as compared with the T4 level in the controls ($P<0.001$). The plasma T3 concentration in animals exposed to 10, 50 and 100 mg bromide/L for 66 days was significantly lowered. Treatment for 16 days did not bring about significant changes in the T3 plasma concentration. The administration of increased bromide concentrations brought about only a slight and statistically insignificant increase in the TSH level after 66 days at 10-100 mg bromide/L. Furthermore, no significant change in the TSH level was observed after a 16-day administration of bromide.

4.5.3 Urinalysis No urinalysis was performed during this investigation.

4.6 Sacrifice and pathology

4.6.1 Organ weights Thyroid dry weights were taken for determination of I/BR concentration ratio. No changes in thyroid weights is reported in the investigation.

4.6.2 Gross and histopathology

The administration of bromide resulted in definite morphological changes in the thyroid gland, in contrast to the control animals. The extent of the changes rose with the concentration of bromine. The extent of changes after 16- and 66- days treatment did not differ conspicuously for any given concentration of bromide.

The tissue of the thyroid gland of animals exposed to bromide displayed a marked growth activation of the follicular epithelial component, and mitoses in the follicular cells were more frequent. Microfollicular reorganisation, increased height of the follicular cells and increased vascularisation of the parenchyma were observed.

Image analysis demonstrated a significant lowering in the proportion of colloid in the thyroid tissue of rats treated with potassium bromide.

4.7 Other Immunohistochemistry:

The assay of thyroglobulin (Tg) in the colloid of most of the follicles in the control animals over the 16- and 66-day periods was strongly or moderately positive, whereas the colloid in the smaller follicles in the central parts of the tissue exhibited moderate but definitely perceptible positivity. Tissue concentration of bromine and iodine:

The increasing bromide intake resulted in a concentration and/or duration of treatment-dependent rise in the bromine level in the thyroid tissue and a concomitant decrease in the I/Br molar concentration ratio.

Conclusions

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In order to establish the functional and morphological effects of bromine on the thyroid, experiments were performed on male rats, which in addition to a standard diet with an estimated iodine/bromine content, were fed for periods of 16 and 66 days with the small quantities of bromide expected to be encountered in the environment (10, 50 and 100 mg/L drinking water). Animals were divided into 6 experimental and two control groups, each containing 6 animals. Rats were fed 0, 10, 50 or 100 mg of bromide/L in drinking water for 16 or 66 days. Important to note is the fact, that each animal consumed approximately 15 mL water per day, corresponding to bromide amounts of 0, 150, 750 and 1500 µg bromide/animal/day or 1.25, 6.25 and 12.5 mg bromide/kg bw/day. The diet fed during the investigation contained 10.04 mg bromine/kg and 0.52 mg iodine/kg; since the animals consumed 20 g food/day, this corresponds to 200 µg bromine/animal/day (about 1.7 mg/kg bw/day) and 10.4 µg iodine/animal/day (about 0.087 mg/kg bw/day). After the experiments were completed, the animals were killed and both thyroid lobes were excised. The tissue of one part of the lobe was fixed and embedded in paraffin, and used for preparation of serial sections which were stained with haematoxylin-eosin and processed by the PAS method for morphometric assessment. The tissue of the other part of the lobe was fixed and further processed for electron microscopy.

Immunohistochemistry was carried out with an anti-human-thyroglobulin primary antibody. For morphometric analysis the proportion of the colloid area relative to the remaining parenchyma, as well as the size distribution of colloid deposits in the follicular lumen and the circularity of the colloid deposits, were measured and evaluated. Radioimmunassay for determination of thyroid hormone concentrations (T3, T4, TSH) in plasma were carried out in all animals.

Instrumental neutron activation analysis (INAA) was performed to determine the bromine and iodine content of the thyroid glands. For determination of the bromine content in the diet, also INAA was performed; and the iodine levels were determined by means of kinetic photometry.

5.2 Results and discussion

Morphology:

The administration of bromide resulted in definite morphological changes in the thyroid gland, in contrast to the control animals. The extent of the changes rose with the concentration of bromide. It was largest at a concentration of 100 mg/L, but changes induced by 50 and 10 mg/L were also well pronounced. The extent of changes after 16 and 66 days treatment did not differ conspicuously for any given concentration of bromide. The tissue of the thyroid gland of animals exposed to bromide displayed a marked growth activation of the follicular epithelial component, and mitoses in the follicular cells were more frequent. Microfollicular reorganisation occurred in up to more than two thirds of the parenchyma, and was accompanied by the formation of minute follicles with diameters ranging from 6 to 20 µm. The strongly reduced slit-like constricted or round lumina exhibited a substantially reduced amount of PAS positive colloid, frequently non-homogeneous in nature. The walls of mediumsized follicles sometimes exhibited very small daughter follicles, which retained PAS positive colloid in their lumina. In most animals, the microfollicles were also found in the peripheral parts of the lobe tissue. The height of the follicular cells was increased relative to the diminished lumina. In a number of medium sized and larger follicles, groups of follicular cells with increased height had formed protuberances within the lumen. The cytoplasm of follicular cells featured multiple PAS positive spherical vacuoles. Increased vascularisation of the parenchyma was

observed.

The electron microscopic findings in the thyroid after 16 and 66 days exposure revealed bromide concentration-dependent changes in the configuration of the follicles. Within the follicular cells, changes of the rough endoplasmic reticulum and lysosomes were particularly apparent.

Image analysis:

Image analysis demonstrated a significant lowering in the proportion of colloid in the thyroid tissue of rats treated with potassium bromide. During the 16-day experiment, the proportion of the colloid dropped from 15 ± 3% to 11 ± 3%, 10 ± 4% and 9 ± 3% for bromide concentrations of 10, 50 and 100 mg/L respectively. The corresponding values found in the 66-day experiment were 23 ± 6% in the controls and 12 ± 4%, 7 ± 2% and 8 ± 3% in experimental animals. The lowest concentration of administered bromide during the 16-day experiment evoked a response comparable to that found with higher concentrations. During the 66-day experiment this concentration induced a response similar to that found in the 116-day experiment but substantially different from the response elicited by higher concentrations. Statistical analysis showed the differences in the proportions of the colloid-occupied area to be significant in the 16-day experiment ($P<0.05$) and highly significant in the 66-day experiment ($P<0.001$). The lowering of the amount of colloid in the tissue was accompanied by microfollicular reorganisation of the tissue. The size distribution of the colloid deposits in the follicular lumen exhibited a conspicuous rise in the number of the smallest-lumen follicles with colloid in all experimental groups of animals exposed to bromide for 16 and 66 days. The colloid deposits with the largest area were fewer. The variations in the effect, depending on the bromide concentration and length of administration in these groups were obviously due to the generally small number of large-area colloid deposits. An evaluation of the circularity of the colloid deposits based on image analysis did not show any significant difference between bromide-exposed rats and control animals. The occurrence of slit-like follicular lumina was accompanied, in terms of the microfollicular reorganisation of the tissue, by an increase in the number of almost completely circular follicular lumina.

Immunohistochemistry:

The assay of thyroglobulin (Tg) in the colloid of most of the follicles in the control animals over the 16- and 66-day periods was strongly or moderately positive, whereas the colloid in the smaller follicles in the central parts of the tissue exhibited moderate but definitely perceptible positivity. Animals exposed to 10 mg bromide/L for 16 or 66 days exhibited mildly positive evidence of Tg in the colloid of microfollicles, and in medium-sized and large follicles. After 16 days of 50 mg/L of bromide administration, the Tg in the colloid of the microfollicles was mildly positive, whereas the colloid of the medium-sized and large follicles was moderately positive. After 66 days the Tg assay in the colloid of microfollicles was moderately or strongly positive, but only in a narrow peripheral rim of the colloid. The colloid of most larger follicles showed no substantial reduction in positivity. Animals exposed to 100 mg bromide/L for 16 days exhibited a weakly positive or even negative reaction to Tg in the colloid of the microfollicles, whereas in the colloid of the larger follicles it was weakly or moderately positive. Animals exposed for 66 days exhibited moderately or strongly positive evidence of Tg in the colloid of some follicles, but predominantly in the narrow peripheral rim adjacent to the apical surface of follicular cells only. The demonstration of Tg in the larger follicles was mainly moderately positive.

Radioimmunoassay:

The plasma thyroxin concentration was significantly decreased in animals exposed to all bromide concentrations as compared with the T4 level in the controls ($P<0.001$). The plasma T3 concentration in animals exposed to 10, 50 and 100 mg bromide/L for 66 days was significantly lowered. Treatment for 16 days did not bring about significant changes in the T3 plasma concentration. The administration of increased bromide concentrations brought about only a slight and statistically insignificant increase in the TSH level after 66 days at 10-100 mg bromide/L. Furthermore, no significant change in the TSH level was observed after a 16-day administration of bromide.

Tissue concentration of bromine and iodine: The increasing bromide intake resulted in a concentration and/or duration of treatment-dependent rise in the bromine level in the thyroid tissue and a concomitant decrease in the I/Br molar concentration ratio.

5.3 Conclusion The results of this investigation indicate that even the lowest amount of bromide administered (10 mg/L corresponding to 150 µg/animal/day or 1.25 mg/kg bw/day) can induce changes comparable with parenchymatous goitre in humans. The decrease in thyroid hormone level

(T3, T4) detected after 16 and 66 days of treatment was accompanied by definite morphological changes. The plasma TSH level of bromide-exposed animals did not significantly differ from that in the controls after administration for a period of 16 days while after 66 days TSH was statistically significantly increased. The results of this investigation demonstrate that rats appear to react in a sensitive manner to the administration of even low amounts of bromide with the drinking water as demonstrated by a modulation of thyroid hormones and thyroid morphology.

5.3.1 LO(A)EL 10 mg/L corresponding to 1.25 mg/kg bw based on morphological changes in the thyroid gland and decreased serum thyroxine and triiodithyronine levels.

5.3.2 NO(A)EL < 10 mg/L

3.9.3.2 [Study 2] 16, 66 or 133 days repeated dose toxicity study of potassium bromide to investigate effects on the thyroid gland of the rat

Reference	A6.10/10, Doc. No. 592-039 Velický J., Titlbach M., Lojda Z., Jelinek F., Vobecký M. and Raska I. (1997b). Expression of the Proliferating Cell Nuclear Antigen (PCNA) in the Rat Thyroid Gland After Exposure to Bromide. Acta histochemica, Vol. 99, No. 4, pp. 391-9.
Guideline	2.1 Guideline study No, there is no guideline available for this special investigation and study was performed according to good experimental practice. 2.2 GLP No. Study is a publication and was performed according to good experimental practice. 2.3 Deviations Not applicable; no guideline available for mechanistic studies
Reliability	2
Species / strain	3.2.1 Species Rat 3.2.2 Strain Wistar 3.2.4 Sex male
Test material	Potassium bromide
Study design	3.2 Test Animals 3.2.5 Age/weight at study initiation: Animals were 41 days of age and within a weight range of 100-120 g. 3.2.6 Number of animals per group: 10/group 3.2.7 Control animals Yes 3.3 Administration/Exposure 3.3.1 Duration of treatment: 16, 66 or 133 days 3.3.2 Frequency of exposure: daily 3.3.3 Postexposure period: No postexposure period. The animals were sacrificed after the treatment period (16, 66 or 133 days) and thyroid lobes were removed for examination. 3.3.4 Oral: in drinking water 3.3.4.2 Concentration/Dosage: 0, 0.15, 0.75 and 1.5 mg Br-/day for 16 and 66 days, 0, 1.5, 3 or 6 mg Br-/day for 133 days (based on a measured water intake of 15 mL/animal/day) 3.3.4.3 Vehicle Drinking water 3.3.4.4 Concentration in vehicle: 0, 10, 50 and 100 mg Br-L for 16 and 66 days; 0, 100, 200 or 400 mg Br-/L for 133 days 3.3.4.6 Controls Vehicle (drinking water)

3.4 Examinations

3.4.1 Observations

3.4.1.1 Clinical signs Not examined

3.4.1.2 Mortality Not examined

3.4.2 Body weight Not examined

3.4.3 Food consumption: 20 g/animal/day

3.4.4 Water consumption: Approximately 15 ml/animal/day

3.4.5 Ophthalmoscopic examination: Not examined

3.4.6 Haematology Not examined

3.4.7 Clinical Chemisty: Not examined

3.4.8 Urinalysis Not examined

3.5 Sacrifice and pathology

3.5.1 Organ Weights Weights of thyroid lobes were taken only

3.5.2 Gross and histopathology: Both thyroid lobes were stained by haematoxylin und eosin and by the PAS reaction

3.5.3 Other examinations

Mitotic activity of follicular cells was evaluated by the immunohistochemical assay of PCNA on sections from the paraffin-embedded thyroid. Sections were transferred to microscopic slides coated with 0.1 % gelatine in water containing 0.01 % chromium potassium sulphate and dried in a thermostat at 37°C for 20 hours. Paraffin was removed by xylene (2-15 min) and the slides were transferred through an alcohol series into water. The endogenous peroxidase activity was blocked with 0.3 % H_2O_2 for 30 minutes. Slides were then washed in distilled water and incubated in 0.05 M Tris-HCl buffer, pH 7.6 containing 1 % bovine albumin, monoclonal antibody mouse anti-PCNA diluted 1:50 and sodium azide (50 mg/50 ml) in a moist chamber at 4°C for 16-24 hours. After washing with Tris-buffered saline (TBS, pH 7.6; 3x5 min) the sections were overlaid with swine-anti-mouse-gamma-globulin labelled with peroxidase (diluted 1:500) and incubated in a moist chamber at room temperature for 60 minutes. After washing in TBS (3x5 min) the reaction was visualized by a solution 3,3´-diaminobenzidinetetrahydrochloride. Thereafter 20 µl of 30% H_2O_2 were added. After 10-minutes incubation at room temperature the specimens were washed in distilled water, counter-stained with hematoxylin, dehydrated and mounted in Entellan

3.5.4 Statistics Statistical evaluation for proliferation activity test results was done by the Kruskal-Wallis test.

3.6 Further remarks The mean iodine content of the diet was determined by kinetic photometry ans amounted to 0.52 mg/kg diet. The mean bromide content of the diet was assayed by determining the radionuclide 82Br by instrumental neutron activation analysis (INAA) in a long-term activation regime and amounted to 10.04 mg/kg diet.

Findings

4.1 Observations

4.1.1 Clinical signs No clinical signs were reported in this investigation

4.1.2 Mortality No mortalities were reported in this investigation.

4.2 Body weight gain No analysis of body weight gain was performed in this investigation.

4.3 Food consumption and compound intake

The average amount of consumed diet was 20 g/animal/day. The amount of consumed water was measured and corresponded approximately to 15 ml/animal/day. Bromide intake was calculated to be 0.15, 0.75, 1.5, 3 and 6 mg bromine/animal/day or 0.5, 2.5, 5, 10 and 20 mg Br-/kg bodyweight based on an assumed average bodyweight of 300 g/rat over the study period

4.4 Ophtalmoscopic examination

No ophthalmoscopic examination was performed in this investigation.

4.5 Blood analysis

4.5.1 Haematology No haematological analysis was performed in this investigation.

4.5.2 Clinical chemistry

No clinical chemistry was performed in this investigation.

4.5.3 Urinalysis No urinalysis was performed in this investigation.

4.6 Sacrifice and pathology

4.6.1 Organ weights No analysis of organ weights was performed in this investigation.

4.6.2 Gross and histopathology

The thyroid of rats exposed to bromide exhibited microfollicular rearrangement of the follicular epithelium and a reduction of the amount of colloid. Control animals showed follicles of varying size the lumina of which were filled with PAS (periodic acid Schiff stain) -positive colloid. PCNA positive nuclei were stained diffusely brown and were larger in size. PCNA-L1 is higher in experimental animals. The values of mitotic index increased with increasing bromide concentrations. The square area in the thyroid sections from control animals contained maximally one PCNA-positive nucleus only. The thyroid of young rats after 16 days of bromide application contained more positive nuclei in the square.

4.7 Other None

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Conclusions 5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Preceding experiments had shown that microfollicular rearrangement of the rat thyroid, accompanied by enlargement of follicular cells, can be induced by low bromine concentrations (10-100 mg Br-/L drinking water).The aim of the study was to find out to which extent the hyperplasia resulting from increased mitotic activity of follicular cells participates in the changes observed in these previous experiments.

The experiments were carried out in three series: (1) Four groups of ten animals each received 0, 10, 50 and 100 mg Br-/L drinking water (corresponding to 0, 0.5, 2.5, 5, 10 and 20 mg Br-/kg bodyweight based on an assumed average bodyweight of 300 g/rat over the study period and taking into account a water consumption of 15 mL/animal/day). Exposure time 16 days.

(2) Four groups of ten animals each received 0, 10, 50 and 100 mg Br-/L drinking water. Exposure time 66 days. (3) Four groups of ten animals each received 0, 100, 200 and 400 mg Br-/L drinking water. Exposure time 133 days. After termination of potassium bromide administration, the animals were sacrificed and thyroid lobes were excised, weighed and fixed in Bouin´s fluid for 24 hours.

Mitotic activity of follicular cells was evaluated by the immunohistochemical assay of PCNA on sections from the paraffin-embedded thyroid. Sections incubated without anti-PCNA were used as negative controls.

Proliferation activity was evaluated by counting diffusely brown-coloured nuclei and PCNA-L1 index calculated according to the following formula: number of PCNA-positive nuclei x 100/total number of nuclei in the square. The number of nuclei evaluated in each specimen was 4025-6407.

5.2 Results and discussion

The thyroid tissue of control animals contained follicles of varying size, the lumina of which were filled with a PAS (periodic acid Schiff stain)-positive colloid. The follicles were lined with cuboid or low-cylindrical epithelium. By contrast, the thyroid of animals exposed to bromide exhibited microfollicular rearrangement of the follicular epithelium and a reduction of the amount of colloid. PCNA positive nuclei were stained diffusely brown and were larger in size. PCNA-L1 is higher in experimental animals. The values of mitotic index increased with increasing bromide concentrations. The Kruskal-Wallis test showed that the PCNA-L1 differences were significant. The square area in the thyroid sections from control animals contained maximally one PCNA-positive nucleus only. The thyroid of young rats after 16 days of bromide application contained more positive nuclei in the

square.

In the synthesis of thyroid hormones a conversion of inorganic iodine into an organic form occurs. The transport of iodine and its organification can be blocked by Br- ions due to a mutual competition between bromide and iodide anions. This results in a thyroxin and triiodothyronine deficiency which in turn increases secretion of TSH (thyrotropic hormone). Previous as well as present experiments also demonstrated an increase in bromine content and a concomitant decrease of the I/Br molar ratio in the thyroid tissue after administration of Br- in drinking water.

This finding is important in connection with the data showing an increasing exposure of living organisms to environmental bromine which represents an important environmental factor contributing to the development of endemic goitre; this is caused by a decreased utilization of the consumed iodine which produces a relative iodine deficiency even when the iodine intake is sufficient.

The experiments of this study demonstrated that the goitrogenic action is exerted even by low concentrations of bromine in drinking water. Changes of the thyroid had the character of a parenchymal goitre and were thus similar to the findings described previously by other groups.

The extent and degree of morphological and functional changes found in this investigation correlates with the bromide concentration and the length of period of its administration. The immunohistochemical demonstration of PCNA also correlated with the changes in the thyroid tissue and showed an increased mitotic activity of follicular cells. The values of the PCNA-L1 index in rats exposed to bromide for 16 days were higher than those in animals exposed to bromide for 66 and 133 days. A decrease of mitoses with increasing age was found also in control groups of animals.

5.3 Conclusion Based on the findings made in this investigations it was concluded that morphological and functional changes in the thyroid correlated with concentration and length of period of bromide treatment when administered via the drinking water. Thyroids from rats treated with bromide showed increased mitotic activity of follicular cells. In addition, an increase in bromine content and a concomitant decrease of the I/Br molar ratio in the thyroid tissue after administration of Br- in drinking water was demonstrated.

5.3.1 LO(A)EL 10 mg bromide/L (corresponding to 0.5 mg/kg bw)

5.3.2 NO(A)EL Not applicable

3.9.3.3 [Study 3] 133 days repeated dose toxicity study of potassium bromide to investigate effects on the thyroid gland of the rat

Reference	A6.10/13, Doc. No. 592-034 Velický J, Titlbach M, Lojda Z, Dusková J, Vobecký M, Strbák V, Raska I. (1998). Longterm action of potassium bromide on the rat thyroid gland. Acta histochem. 100, 11-23.
Guideline	2.1 Guideline study No. Study is a publication and was performed according to good experimental practice. 2.2 GLP No: Study is a publication and was performed according to good experimental practice. 2.3 Deviations Not applicable; no guideline available for this special mechanistic investigation
Reliability	2
Species / strain	3.2.1 Species Rat 3.2.2 Strain Wistar 3.2.3 Source Top Velaz, Prague

	3.2.4 Sex Male
Test material	Potassium bromide
Study design	3.2 Test Animals
	3.2.5 Age/weight at study initiation: Animals were 42 days of age and within a weight range of 100-130 g.
	3.2.6 Number of animals per group: 10/group
	3.2.7 Control animals Yes
	3.3 Administration/Exposure
	3.3.1 Duration of treatment: 133 days
	3.3.2 Frequency of exposure: daily
	3.3.3 Postexposure period: No postexposure period; animals were sacrificed at the end of the treatment period.
	3.3.4 Oral: in drinking water
	3.3.4.2 Concentration drinking water: 100, 200 or 400 mg/L corresponding to 11.6, 23.1 and 46.2mg/kg bw/day with the following assumptions: drinking water consumption: 15 mL/animal, average animal weight: 130 g/animal
	3.3.4.3 Vehicle drinking water
	3.3.4.4 Concentration in vehicle: Not applicable; KBr was administered via the drinking water at 100, 200 and 400 mg/L
	3.3.4.5 Total volume applied: 10-15 mL/animal/day
	3.3.4.6 Controls water
	3.4 Examinations
	3.4.1 Observations
	3.4.1.1 Clinical signs No
	3.4.1.2 Mortality Yes
	3.4.2 Body weight Yes; at termination of the study X3
	3.4.3 Food consumption Yes
	3.4.4 Water consumption Yes; measured in a random manner by weighing the drinking vessel after
	filling and 24 hours later.
	3.4.5 Ophthalmoscopic
	examination
	Not examined
	3.4.6 Haematology Not examined.
	3.4.7 Clinical Chemistry The concentration of thyroid hormones and TSH in plasma was determined by radioimmunoassay (RIA). Blood was collected from animals of all experimental groups at the end of the treatment period by cardiac puncture. No further clinical chemical parameters were determined in this investigation.
	3.4.8 Urinalysis Not examined.
	3.5 Sacrifice and pathology
	3.5.1 Organ Weights Yes; at termination of the investigation.
	3.5.2 Gross and histopathology
	Yes; thyroid lobes were examined only. Sections were stained with haematoxylin-eosin and by the PAS method. Small samples from the other part of the lobe were fixed in 2,5% glutardialdehyde/0.1

M phosphate buffer for 2 hours. After washing with 5% glucose/0.1 M phosphate buffer and postfixation in 2% osmium tetroxide for 1 hour, the material was dehydrated and embedded in Durcupan and further processed for electron microscopy.

3.5.3 Other examinations Immunohistochemistry:

The assay of thyroglobulin (Tg) was carried out in paraffin sections by an indirect immunoperoxidase technique followed by 3,3´-diaminobenzidine tetrahydrochloride visualisation. Rabbit anti-human thyroglobulin was used as primary, and swine-anti-rabbit as secondary antibody.

Morphometry:

The morphometric and immunohistochemical evaluation was performed by using the Nikon microphot FXA microscope and the Lucia M image analysis programme Lucia 3.5.

Determination of tissue and diet bromine and iodine concentrations:

Bromine and iodine levels in the thyroid gland dry weight were determined by instrumental neutron activation analysis (INAA) in a short-term irradiation regime applied to dried thyroid glands in a nuclear reactor. The bromine level in the diet was assessed by INAA in a long-term irradiation regime using the 82Br radionuclide and the iodine level by kinetic photometry.

3.5.4 Statistics Differences between mean values were tested by variance analysis followed by F-test and Kruskal-Wallis test.

3.6 Further remarks None

Findings

4.1 Observations

4.1.1 Clinical signs There were no clinical signs reported in this investigation.

4.1.2 Mortality There were no premature descendents during the treatment period.

4.2 Body weight gain The bodyweight taken at termination of the study is not further specified in the

investigation.

4.3 Food consumption and compound intake: Food consumption was approximately 20 g/animal/day. Consumption of drinking water, and with that intake of the test substance, was approximately 10-15 mL/animal/day.

4.4 Ophthalmoscopic examination: No Ophthalmoscopic examination was performed during the investigation.

4.5 Blood analysis

4.5.1 Haematology No haematological analysis was performed during the investigation.

4.5.2 Clinical chemistry Exposure of the rats to 100 mg bromide/L evoked a slight increase in TSH level after 133 days. Treatment with 200 and 400 mg bromide/L did not increase TSH concentration but led rather to a slight decrease. The thyroxin (T4) concentration in the plasma of animals exposed to different bromine concentrations decreased significantly as compared to the T4 level in controls. The plasma concentration of 3,5,3´-triiodothyronine (T3) in bromine-exposed animals did not differ from control values.

4.5.3 Urinalysis No urinalysis was performed in this investigation.

4.6 Sacrifice and pathology

4.6.1 Organ weights Dry weights from thyroid lobes were taken only and used for calculation of bromine and iodine content.

4.6.2 Gross and histopathology

In comparison to control rats the thyroid glands of animals exposed to a concentration of 100 mg bomide/L dinking water for 133 days exhibited a striking follicular rearrangement characterized by the presence of a large number of very small follicles (6-20 μm) with round lumina of very small diameter (1-10 μm) or with slit-like constricted lumina.

The height of the thyrocytes was increased in medium-sized follicles at the thyroid periphery.

Mitoses of thyrocytes were more frequent than in the controls. Animals exposed to 200 and 400 mg/L showed the same changes. In addition, the microfollicular tissue of the 200 mg-group occupied a smaller part of the lobe and the proportion of medium-sized follicles appeared larger. Electron microscopy revealed changes in the localisation and extent of the golgi apparatus, rough endoplasmic reticulum, lysosomes and microvilli in all treatment groups.

4.7 Other Immunohistochemistry of thyroglobulin (Tg): Animals exposed to 100 mg bromide/L drinking water for 133 days exhibited an overall decrease in Tg immunoreactivity.

A complete loss of Tg immunoreactivity occurred in animals exposed to a bromide concentration of 400 mg/L.

Morphometry:

The amount of intrafollicular colloid was significantly decreased in thyroids of rats treated with bromide in comparison with the control animals. Surprisingly, the lowest concentration of the bromide ion administered in this experiment (100 mg/L) lowered the colloid volume more than the next higher dose (200 mg/L). The changes obtained with 100 and 400 mg/L were similar.

Bromine and iodine tissue concentrations:

Animals exposed to bromine for 133 days exhibited changes in bromine and iodine concentration in the thyroid tissue. With an increasing concentration of bromide, the amount of bromine in the thyroid dry weight showed a concomitant decrease in the molar I/Br ratio from 17.9 to 0.39, and the extent of the decrease were again dependent on bromide concentration.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This investigation was a continuation of previous experiments which revealed a positive effect of 16- and 66-day administration of low bromide concentrations on the rat thyroid which displayed a marked growth activation of the follicular epithelial component, and in addition, mitoses in the follicular cells were more frequent. Also, microfollicular reorganisation occurred. The aim of this investigation was to show if the most effective concentration used in the previous study (100 mg bromide/L) and further multiples of this concentration would evoke morphological and functional changes in the rat thyroid after a long-term administration.

Male rats were divided into four groups, each consisting of 10 animals, and treated for 133 days with 0, 100, 200 or 400 mg bromide/L drinking water corresponding to 0, 1-1.5, 2-3 and 4-6 mg/animal/day (consumption of drinking water: 10-15 mL/animal/day) or 0, 7.7-11.5, 15.4-23.1 and 30.8-46.2 mg/kg bw/day (based on a weight of 130 g/rat).

After the termination of the experiments the animals were killed, weighed, and the thyroid lobes excised. A portion of one lobe (fixed in Bouin´s fluid and embedded in paraffin) was used for preparation of serial sections (200 sections/animal). These sections were stained, fixed and further processed for electron microscopy.

The assay of thyroglobulin (Tg) was carried out in paraffin sections by an indirect immunoperoxidase technique followed by 3,3´-diaminobenzidine tetrahydrochloride visualisation. Rabbit anti-human thyroglobulin was used as primary antibody.

The concentration of thyroid hormones and TSH in plasma was determined by radioimmunoassay (RIA). Bromine and iodine levels in the thyroid gland dry weight were determined by instrumental neutron activation analysis (INAA) in a short-term irradiation regime. The bromine level in the diet was assessed by INAA in a long-term irradiation regime using the 82Br radionuclide and the iodine level by kinetic photometry.

5.2 Results and discussion

Light microscopy:

In comparison to control rats the thyroid glands of animals exposed to a concentration of 100 mg bomide/L dinking water for 133 days exhibited a striking follicular rearrangement characterized by the presence of a large number of very small follicles (6-20 μm) with round lumina of very small diameter (1-10 μm) or with slit-like constricted lumina. The lumina contained a small amount of PAS-positive colloid. Some follicles had no lumina. In other follicles, the thyrocytes appeared higher. These changes were usually diffuse and occurred less frequently in foci, usually in the central

part o the lobe. Their presence in the periphery of the lobe, however, was also found. The height of the thyrocytes was increased in medium-sized follicles at the thyroid periphery. Groups of such cells protruded into the lumina of the follicles. The apical cytoplasm of thyrocytes showed multipled PAS-positive spherical vacuolesMitoses of thyrocytes were more frequent than in the controls. The capillaries were numerous and dilated.

The thyroid of animals exposed to 200 mg/L for 133 days exhibited a similar histological picture but the microfollicular tissue occupied a smaller part of the lobe, and the proportion of medium-sized follicles appeared larger. In animals exposed to 400 mg bromide/L drinking water for 133 days the pattern was essentially identical with that in animals receiving 100 mg/L.

Electron microscopy:

Electron microscopy revealed changes in the localisation and extent of the golgi apparatus, rough endoplasmic reticulum, lysosomes and microvilli in thyrocytes of all treatment groups.

Immunohistochemistry of thyroglobulin:
In control animals thyroglobulin (Tg) immunoreactivity in the colloid of most follicles was medium to strongly positive particularly in the peripheral rim adjacent to the apical pole of the thyrocytes. By contrast, animals exposed to 100 mg bromide/L drinking water for 133 days exhibited an overall decrease in Tg immunoreactivity. In some follicles a medium to strong immunoreaction was concentrated partly at the border between the thyrocyte and peripheral rim of colloid of both microfollicles and larger follicles. In some follicles immunoreactivity was absent. A complete loss of Tg immunoreactivity occurred in animals exposed to a bromide concentration of 400 mg/L. The central part of the thyroid tissue of these animals had medium Tg reactivity concentrated mostly in the narrow peripheral colloid rim in the lumina of part of the follicles, while other parts were negative. The periphery of the thyroid reacted less intensively.
Morphometry:
The amount of intrafollicular colloid was significantly decreased in thyroids of rats treated with bromide in comparison with the control animals. Surprisingly, the lowest concentration of the bromide ion administered in this experiment (100 mg/L) lowered the colloid volume more than the next higher dose (200 mg/L). The changes obtained with 100 and 400 mg/L were similar.
Statistical evaluation showed differences in the volume proportion of colloid in the thyroid tissue of rats exposed to 100, 200 and 400 mg bromide/L for 133 days relative to controls that were highly significant ($P<0.001$). The distribution of colloid deposits in the follicular lumina in sections showed a marked increase in the number of the smallest follicles (colloid area in the section up to 100 and 100-300 μm^2) in all groups of animals exposed to bromine. By contrast, the proportion of larger-area colloid deposits (500-1000 μm^2) was lower. The form of follicular lumina of bromine-exposed rats did not differ from that in controls.
RIA determination of T4, T3 and TSH:
Exposure of the rats to 100 mg bromide/L evoked a slight increase in TSH level after 133 days. Treatment with 200 and 400 mg bromide/L did not increase TSH concentration but led rather to a slight decrease. The thyroxin (T4) concentration in the plasma of animals exposed to different bromine concentrations decreased significantly as compared to the T4 level in controls.
The plasma concentration of 3,5,3´-triiodothyronine (T3) in bromine-exposed animals did not differ from control values. The T4 decrease in the plasma of bromine-exposed animals correlated well with the persisting morphological changes and with changes in the thyroglobulin content of follicles.
Bromine and iodine tissue concentrations:
Animals exposed to bromine for 133 days exhibited changes in bromine and iodine concentration in the thyroid tissue. With an increasing concentration of bromide, the amount of bromine in the thyroid dry weight showed a concomitant decrease in the molar I/Br ratio from 17.9 to 0.39, and the extent of the decrease were again dependent on bromide concentration.
5.3 Conclusion Bromide administration for 133 days caused an increased growth activity of the thyrocytes accompanied by symptoms of hypothyroidism, decreased T4 plasma concentration, lowered Tg immunoreactivity and a decrease in the I/Br molar ratio in the thyroid. The results of this investigation demonstrate that rats appear to react in a sensitive manner to the administration of even low amounts of bromide with the drinking water as demonstrated by a modulation of thyroid hormones in the plasma and changes in thyroid morphology.

5.3.1 LO(A)EL LO(A)EL was estimated to be 100 mg/L (about 12.5 mg/kg bw/day) based on follicular rearrangements in the thyroid and decrease of TSH, T4 and Tg immunoreactivity

5.3.2 NO(A)EL NO(A)EL was estimated to be < 100 mg/L

5.3.3 Other None

Conclusions

3.9.3.4 [Study 4] 16, 66 or 133 days repeated dose toxicity study of potassium bromide to investigate effects on the thyroid gland of the rat

Reference A6.10/12; Doc. No. 592-038

Velický J, Titlbach M, Lojda Z, Dusková J, Vobecký M, Raska I. (2004). The effect of bromide on the ultrastructure of rat thyrocytes. Ann Anat 186, 209-216.

Guideline 2.1 Guideline study No guideline available for this special investigation; study was performed according to good experimental practice.

2.2 GLP No. Study is a publication and was performed according to good experimental

practice.

2.3 Deviations Not applicable; no guideline available for mechanistic studies

Reliability 2

Species / strain 3.2.1 Species Rat

3.2.2 Strain Wistar

3.2.4 Sex male

Test material Potassium bromide

Study design 3.2 Test Animals

3.2.5 Age/weight at study initiation: Animals were 41 days of age and within a weight range of 100-120 g.

3.2.6 Number of animals per group: 10/group

3.2.7 Control animals: Yes

3.3 Administration/Exposure

3.3.1 Duration of treatment: 16, 66 or 133 days

3.3.2 Frequency of exposure daily

3.3.3 Postexposure period: No postexposure period. The animals were sacrificed after the treatment period (16, 66 or 133 days) and thyroid lobes were removed for electron microscopic examination.

3.3.4 Oral

3.3.4.1 Type in drinking water

3.3.4.2 Concentration/dosage: 0, 0.15, 0.75 and 1.5 mg Br-/day for 16 and 66 days

0, 1.5, 3 or 6 mg Br-/day for 133 days (based on a measured water intake of 15 mL/animal/day)

3.3.4.3 Vehicle Drinking water

3.3.4.4 Concentration in vehicle: 0, 10, 50 and 100 mg Br-L for 16 and 66 days

0, 100, 200 or 400 mg Br-/L for 133 days

3.3.4.5 Total volume applied: Not applicable; substance was administered via the drinking water

3.3.4.6 Controls Vehicle (drinking water)

3.4 Examinations

3.4.1 Observations

3.4.1.1 Clinical signs Not examined

3.4.1.2 Mortality Not examined

3.4.2 Body weight Not examined

3.4.3 Food consumption: Yes

3.4.4 Water consumption Yes

3.4.5 Ophthalmoscopic examination: Not examined

3.4.6 Haematology Not examined

3.4.7 Clinical Chemisty Not examined

3.4.8 Urinalysis Not examined

3.5 Sacrifice and pathology

3.5.1 Organ Weights Weights of thyroid lobes were taken only

3.5.2 Gross and histopathology: Yes; Both thyroid lobes were stained by haematoxylin und eosin and by the PAS reaction.

3.5.3 Other examinations None

3.5.4 Statistics Statistical evaluation for proliferation activity test results was done by the Kruskal-Wallis test.

3.6 Further remarks Electron microscopy:

Thyroid glands of rats were dissected free of fibrous fatty tissue and muscles; small pieces of tissue (approximately 1 mm3) were fixed in 2.5 % glutardialdehyde in a 0.3 M phosphate buffer, pH 7.2 at 4°C for 2 hours, rinsed in the same buffer with 5 % glucose, and post-fixed in 2 % osmium tetroxide in the same buffer at room temperature for 1 hour. Afterwards the material was dehydrated in ethanol, embedded in Durcupam ACM and processed for electron microscopy. Semithin and ultrathin sections were cut using ultramicrotomes and Ultracut. Semithin sections were stained in aqueous solution of Azur B in borax buffer, pH 8. Ultrathin sections were contrasted using uranyl-acetate, lead-citrate and examined using electron microscope.

The mean content of bromide and iodide in the diet was 10.04 mg bromide/kg and 0.52 mg iodide/kg respectively.

Findings

4.1 Observations

4.1.1 Clinical signs There were no clinical signs described in this investigation.

4.1.2 Mortality There were no mortalities described in this investigation.

4.2 Body weight gain No analysis of body weight gain was performed in this investigation.

4.3 Food consumption and compound intake

The diet was available ad libitum. The mean amount of food consumed was approximately 20 g/animal/day. Water consumption was measured to be approximately 15 mL/animal/day.

4.4 Ophtalmoscopic examination

No ophthalmoscopic examination was performed in this investigation.

4.5 Blood analysis

4.5.1 Haematology No haematological analysis was performed in this investigation.

4.5.2 Clinical chemistry No clinical chemistry was performed in this investigation.

4.5.3 Urinalysis No urinalysis was performed in this investigation.

4.6 Sacrifice and pathology

4.6.1 Organ weights No analysis of organ weights was performed in this investigation.

4.6.2 Gross and histopathology

The thyroid gland was examined in detail via electron microscopy. No other organs were investigated for changes.

The thyroid gland of the control animals consisted of follicles predominantly round in shape, containing colloid in their lumen. The follicular size varied to some extent and measured 30-50 µm on average. The walls of these follicles were lined with a simple cuboid epithelium of thyrocytes. The nucleus was usually round or ovoid and the nuclear chromatin of medium density, the medium and basal parts of the cytoplasm contained a well developed granular endoplasmic reticulum (ER) and numerous of free ribosomes. The Golgi complex consisted of a varying number of flattened cisterns with small pockets and vacuoles at the periphery and was usually located between the nucleus and luminal plasma membrane. Oval and elongated mitochondria with marked cristae were scattered thoughout the entire cytoplasm. In the luminal cytoplasm dark or medium density granules with a homogenous core were found close to the surface membrane. The cytoplasmic matrix also contained numerous primary and secondary lysosomes, including polyphagosomes, colloid droplets and residual myelin corpuscles. The luminal plasma membrane of the thyrocytes showed coated pits, and branching microvilli. Between the lateral membranes of the thyrocytes, zonulae occludents, zonulae adhaerentes, desmosomes and nexus were found. After 16 days of 10 mg bromide application the size of follicles varied, many small follicles with very small lumina were found. Thyrocytes with hypertrophic granular ER and dilated cisterns with light material were observed. Proliferated microvilli protruded into the luminal colloid. The nuclei were often irregular in shape with incisions, and the density of nuclear chromatin was higher than in the controls. The well developed Golgi apparatus showed small, transport granules of a density similar to that of some subapical granules and minute spherical structures and was mostly enlarged. The number of secondary lysosomes including phagolysosomes was increased only in some cells. Colloid droplets were rarely found. Sporadic thyrocytes with signs of necrosis were seen. Complex intercellular contacts were typical between membranes of adjacent cells even in very small follicles. Similar findings were obtained after 66 days administration of bromide. After 16 days of 50 mg bromide application the overall picture of the tissue was dominated by numerous very small follicles with very small lumen containing a smaller amount of colloid. ER was markedly increased with highly dilated cisterns not located only in central and basal parts of cytoplasm but also in the subapical region of many cells. Microvilli protruding into the luminal colloid were significantly increased. The nuclei were of irregular shape with condensed nuclear chromatin. In the supranuclear cytoplasm of the follicular cells the small, subapical granules wth dark or medium-dark homogeneous core substance and coated pits were increased. Lysosomes (size: 400-1500 nm)filled with a fine granulated substance were also present sometimes fusing with small granules. Colloid droplets were rare. Similar findings were obtained after 66 days of 50 mg bromide administration.

After 16 days of 100 mg bromide administration the thyroid gland showed the highest proliferation rate of the thyrocytes. Newly formed intracellular cavities resembling microfollicles were frequently found containing normal colloid or fragments of degraded follicular cells separated from the wall of the follicle.

The cytoplasm was filled with dilated ER cisterns. Remnants of former thyrocytes were also found in the colloid of some follicles after their separation from the follicular wall. The nucleus of the thyrocytes was usually smaller, often of irregular shape, with increased chromatin density. Even in apical parts of the cytoplasm the ER cisterns were significantly delated. Golgi complex was usually enlarged. The number of larger round lysosomes including phagolysosomes appeared to be unchanged; however, their contents were mostly more dense. Colloid droplets were rarely found. In some cases clusters of cells with spongy cytoplasm and bizarre-shaped nuclei were seen. Two types of cells were present in the follicular epithelium. One type was characterized by many small round subapical granules of medium and small density, and flat ER cisterns. The other type had only a few subapical granules and dilated ER cisterns with a low density content. Similar results were obtained 66 days after administration of 100 mg bromide.

In the group receiving 100 mg bromide over 133 days the overall picture micro-follicular restructure predominated and the mean follicular size was around 8-20 µm and less. Nuclei were of irregular shape with higher density chromatin. In the basal parts of the thyrocytes various amounts of dilated ER cisterns, often parallel in arrangement were found. Dense bodies were also present. As in the previous groups of animals (receiving 100 mg bromide/L for 16 and 66 days), two types of thyrocytes were present. Between the luminolateral plasma membranes, well developed junctional complexes were found and signs of necrosis were observed in the cytoplasm of some thyrocytes. In

the group treated with 200 mg bromide/L for 133 days, microfollicles predominated. The amount of colloid in the small lumina was markedly reduced in most follicles. An increased number of microvilli and coated pits were frequently seen. The luminal cytoplasm contained more spherical structures and granules, cisterns of ER and dense bodies (lysosomes). The nucleus was of irregular shape and with higher density chromatin. The golgi apparatus, desmosomes and junctional complexes between the neighbouring thyrocytes were usually well developed. The thyroid gland of the 400 mg/L-group (133 days) was characterized by small follicles with reduced lumina. Their wall consisted of thyrocytes, the ultrastructure of which displayed smaller changes than in the other groups of bromide-treated rats. One common finding were medium dilated cisterns of ER and increased number of spherical structures and granules in the subapical cytoplasm.

Conclusions 5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The present investigation is a continuation of previous investigations in which environmental concentrations of bromide have been used. It extends the earlier findings on the rat thyroid gland to the electron microscopic level using the same experimental design. Male Wistar rats were divided into 9 experimental and 3 control groups, consisting of 10 animals each. The animals received bromide in drinking water: 0, 10, 50 and 100 mg/L for 16 and 66 days and 0, 100, 200 and 400 mg/L for 133 days. The amount of water consumed by the animals was measured and was approximately 15 ml/animal/day.

5.2 Results and discussion

The most important finding in the cytoplasm of thyrocytes was the hypertrophy and hyperplasia of ER, combined with dilated cisterns and tubules not only in the central and basal but also in the apical cytoplasm where, in addition, the dilated cisterns were often of ovoid shape. The hypertrophic ER, together with other ultrastructural findings, may indicate the degradation of protein biosynthesis of components of iodine containing thyroglobulin in thyrocytes. The shape, size and number of mitochondria did not significantly differ from the control groups, with the exception of those animals receiving 100 mg Br-/L for 133 days. In these rats the mitochondria were markedly enlarged. Colloid droplets indicating an increased resorption of the colloid from the lumen were hardly seen in the test material. This finding was also described by other groups after high bromide doses.

The dilatation or vesiculation of ER may relect enzymatic or mechanical defects in the ER of neoplastic cells, which was noticed for thyroid tumours. The cistern patterns in he ER are comparable to observations in follicular cells of experimentally induced goitre by low-iodine diet. The thyrocytes also showed nuclei with irregular shape and condensed chromatin. This was also found in hamsters fed a low-iodine diet.

5.3 Conclusion The ultrastructural findings in this investigation after bromide administration were similar to those caused by well known goitrogens used in experiments and clinical practice. It may be suggested that the changes caused by bromide treatment of rats in the thyrocytes point to a defect in transport and probably also synthesis of thyroidal hormones caused by increased bromide levels which inhibit active absorption of iodide by the thyroid.

5.3.1 LO(A)EL 10 mg/L drinking water/day, corresponding to 0.15 mg/animal/day or 0.6 mg/kg bw/day

5.3.2 NO(A)EL Not applicable

3.9.3.5 [Study 5] Repeated dose toxicity study of potassium bromide to investigate effects on the thyroid gland of the dog

Reference A6.10/18, Doc. No. 592-022

Paull, L. C. et al (2003). Effect of Anticonvulsant Dosages of Potassium Bromide on Thyroid

	Function and Morphology in Dogs. Journal of the American Animal Hospital Association, 39, 193-202.
Guideline	2.1 Guideline study Not applicable, no guideline available for this type of special investigations.
	2.2 GLP No. Study is a publication and was performed according to good experimental practice.
	2.3 Deviations Not applicable, no guideline available for this type of special
	investigations
Reliability	2
Species / strain	3.2.1 Species Dog
	3.2.2 Strain Laboratory hound dog
	3.2.4 Sex male and female
Test material	Potassium bromide
Study design	3.2 Test Animals
	3.2.5 Age/weight at study initiation: Animals were approximately 1-2 years of age, mean bodyweight at study initiation was 24 kg
	3.2.6 Number of animals per group: 2 males and 3 females for treatment and control group each.
	3.2.7 Control animals Yes
	3.3 Administration/Exposure
	3.3.1 Duration of treatment: 182 days
	3.3.2 Frequency of exposure: daily
	3.3.3 Postexposure period: None; animals were killed on termination of the study on Day 182
	3.3.4 Type in drinking water
	3.3.5 Concentration Loading dose: - 100 mg/kg bw over 12 hours for a period of 2 days
	Maintenance dose: - 30 mg/kg bw over 24 hours for a period of 180 days - adjustment on Day 120; if serum bromide concentrations were less than 250 mg/dL, dose was increased by 5 mg/kg bw for the remaining study
	3.3.6 Vehicle distilled water
	3.3.7 Concentration in vehicle: 200 mg/mL
	3.3.8 Total volume applied: Not applicable, drinking water study
	3.3.9 Controls Placebo-control (distilled water)
	3.4 Examinations
	3.4.1 Observations
	3.4.1.1 Clinical signs Yes; daily
	3.4.1.2 Mortality Not examined
	3.4.2 Body weight Yes; at study initiation and on Day 177; no subsequent body weight determinations were performed.
	3.4.3 Food consumption Not examined
	3.4.4 Water consumption: Not examined
	3.4.5 Ophthalmoscopic examination: Not examined
	3.4.6 Haematology Yes; at start and termination of study haematological examination was undertaken. The parameters examined are not further specified in the report since no differences were observed between values at start and termination of the study.

3.4.7 Clinical Chemisty Yes; on Days 3, 30, 120 and 177 serum bromide concentration, T4, free T4 and TRH levels were examined and serum biochemical analysis was performed (the details of parameters determined were not further specified in the report sine no differences were observed at start and termination of the study in serum biochemical parameters). Total T4 and TSH concentrations were measured using chemiluminescent enzyme immunoassays validated for use in the dog. Free T4 concentrations were measured by equilibrium dialysis validated for use in dogs.

Serum bromide concentration was measured using a gold colorimetric assay. All blood samples were processed on the day of collection. Serum was removed from each blood tube within 45-60 minutes. Serum samples were divided into 4-5 aliquots and stored at -20°C until analysis was performed.

3.4.8 Urinalysis Yes; at start and termination of study (urine specific gravity, USG)

3.5 Sacrifice and pathology

3.5.1 Organ Weights Only thyroid tissue wet weights were recorded immediately after removal and expressed as g/kg bodyweight. No other organ weights were taken.

3.5.2 Gross and histopathology

Thyroids were subjected to gross and histopathological examinations. Hematoxylin and eosin-stained sections of all thyroids were evaluated by light microscopy. No further organs/tissues were examined. Thyroidectomy was performed as follows:

Dogs were premedicated with butorphanol tartrate (0,3 mg/kg bw intramuscularly) and acepromazine maleate (0.03 mg/kg bw intramuscularly). Anesthesia was induced with intravenous injection of sodium pentothal and maintained with isoflurane through a semiclosed rebreathing system. The right lobe of the thyroid gland was approached through a midline skin incision. Unilateral thyroidectomy was performed using an extracapsular technique.Tissue was fixed in 10 % neutralbuffered formalin, paraffin-embedded and 5-6 µm sections were prepared.

3.5.3 Other examinations

Physical examinations were performed daily for the first week, every 2 weeks for the first 28 days, and then once every 30 days until completion of the study.

Serum basal total T4 (TT4) and thyroid stimulating hormone (TSH) concentrations, serum free T4 (fT4) concentration, anticanine thyroglobulin antibody (TgA) optical density (OD) and a thyrotropinreleasing hormone (TRH) stimulation test were evaluated in each dog at baseline. Basal TT4 and TSH concentrations were measured in addition on Days 3, 30, 120 and 177. A fT4 by equilibrium dialysis concentration and TRH stimulation test were repeated on Day 177.

3.5.4 Statistics The independent and joint influences of group (experimental versus control) and time on serum hormone concentration or body weight were evaluated by repeated measures analysis of variance (ANOVA). Thyroid wet-weights and thyroid histopathology scores were analyzed by the Wilcoxon´s signed rank sum test. Significance was defined as $P< 0.05$ for all statistical analysis.

3.6 Further remarks Thyroid tissue was assessed for activation referring to the following criteria that occur in response to hormone stimulation: enhanced vascularisation, increased microfollicular development (MFD), decreased intrafollicular colloid staining (IFC), increased number of columnar follicular epithelial cells, and increased follicular epithelium mitotic figures.

Findings

4.1 Observations

4.1.1 Clinical signs Neither clinical signs of hypothyroidism nor evidence of bromism were identified in any of the dogs.

4.1.2 Mortality No mortalities were reported in this study.

4.2 Body weight gain There was a significant ($P< 0.0001$) gain in body weight over time, but the weight change did not differ between experimental and control groups. However, the interaction for group x time was significant ($P< 0.01$), reflected by more rapid weight gain of dogs in the experimental group.

4.3 Food consumption and compound intake: No analysis of food consumption and compound intake was performed in this study.

4.4 Water Consumption: No analysis of water consumption was performed in this study.

4.5 Ophthalmoscopic examination: No ophthalmoscopic examination was performed in this study.

4.6 Blood analysis

4.6.1 Haematology Hematological analysis was within reference ranges in each dog at

initiation and termination of the study.

4.6.2 Clinical chemistry Serum biochemical analysis was within reference ranges in each dog at initiation of the study. Abnormalities identified in KBr-treated dogs were consistent with those expected due to administration of KBr (falsely elevated chloride and low anion gap).

From day 30 to completion of the study, all serum bromide concentrations in KBr-treated dogs were within or exceeded the therapeutic range (88- 300 mg/dL) recommended for epileptic dogs treated with KBr monotherapy. Three dogs exceeded the target serum bromide concentration (250-300 mg/dL) on Day 120. One of theses dogs was within the target range, one exceeded it on Day 177 and the third one had a serum bromide concentration within the therapeutic range but below the target range. At the end of the study, four of five dogs were within the therapeutic range, one of which was within the target range (250- 300 mg/dL). The remaining dog had a serum bromide concentration of 313 mg/dL. On Day 177, serum bromide concentrations in three of five control dogs measured 0 mg/dL. Two control dogs had serum bromide concentrations of 8 and 9 mg/dL, but on repeated analysis bromide concentrations were 0 mg/dL in both dogs.

4.6.3 Urinalysis Urine specific gravity (USG) was within reference ranges in each dog at initiation of the study.

4.7 Sacrifice and pathology

4.7.1 Organ weights No significant difference in thyroid wet-weight ($P > 0.05$) was found between experimental and control dogs. Thyroid wet-weights of the experimental group were 0.037 ± 0.017 g/kg bodyweight and for the control group 0.045 ± 0.015 g/kg bodyweight.

4.7.2 Gross and histopathology

In the scored categories of microfollicular development (MFD) and intrafollicular colloid staining (IFC), no significant difference was found between the KBr-treated and control dogs. However, most dogs (9/10 for MFD, 8/10 for IFC) scored outside of normal for these categories. The degree of vascularity identified in thyroid sections was considered appropriate and as not different between the treatment and control groups. Calculation of a mitotic index for thyroid sections was not possible because of the difficulty of differentiating apoptotic cells from mitotic cells. In general, mitotic figures appeared primarily in areas of microfollicular development; however, they were rarely seen in these regions. With respect to the prevalence of columnar epithelium in thyroid sections, no morphological differences were identified when microfollocular structures were compared with normal-appearing follicles. Neither normal follicular nor microfollicular regions of thyroid sections were different between the treatment and control groups. No significant inflammatory infiltrates consistent with thyroiditis were found in any of the thyroid sections.

4.8 Other The median anticanine thyroglobulin antibody (TgA) OD for all dogs was 0.037 (range 0.009-0.153). Eight dogs were negative for TgA, having ODs less than twice the OD of the negative control. Two dogs (one each fro the treatment and control groups) were weakly positive for TgA.

There was no significant difference between experimental and control groups for serum basal TT4, basal TSH or results of TRH (thyrotropinreleasing hormone) stimulation tests. There were significant decreases in TT4 ($P < 0.0001$) and fT4 ($P = 0.0002$) over time, but these changes did not differ between the experimental and control groups. At baseline, one control dog had a basal TT4 concentration less than the established reference range (1.3-4 µg/dL) and a TSH concentration in the reference range (0-0.65 ng/mL). The postTRH TT4 concentration in this dog was consistent with euthyroidism (1.7 µg/dL). On Day 177, five dogs (three KBr-treated and two control dogs) had basal TT4 concentrations below the reference range. The fT4 of one KBr-treated dog was below the reference range (9-40 pmol/L) on Day 177. Thyroid-stimulating hormone concentrations in all dogs were within the reference range (0-65 ng/mL).

Four of the five KBr-treated dogs gad postTRH TT4 concentrations > 1.5 µg/dL. One KBr-treated dog had a postTRH TT4 concentration of 1.3 µg/dL. On Day 0, percent TSH concentration change following TRH stimulation was < 100 % in all dogs. All KBr-treated dogs and four of five control dogs had a percent TSH concentration change following TRH stimulation > 100 % on Day 177.

Conclusions

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A placebo-controlled experiment was performed to evaluate the effect of potassium bromide on the canine thyroid gland. Basal total thyroxine (TT4), free thyroxine (fT4), and basal thyrotropin serum (TSH)7.1 Organ weights No significant difference in thyroid wet-weight (P> 0.05) was found concentrations were evaluated over a 6-month period in potassium bromide-treated and control dogs. A thyrotropin-releasing hormone (TRH) stimulation test was also performed in all dogs at the beginning and conclusion of the study. Thyroid histopathology was compared between treated and control dogs at the end of the study.

Serum bromide levels were collected from KBr-treated dogs on Days 3, 30, 120 and 177, and from control dogs on Day 177.
Unilateral thyroidectomy was performed in all dogs on Day 182. Thyroid tissue wet weights were recorded and expressed as g/kg bodyweight.
Hematoxylin and eosin-stained sections were evaluated and each slide assessed for evidence of thyroid activation. Increased microfollicular development (MFD) and decreased intrafollicular colloid staining (IFC) were scored for each slide on a scale of 1 to 4. Vascularity was scored by averaging the number of intermediate-sized blood vessels. To evaluate the prevalence of mitotic figures in thyroid sections, calculation of a mitotic index (number of mitotic figures per follicle/total number of epithelial cells per follicle) was attempted. The predominance of columnar follicular epithelial cells was subjectively assessed.
5.2 Results and discussion
Neither clinical signs of hypothyroidism nor evidence of bromism were identified in any of the dogs. From day 30 to completion of the study, all serum bromide concentrations in KBr-treated dogs were within or exceeded the therapeutic range (88- 300 mg/dL) recommended for epileptic dogs treated with KBr monotherapy. Three dogs exceeded the target serum bromide concentration (250-300 mg/dL) on Day 120. Hematological and serum biochemical analyses and USG were within reference ranges in each dog at initiation and as well at termination of the study. Abnormalities identified on serum biochemical analyses of KBrtreated dogs were consistent with those expected due to administration of KBr (falsely elevated chloride and low anion gap). The ability of the thyroid to concentrate iodide and subsequently incorporate this halide into thyroglobulin is essential to thyroid hormone synthesis. Experimental studies *in vitro* demonstrated that the thyroid was able to concentrate other ions of the group IV elements (*ie* fluorine, chlorine, bromine, iodine, astatine). In addition to the relative lack of anion specificity of the iodide uptake mechanism, halogen ions were also found to act as competitive inhibitors of iodide accumulation and cause release of accumulated iodide. Based on these findings it was hypothesized that bromide treatment might decrease thyronine synthesis. Experimental studies performed in rats confirmed that administration of bromide salts resulted in significantly decreased serum T4 and triiodothyronine (T3) concentrations with concurrent goitrous alterations of the thyroid gland. Further investigations suggested that in addition to inhibiting iodide uptake, bromide strongly inhibited the oxidation of iodide to iodine by hydrogen peroxide and, to a lesser degree, inhibited iodinated tyrosine residue coupling to thyronine. For the present study a target serum concentration of 250-300 mg/dL was chosen to ensure maximal effect on thyroid function. There was no significant difference between experimental and control groups for serum basal TT4, basal TSH or results of TRH (thyrotropin-releasing hormone) stimulation tests. This is in agreement with a previous study (Kantrowitz, et al, 1992) that also failed to find an effect of bromide administration on canine thyroid function. Bromide is a relatively weak competitive inhibitor of iodide uptake by the thyroid gland, and compared to iodide, it is concentrated by the thyroid to a much smaller degree. The figures measured in KBrtreated and control dogs for total and free T4 are in line with the background figures reported elsewhere for dogs (total T4 levels in the range of 1.5-4.5 µg/dL and free T4 levels of 7.7-47.6 pmol/L are reported as reference ranges in a textbook of veterinary medicine (“Klinische Labordiagnostik in der Tiermedizin”, Schattauer Verlag, 5.Auflage)). Although in a different study, bromide administration in rats caused depression of thyroid hormone production, this was not observed in dogs in this study. Reasons for this may include species differences in iodide uptake, thyroglobulin organification, tyrosine residue coupling, or thyroid hormone metabolism. The duration of exposure to bromide may have been inadequate and in addition, most studies in rats utilized higher doses of bromide than were used in this study. Another possible explanation for the differences in the effect of bromide on the thyroid function between rats and dogs is the fact that the majority of thyroid-hormones in dogs are plasmaprotein-

bound which is not the case in rats. Thyroid hormones in rats are found free in the plasma and might, thus, be more sensitive to bromide than when protein-bound. Both control and experimental groups of dogs of the study described herein developed a statistically significant decrease in serum TT4 and fT4 concentrations over time but were within the reference ranges, consistent with euthyroidism. No significant difference in thyroid wet-weight (P> 0.05) and in thyroid (histo)pathology was found between experimental and control dogs and no significant inflammatory infiltrates consistent with thyroiditis were found in any of the thyroid sections.

The difference in body weights between dogs in the experimental and control groups at day 177 was not significant (P= 0.15).

5.3 Conclusion In this study, KBr administration for 6 months to young, healthy adult dogs did not have a significant effect on the function or morphology of the canine thyroid gland compared to the control group. Although there was no difference in thyroid function between the groups at any time, the fact that both control dogs and KBr-treated dogs exhibited a significant decline in TT4 and fT4 over time complicated the results of this study. It was not possible to determine the cause of the decreased thyroid hormone concentration over the course of the study; however, because the decrease occurred in both the control and the experimental group, the change did not appear to be related to bromide administration. Based on the results of this investigations it may be further concluded that serum bromide levels in the therapeutic range of 250 – 300 mg/dL are not associated with adverse side effects.

5.3.1 LO(A)EL > 300 mg bromide/dL (serum concentration)

5.3.2 NO(A)EL 300 mg bromide/dL (serum concentration) corresponding to about 240 mg/kg bw/day assuming a body weight of 25 kg/dog and a (total) blood volume of about 2 L.

3.9.3.6 [Study 6] 4 or 12 weeks feeding study of sodium bromide in rat to investigate alterations in the endocrine system

Study reference:

A6.10/09; Doc. No. 592-036

Loeber J. G., Franken M. A. M. and van Leeuwen F. X. R. (1983). Effect of Sodium Bromide on Endocrine Parameters in the Rat as Studied by Immunocytochemistry and Radioimmunoassay. Fd. Chem. Toxic. 21 (4), 391-404.

3.9.3.7 [Study 7] 4 weeks dietary study of sodium bromide in rat to investigate effects on the thyroid on low iodine diet

Reference	A6.10/14; Doc. No. 592-041 Buchberger W., Holler W. and Winsauer K. (1990). Effects of Sodium Bromide on the Biosynthesis of Thyroid Hormones and Brominated/Iodinated Thyronines. J. Trace Elem. Electrolytes Health Dis 4 (1), 25-30.
Guideline	Guideline study No Study is a publication and there is no guideline available for this special mechanistic investigation GLP No Study is a publication and was performed according to good experimental practice. Deviations Not applicable; no guideline available for this special mechanistic investigation
Reliability	2
Species / strain	Species Rat Strain Sprague-Dawley [Crl: CD (SD) BR] Sex males and females

Test material	Sodium bromide
	Purity Not examined
	Stability Not examined
Study design	3.2 Test Animals
	Age/weight at study initiation
	Animals were approximately 11 weeks of age
	Number of animals 12/sex/group
	Control animals Yes
	3.3 Administration/
	Exposure: Oral In food
	Duration of treatment: 4 weeks
	Frequency of exposure: daily
	Postexposure period: No postexposure period; animals were sacrificed at termination of the treatment period
	Concentration food: 4, 8 and 16 g/kg (corresponding to 200, 400 and 800 mg/kg bw/day with 1 ppm = 0.05 mg/kg bw/day for older rats)
	Vehicle No vehicle used, test substance was mixed with the diet
	Controls Two control groups receiving either plain diet or low iodine diet
	3.4 Examinations
	Observations
	Clinical signs Yes; twice daily
	Mortality Yes
	Body weight Yes; weekly
	Food consumption Yes; weekly
	Water consumption No; ad libitum
	Ophthalmoscopic examination: No
	Haematology No
	Clinical Chemisty Yes; blood samples were taken on Day 1, 14, 22, 29, 36 and 43 and analysed for bromide and iodide content and T4 and TSH levels. No other clinicalchemical parameters were investigated.
	Urinalysis Yes; urine samples were taken on Day 1, 14, 22, 29, 36 and 43 and analyzed
	for iodide content.
	3.5 Sacrifice and pathology
	Organ Weights Yes; Thyroid and parathyroid weights were taken only
	Gross and histopathology: No
	Other examinations For determination of bromo- and iodosubstituted thyronines in the thyroid these compounds were quantitatively released from thyroglobulin by digestion of the tissue with pronase-E and subsequently analyzed by HPLC combined with UV detection at 230 nm and with off-line radioimmunoassay detection.
	3.5.4 Statistics Statistics were performed with the Kruskal- Wallis test for differences between more than two groups of non-normally distributed data.
	3.6 Further remarks The animals underwent a 2-week pre-treatment period and a 4-week treatment period. During these periods animals were fed either a normal laboratory rodent diet or a low iodine

diet with or without varying amounts of sodium bromide. Total T4 and free T4 in serum were measured by radioimmunoassay. Thyroid stimulating hormone (TSH) in serum was measured by an immunoradiometric assay.

Findings

4.1 Observations

4.1.1 Clinical signs For animals in group 5 (low-iodine diet, 16 g sodium bromide/kg) hypoactivity, ruffled fur and emaciation were noticed in Week 3. From Week 4, hypoactivity was found in group 4 (low-iodine diet, 8 g/kg of sodium bromide). No abnormal clinical signs were observed in groups 1-3.

4.1.2 Mortality All animals of the highest dosage group (16 g/kg) fed a low-iodine diet, were either found dead or had to be sacrificed because of poor general appearance. Most of the animals of this group were dead on Day 37 of the investigation. From the 8 g/kg group, one male was found dead on Day 33 and another male was dead by Day 42 of the study.

4.2 Body weight gain Analysis of bodyweight showed dramatically reduced bodyweights for the animals receiving 16 g sodium bromide/kg from Day 29 onwards. Bodyweight gain in group 4 (8 g/kg) was also reduced from Day 29 on. Animals receiving the lowest sodium bromide concentration used (4 g/kg) had bodyweights comparable to the control groups (normal diet and low-iodine diet).

4.3 Food consumption and compound intake

Food consumption was reduced in animals treated with 8 and 16 g/kg.

4.4 Ophtalmoscopic examination

No ophthalmoscopic examination was performed in this investigation.

4.5 Blood analysis

4.5.1 Haematology No haematological analysis was performed in this investigation.

4.5.2 Clinical chemistry For determination of bromide, total T4, free T4 and TSH in serum the samples of four animals of each cage were pooled. No differences in the results of both sexes could be detected, so the data were combined. With increasing serum bromide concentrations free T4 and total T4 levels decreased in all treatment groups. The higher the sodium bromide concentration in food, the higher was the observed serum bromide level and the lower the T4 levels. Group one, which was fed normal rodent diet, had higher serum bromide concentrations and higher T4 levels than the animals treated with low-iodine diet but without sodium bromide.

4.5.3 Urinalysis Urine was analyzed for iodide content. There were no differences found between male and female animals and for that reason, the data were combined for both sexes. The results show iodine deficiency for all animals receiving the iodine-poor diet. Between the groups of animals treated with different amounts of sodium bromide, there were no differences observed.

4.6 Sacrifice and pathology

4.6.1 Organ weights Thyroid weights were increasing with increasing sodium bromide content in food with exception of the highest dosage group which showed organ weights being lower than the ones from the lowest treatment group. Thyroid weights of animals fed iodine-poor diet were higher than the ones from the animals receiving the normal rodent diet.

4.6.2 Gross and histopathology

No gross and histopathology was performed in this investigation.

4.7 Other The concentrations of T4, T3 and 3,3´,5´-triiodothyronine (reverse T3) in the thyroid gland decreased with increasing bromide concentrations and were lower in animals fed the low-iodine diet compared to the animals receiving the normal rodent diet (control). T3, reverse T3 and T4 levels decreased from 19.6, 5.5 and 143 from control to <2 for all parameters in bromide treated animals respectively. Trisubstituted bromo/iodothyronines were detected in the thyroids of all groups treated with bromide, but not in groups one and two fed a normal or iodine-poor diet. The amount of these bromo/iodosubstituted thyronines was at least one order of magnitude lower than that of the thyroid hormone T3 in normal rats.

Conclusions

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The investigation was performed to study the influence of bromide on the thyroid of rats, paying special attention to the possibility of biosynthesis of brominated thyroid hormone analogues. Bromide doses were chosen where effects on the thyroid were to be expected. Animals were treated with 4-16 g sodium bromide/kg diet for four weeks (corresponding to 200, 400 and 800 mg/kg bw/day). Rats were separated into two groups; one group receiving the normal rodent diet and the other group was fed a low iodine diet. Within these two groups animals were given varying amounts of sodium bromide within the diet.

At Day 1, 14, 22, 29, 36 and 43 urine samples were collected and analyzed for iodide content. On the same days, blood samples were taken and analyzed for bromide content; in addition total and free T4 and TSH levels were determined in serum.

At the end of the treatment period all rats were sacrificed, thyroid glands and parathyroid glands removed and organ weights taken. Bromo- and iodosubstituted thyronines were determined.

5.2 Results and discussion

All animals of the highest dosage group (16 g/kg) fed a low-iodine diet, were either found dead or had to be sacrificed because of poor general appearance.

Most of the animals of this group were dead on Day 37 of the investigation. From the 8 g/kg group, one male was found dead on Day 33 and another male was dead by Day 42 of the study. There were no premature decedents within the animals receiving either the standard diet or the low-iodine diet or the iodine-poor diet in combination with the lowest amount of sodium bromide used (4 g/kg).

For animals in group 5 (low-iodine diet, 16 g sodium bromide/kg) hypoactivity, ruffled fur and emaciation were noticed in Week 3. From Week 4, hypoactivity was found in group 4 (low-iodine diet, 8 g/kg of sodium bromide). No abnormal clinical signs were observed in groups 1-3.

Animals in the highest dosage group (16 g/kg) had dramatically reduced bodyweights from Day 29 onwards. Also the animals treated with 8 g/kg showed reduced bodyweight gain from Day 29 on. The bodyweights from the lowest treatment group were comparable to control groups.

Food consumption was reduced in the 16 and 8 g/kg groups. The actual iodine intake was checked by determining the urinary iodide secretion. There were no differences for male and female animals; for this reason, the data of both sexes were combined. The results obtained show a iodine deficiency for all animals receiving the low-iodine diet. The values between the different sodium bromide concentrations did not show differences in iodide excretion.

For determination of bromide, total T4, free T4 and TSH in serum, the samples of four animals of each cage were pooled. Since no differences in the results of both sexes could be detected, the data were combined. With increasing serum bromide concentrations free T4 and total T4 levels decreased in all treatment groups. The higher the sodium bromide concentration in food, the higher was the observed serum bromide level and the lower the T4 levels. Group one, which was fed normal rodent diet, had higher serum bromide concentrations and higher T4 levels than the animals treated with low-iodine diet but without sodium bromide. The differences in the concentrations of free T4 of the iodinedeficient groups were statistically highly significant ($p < 0.001$) on Day 22, 29, 36 and 43.

The wet weights of the thyroid glands at the end of the study showed increasing absolute and relative organ weights with increasing sodium bromide concentrations in food. Exception of this rule were the animals receiving the highest sodium bromide amount; absolute and relative thyroid weights were lower than from the lowest treatment group (4 g/kg). Thyroid weights of animals fed iodine-poor diet were higher than the ones from the animals receiving the normal rodent diet.

The concentrations of T4, T3 and 3,3′,5′-triiodothyronine (reverse T3) in the thyroid gland decreased with increasing bromide concentrations and were lower in animals fed the low-iodine diet compared to the animals receiving the normal rodent diet (control). Trisubstituted bromo/iodothyronines were detected in the thyroids of all groups treated with bromide, but not in groups one and two fed a normal or iodine-poor diet. The amount of these bromo/iodosubstituted thyronines was at least one order of magnitude lower than that of the thyroid hormone T3 in normal rats.

5.3 Conclusion All animals fed with an iodine-poor diet were in a state of hypothyroidism (decrease of total and free T4 and increase of TSH in blood). This was further enhanced by intake of sodium bromide. Effects on thyroid hormone and bromide levels in serum followed a dose-response

relationship. Based on the findings made, a NOAEL could not be derived in this study.

3.9.3.8 [Study 8] 2 weeks dietary study in rat to investigate the effect of sodium bromide on thyroid function

Reference	A6.10/15; 592-042
	Van Leeuwen F. X. R., Hanemaaijer R. and Loeber J. G. (1988). The Effect of Sodium Bromide on Thyroid Function, The Target Organ and the Toxic Process. Arch. Toxicol. Suppl. 12, 93-97.
Guideline	Guideline study No; , no guideline available for this special investigation
	GLP No
	Study is a publication and was performed according to good experimental practice.
	Deviations Not applicable, no guideline available for this special investigation
Reliability	2
Species / strain	Species Rat
	Strain Wistar
	Sex male
Test material	Sodium bromide
	Purity Not examined
	Stability Not examined
Study design	3 MATERIALS AND METHODS
	3.2 Test Animals
	3.2.5 Age/weight at study initiation: Animals were within a weight range of 200-300 g. Age not indicated, based on the weight animals should have been about 8-10 weeks of age.
	3.2.6 Number of animals per group: 8/group
	3.2.7 Control animals Yes
	3.3 Administration/
	Exposure: Oral in food
	3.3.1 Duration of treatment 14 days
	3.3.2 Frequency of exposure daily
	3.3.3 Postexposure period: No postexposure period; animals were sacrificed at the end of the treatment period.
	3.3.4.2 Concentration Food: 19 g NaBr/kg (corresponding to 950 mg/kg bw/day using a default conversion of 1 ppm = 0.05 mg/kg bw for older rats)
	3.3.4.3 Vehicle No vehicle used; test substance was mixed with diet
	3.3.4.6 Controls Plain diet and 11 g NaCl/kg in diet
	3.4 Examinations
	3.4.1 Observations
	3.4.1.1 Clinical signs No
	3.4.1.2 Mortality No
	3.4.2 Body weight Yes; at the and of the investigation

3.4.3 Food consumption No; food was administered ad libitum

3.4.4 Water consumption No

3.4.5 Ophthalmoscopic examination: No

3.4.6 Haematology No

3.4.7 Clinical Chemisty Yes; T4 and TSH levels were determined in serum; no other clinical chemical parameters were investigated

3.4.8 Urinalysis No

3.5 Sacrifice and pathology

3.5.1 Organ Weights Yes; thyroid weights were taken only.

3.5.2 Gross and histopathology: No

3.5.3 Other examinations Thyroid peroxidase (TPO) activities were determined. Thyroids were homogenized in 50 mM phosphate buffer, pH 7.4 (4 ml/g tissue). Homogenates were centrifuged (15 min, 1000 g) and the supernatant diluted 10-times in buffer. TPO activity was determined using iodide as substrate. The incubation mixture consisted of 50 mM phosphate buffer, pH 7.4; 0.135 mM H2O2, 5 mM KI and 50 µl diluted thyroid homogenate in a total volume of 1 ml. tHe reatio was followed spectrophotometrically at 353 nm.

For determination of peroxidise activity, guaiacol as used as substrate. Final concentrations in the incubation mixture were: 50 mM phosphate buffer, pH 7.4, 0.27 mM H2O2, 30 mM guaiacol and 50 µl diluted homogenate in a total volume of 1 ml. The oxidation of guaiaol was followed at 470 nm.

The activity of NADH and NADPH was determined using the following assay conditions: 80 mM phosphate buffer, pH 7.6, 6.07 mM sodium azide, 50 µl diluted homogenate and 100 nM NADH/25 nM cytochrome c or 100 nM NADPH/100 nM cytochrome c. Cytochrome c reduction was monitored at 550 nm.

Additionally, animals held on the same regimen were intubated after two weeks with 0.25 ml phosphate buffered saline (0.1 mM, pH 7.2) containing 28 µg NaI/mL and 2.4 µCi 125-I/mL. After 24 hours the animals were desanguinated and the thyroid glands were excised for the determination of 125-I with a gamma-scintillation counter.

3.5.4 Statistics No

Findings

4.1 Observations

4.1.1 Clinical signs No clinical signs were reported within this investigation.

4.1.2 Mortality There were no premature decedents during the treatment period.

4.2 Body weight gain Bodyweight of animals treated with sodium bromide (19 g/kg = 950 mg/kg bw/day) was lower than in the controls.

4.3 Food consumption and compound intake: Food was administered ad libitum.

4.4 Ophtalmoscopic examination: No ophthalmoscopic examination was performed in this investigation.

4.5 Blood analysis

4.5.1 Haematology No haematological examination was performed in this investigation.

4.5.2 Clinical chemistry In the serum of NaBr-treated rats the concentration of T4 was lower and that of TSH higher than in the serum of control animals. No other clinical chemical parameters were examined in this investigation.

4.5.3 Urinalysis No urinalysis was performed in this investigation.

4.6 Sacrifice and pathology

4.6.1 Organ weights The absolute and relative weight of the thyroid gland was significantly higher in animals treated with sodium bromide compared to controls. No further organ weight determinations were performed.

4.6.2 Gross and histopathology: No gross and histopathological examinations was performed in this

investigation.

4.7 Other I-TPO activity was strongly decreased in NaBr-treated rats compared to control animals, but also the guaiacol-TPO activity was significantly lower.

Hardly any effect was observed in the activity of NADPH cytochrome c reductase. However, both NaCl and NaBr induced an increase in activity of NADH cytochrome c reductase, with NaBr showing the strongest effect.

Conclusions 5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The investigation was performed to clarify the mechanism of action of bromide ion on the thyroid gland. Furthermore, the effects of bromide on active uptake of iodide by the thyroid gland, oxidation of iodide by the thyroid peroxidase enzyme and bromide effects on thyroid hormone biosynthesis were investigated.

Male rats were fed a diet containing 19 g NaBr/kg (corresponding to about 950 mg/kg bw/day based on a default conversion of 1 ppm = 0.05 mg/kg bw/day for older rats) or 11 g NaCl/kg for two weeks.

Animals were sacrificed after the treatment period and blood was colleted for determination of T4 and TSH levels. Thyroid glands were weighed and homogenized.

Thyroid peroxidise (TPO) activities, peroxidise activity and activity of NADH and NADPH were determined. Additionally, animals held on the same regimen were intubated after two weeks with phosphate buffered saline containing 28 µg NaI/mL and 2.4 µCi 125-I/mL. After 24 hours the animals were desanguinated and the thyroid glands were excised for the determination of 125-I with a gamma-scintillation counter.

5.2 Results and discussion

After two weeks feeding of a NaBr-containing diet, bodyweight of animals was lower than in the controls, and the absolute and relative weight of the thyroid gland was significantly higher. In the serum of these rats the concentration of T4 was lower and that of TSH higher than in the serum of control animals.

These findings are in accordance with the earlier findings pointing to a disturbance of thyroid hormone biosynthesis, consequently followed by a compensatory increase in TSH. In addition to this, in NaBr-treated animals the uptake of 125-I by the thyroid gland was significantly lower than in control animals.

Surprisingly, the animals fed an equimolar dose of NaCl also showed an increase in absolute and relative thyroid weight. However, no alterations were found in the concentration of T4 and TSH. So far, the origin of this latter effect is unknown.

NaBr caused a marked effect on the activity of thyroid peroxidase. Particularly the I-TPO activity was strongly decreased compared to control animals, but also the guaiacol-TPO activity was significantly lower. NaCl treatment did not cause any effect on peroxidise activity. I-TPO activity might reflect the oxidase potential of thyroid peroxidise and the ability to incorporate iodine in tyrosine residues, whereas the guaiacol-TPO reaction reflects the activity of the coupling reaction of tyrosine residues in thyroglobulin by peroxidise. Therefore, it can be hypothesized that bromide strongly inhibits the oxidation of iodide into iodine by H2O2, and to a lesser extent inhibits the coupling of iodinated tyrosine residues to thyronines. This inhibition of peroxidise activity, combined with the earlier mentioned reduction in iodide uptake by the thyroid

3.9.3.9 [Study 9] 36 days study of sodium bromide to investigate effects of bromide on behaviour of mice

Reference A6.9.2/01, Doc. 592-016

Hansen K. and Hübner H. (1983). Effects of Bromide on Behaviour of Mice. Fd. Chem. Toxic. Vol.

	21, no. 4, pp. 405-408.
Guideline	2.1 Guideline study No guideline available for the special type of investigations performed; the study was performed according to good experimental practice
	2.2 GLP No; GLP was not mandatory at the time of study conduct
	2.3 Deviations Not applicable; there is no guideline available for the special type of investigations performed and the study was performed according to good experimental practice
Reliability	2
Species / strain	Species Mouse
	Strain NMRI
	Sex male
Test material	Sodium bromide
	Purity 99.5%
	Stability Not examined
Study design	3 MATERIALS AND METHODS
	3.3 Test Animals
	3.3.6 Age/weight at study initiation: Not indicated; according to the descriptions in the study, adolescent animals were used as treatment started on day 43.
	3.3.7 Number of animals per group Not indicated
	3.3.8 Control animals Yes
	3.4 Administration orally with the diet
	3.4.1 Exposure 36 days (Day 43-78 of the experiment)
	3.4.2 Dose Levels 400, 1200, 3600 and 10.800 ppm (corresponding to 60, 180, 540 and 1620 mg bromide/kg bw/day with 1 ppm = 0.15 mg/kg bw/day)
	3.4.3 Vehicle Test substance was dissolved in water, then mixed with the diet
	3.4.4 Concentration in vehicle: Not applicable; substance was irrigated with water and administered with the diet
	3.4.5 Total volume applied: Not applicable; substance was irrigated with water and administered with the diet
	3.4.6 Postexposure period 50 days
	3.4.7 Anticholinergic substances used: Not applicable, no testing of organophosphates or carbamates
	3.4.8 Controls Plain diet
	3.5 Examinations
	3.5.1 Body Weight Yes; daily
	3.5.2 Signs of Toxicity Yes; daily
	3.5.3 Observation schedule: Daily observation for 128 days, with application of test substance during Days 43-78.
	3.5.4 Clinical Chemistry Not performed
	3.5.5 Pathology Not performed
	3.5.6 Histopathology Not performed
	3.6 Further remarks Measurement of activity/inactivity: The equipment used to measure spontaneous motility recorded movements simultaneously for a maximum of 120 individual caged mice. Each cage was controlled by four infra-red light barriers placed independently in the cage. Light transmitters and receivers were arranged with part of the electronics on printed circuits so that normal

animal maintenance and grooming was possible without restriction. The light barriers of the system worked with short light impulses, thus producing a substantially higher light intensity to prevent failures in measurement caused by dust and dirt and to reduce the expenditure on electronics. The frequency of the impulse was fixed so that interruptions of the light barriers were detectable if their duration is not less than 10 msec. Since the signals of the light barriers had to indicate motility, their outputs were logically connected. This was established by counting an impulse of a particular light barrier only if another light barrier has detected a signal before. Thus all movements of an animal that were not motility were ignored. If the signals met this pre-condition, they were registered on a data file.

From the values obtained in the experiment the following variables were processed:

(1) Variables 0-3: The impulses from measurement positions 0-3 totalled over 12 hours

(2) Variables 4-7: The numbers of periods of 5 minutes in which any movement occurs over 12 hours
(3) Variables 8-10: In Sequence, the evasion time, the spontaneous performance on the treadmill in 10 minutes, and the bodyweight as a general basic value.

The evasion time was the time an animal needed to leave a small isolated area where it had been placed. The running behaviour on the treadmill was measured by counting the revolutions of the wheel in 10 minutes.

Statistical analysis was mainly accomplished by multivariante analysis of variance and additionally by univariante analysis of variance. The multivariance was chosen because a mulitidimensional approach to the variables better illustrates their independency.

Findings

4.1 Body Weight There was a decrease in bodyweight in the highest dosage group with a massive drop in bodyweight during the first days of sodium bromide administration. After feeding the plain diet again from Day 78 on, bodyweight steadily increased until the end of the experiment. After

treatment with up to 3600 ppm sodium bromide only small differences in bodyweight compared to control were observed, the control rats exhibiting the highest bodyweight in each case.

4.2 Clinical signs of toxicity

There were no clinical signs other than the changes in activity and bodyweight gain described.

4.3 Clinical Chemistry No clinical chemistry was performed in this investigation.

4.4 Pathology No pathological examination was performed in this investigation.

4.5 Histopathology No histopathology was performed in this investigation.

4.6 Other Measurement of activity/inactivity:

Evaluation of activity measurement revealed that the two lowest dosage groups (400 and 1200 ppm) reacted like the control group, showing two characteristic periods of higher activity each day between 7.30 and 8 am and 4-6 pm. The 3600 ppm-group took a similar course, but the first period was elevated in magnitude and prolonged. In the group of the 10.800 ppm diet, this typical behaviour was absent. The animals moved more continuously, with noticeable resting time, but the total amount of movement was lower. The motility in the two highest dosage groups rose after the beginning of sodium bromide administration, passed a maximum and developed a plateau. A sudden decrease in motility followed the end of treatment but the group on the highest dosage did not return to the original level of activity. The two lower dosages did not have visible effects. Examination of the influence of bromide on the evasion time showed that at all dose levels used, sodium bromide caused a decrease in evasion time. The high-dose group show the most obvious change. An effect on behaviour on the treadmill was evident only in the group on the 10.800 ppm diet. Two marked peaks can be seen after the beginning and the end of bromide administration.

Multivariance analysis of results: The effects of the lowest dose level (400 ppm) were not significant. The significance of the effects of 1200 ppm sodium bromide seen in only some variables could be established by this method, and demonstrates that the “effect limit” based on behavioural variables and bodyweight lies between 400 and 1200 ppm in mice. It can also be seen that the effects of the 10.800 ppm diet are not completely reversible, but on the basis of the results of the statistics, this seems to be largely due to the influence of the retardation in bodyweight.

Conclusions

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In human bromide intoxication, mental and neurological disturbances are the most common and prominent features. There is no objective and quantitative method for recording these effects in standard toxicological feeding studies. It may be possible to observe this type of effects but only at doses that are far from correlating with the therapeutic level in man. But only low doses are of interest in making decisions relating to residue levels.

In the present investigation, bromide orally administered in the form of its sodium salt was used to look at the specific effects of the substance and to test the basic usefulness of the new method used. One aim was therefore to record, in feeding experiments, signs interpretable as indicators of mental or neurological effects. For this purpose, the activity and times of inactivity of the mice during the night were recorded. Further methods were used to obtain correlates for mental or neurological disturbances in the daytime.

The total duration of the experiment was 128 days, the test diets of 0, 400, 1200, 3600 and 10.800 mg sodium bromide/kg diet being administered during Days 43-78 (corresponding to 60, 180, 540 and 1620 mg bromide/kg bw/day with 1 ppm = 0.15 mg/kg bw/day) followed by an exposure-free period of 50 days.

5.2 Results and discussion

The experiments were performed in order to investigate whether behavioural variables are applicable in a standard feeding study. For this purpose it was necessary to be able to quantify these variables. An exact description and performance of an adequate measuring technique that can produce processable data was therefore required. One major consideration was the behavioural characteristics typical of each single animal. The measuring technique used did not make allowance for all difficult aspects (individual and sensitive reaction of the single animal and behavioural manifestations). The substance-related aspects found were therefore interpreted as bein in a way equivalent to mental and neurological disturbances. The reduction in evasion time and the disturbance of the normal nocturnal rhythm of motility indicate a disinhibition. The obvious difference between the two highest dosage groups shows another aspect of bromide intoxication. On the basis on previous results, a comparison of the effects found in the present study with those known to occur in man is made possible by a consideration of the concentrations in the experimental diets. The dietary level of chloride of about 0.8 g/kg (van Logten et al, 1976; please refer to Document IIIA, Section 6.4.1/05, Doc. No. 592-006)correspond to that of the present mice diets. If uptake and resorption of bromide ions are the same in rats and mice, it can be assumed that in mice between 400 and 1200 ppm NaBr would therefore correspond to plasma levels of about 200-500 mg bromide/L on the basis of the results obtained in rats by van Logten (please refer to Document IIIA, Section 6.4.1/05).

Treatment with 1263.6 mg bromide/kg bw resulted in marked effects on bodyweight and behaviour of mice which were not completely reversible. Lower dose levels of sodium bromide used also resulted in a decreased bodyweight and changes in behaviour, like decreased evasion time, but the effects were gone after sodium bromide was withdrawn from the diet. After treatment with 46.8 mg bromide/kg bw no significant effects were observed. Other investigations performed in rats had shown that concentrations of 78 mg bromide/kg bw/day do neither produce clinical signs nor effects on the thyroid gland. The group treated at the next higher dose level applied in this investigation showed effects on bodyweight and behaviour. Since this concentration was 9-times the no effect level no shorter perimeter of bromide concentrations which already have an effect and the ones which still do not affect the animal can be made.

5.3 Conclusion With the method used in this investigation, behavioural effects in mice have been shown to correspond with findings in man. The effects on bodyweight and behaviour after treatment with up to 3600 ppm (corresponding to 421.2 mg bromide/kg bw/day) were completely reversible after sodium bromide was withdrawn from the diet.

Treatment with 10800 ppm (corresponding to 1263.6 mg/kg bw/day) resulted in irreversible effects on bodyweight and behaviour. Taking into account the behavioural effects observed the NOAEL of this study is lying somewhere between 400 and 1200 ppm. Using a conservative approach the NOAEL is derived at the lowest dose level of 400 ppm corresponding to 60 mg/kg bw/day.

5.3.1 LOAEL 1200 ppm (corresponding to 180 mg bromide/kg bw/day)

5.3.2 NOAEL 400 ppm (corresponding to 60 mg bromide/kg bw/day)

3.9.3.10 [Study 10] Prenatal dev elopmental toxicity study to investigate the effect of the administration of sodium bromide to pregnant rats on the learning ability of the offspring

Reference

A6.9.2/02, Doc. No. 592-017

Harned B. K., Hamilton H. C. and Cole V. V. (1944). The Effect of the Administration of Sodium Bromide to Pregnant Rats on the Learning Ability of the Offspring, II. Maze Test. Journal of Pharmacology and Experimental Therapeutics, Vol 82, Issue 3, 215-226.

Guideline

2.1 Guideline study: No guideline available for the special type of investigations performed; the study was performed according to good experimental practice

2.2 GLP: No; GLP was not mandatory at the time of study conduct

2.3 Deviations: Not applicable; there is no guideline available for the special type of investigations performed and the study was performed according to good experimental practice

Reliability

2

Species / strain

3.3.1 Species: Rat

3.3.2 Strain: Wistar

3.3.4 Sex: male and female

Test material

Sodium bromide

Study design

3.3 Test Animals

3.3.6 Age/weight at study initiation: Not indicated; according to the descriptions in the study, adult rats were used as treatment occurred during gestation.

3.3.7 Number of animals per group: Investigation was focused on the offspring, treatment was performed on the dams:

Treatment of dams [mg NaBr/kg bw/day]	No. of offspring investigated	
	male	female
120	16	18
80	18	12
40	20	13
0	17	13

3.3.8 Control animals Yes

3.4 Administration oral by gavage

3.4.1 Exposure Oral administration once daily from Day 3 to Day 20 of gestation

3.4.2 Dose Levels 40, 80 and 120 mg/kg bw/day

3.4.3 Vehicle water

3.4.4 Concentration in vehicle: Not indicated

3.4.5 Total volume applied: Not indicated

3.4.6 Postexposure period Approximately 85 days after pups were born.

3.4.7 Controls Vehicle

3.5 Examinations

3.5.1 Body Weight Yes; in frequent intervals

3.5.2 Signs of Toxicity Yes

3.5.3 Observation schedule: daily observation

3.5.4 Clinical Chemistry Yes;

Blood bromide levels were determined in newborn and at Day 62 after birth. No other clinical-chemical examinations were performed.

3.5.5 Pathology Not performed

3.5.6 Histopathology Not performed

3.6 Further remarks From the age of 57-60 days the rats were prepared for learning in the maze. From the age of 61-85 days each animal was given two trials per day in a five cul-de-sac u-maze.

Bromide concentration was determined in addition to blood in dry tissue.

Findings

4.1 Body Weight Bodyweight analyses of pregnant rats showed that the group treated at 40 mg/kg bw/day was heavier than the control group and the two higher dosage groups were heavier than the 40 mg group. When animals were allowed only 15 minutes of feeding, they all lost weight and put on

weight again when feeding time was extended. This weight loss showed the same relationship among all groups whereas for the 80 and 120 mg groups the weight gain was more slowly compared to the lowest dosage group. All treated animals were heavier at the end of the investigation compared to controls. Pups from the groups treated at 80 and 120 mg/kg bw/day, the growth was subnormal for a few days, but this handicap was temporary and of a minor order. Weight curves of these animals reveal no evidence of any physical handicap.

4.2 Clinical signs of toxicity

Pregnant bromide treated rats offered less resistance to the passage of the stomach tube than did the normal rats, but no evidence of depression could be distinguished. The mortality among the newborns was high and paralleled the doses given the mothers. Pups that died before 20 days of age were 2.3%, 27%, 42% and 58% from animals treated at 0, 40, 80 and 120 mg/kg bw/day, respectively (please refer to table 6.9.2/02-3).

4.3 Clinical Chemistry Multiplying the relative concentrations of blood bromide levels by the mean value in the control group (3.8 mg/100 ml), one calculates that at parturition the values for bromide in the blood serum of the mothers were 152 mg%, 80 mg% and 42 mg% for animals treated at 120, 80 and 40 mg/kg bw/day based on the ratios of bromide content of the control and of the experimental groups in dried tissues which were 1:40 for the group treated at 120 mg/kg bw/day, 1:21 for the 80 mg/kg bw/day group and 1:11 for the 40 mg/kg bw/day group, respectively (please refer to table 6.9.2/02-2). Serum bromide levels of pups from bromide treated dams at Day 62 of age revealed bromide concentrations being all in the same order of magnitude which were approximately 1 mg/100 ml less than that of the control group.

Newborn rats were analysed for bromide concentration 4 days after birth. These analyses showed that the bromide concentrations in the treated groups bore approximately the same quantitative relationship to each other as the dosages administered to the mothers (please refer to table 6.9.2/02-1).

4.4 Pathology No pathological examination was performed in this investigation.

4.5 Histopathology No histopathological examination was performed in this investigation

4.6 Other

Bromide concentration:

Bromide determination in dried tissues revealed that the ratios of bromide content of the control and

of the experimental groups are 1:40 for the group treated at 120 mg/kg bw/day, 1:21 for the 80 mg/kg bw/day group and 1:11 for the 40 mg/kg bw/day group.

Newborn rats were analysed for bromide concentration 4 days after birth. These analyses showed that the bromide concentrations in the treated groups bore approximately the same quantitative relationship to each other as the dosages administered to the mothers.

Learning in the maze:

The criterion of errors shows a positive relationship between the number of errors and the strength of the dosage. The group at the highest dose level (120 mg/kg bw/day) made a significantly greater number of errors than each of the other groups. The performance of the animals treated at 80 mg/kg bw/day was reliably worse than the one from the control group. Other differences were not significant (please refer to table 6.9.2/02-4).

The criterion of time shows that the 120 mg/kg bw/day group was virtually significantly slower than each of the other groups, but the other groups do not differ among themselves.

The error and time curves show that all groups reached essentially the same level of performance before the end of the run. The standard deviations of the groups show, in general, an increase with increasing dosage. The standard deviations computed on the time scores show the same relationship as those for error scores; moreover, all groups differ significantly from one another, with the exception of the 80 and 40 mg/kg bw/day groups.

Conclusions 5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The purpose of the investigation was to study the effects of prenatal administration of sodium bromide to rats by means of tests designed to detect functional damage in the central nervous system of the offspring by studying the learning ability after birth.

Pregnant rats were treated at 40, 80 or 120 mg NaBr/kg bw/day from Day 3 to 20 of gestation. Pups born on Day 22 received bromide only by the milk of their mothers and were weaned until 20 days of age. From Day 20-34 after birth, the drinking water was replaced by a 0.2% solution of sodium chloride, and from 35 days of age on, pups received a 0.5% NaCl solution. Blood bromide concentration in dams, newborns (Day 4 after birth) and pups being 62 days old, was determined, as well as bromide content in dried tissues of the newborn rats. From the age of 57-60 days the rats we re prepared for learning in the maze. From the age of 61-85 days each animal was given two trials per day in a five cul-de-sac u-maze.

5.2 Results and discussion

The reduced serum bromide levels observed in the pups from bromidetreated dams at 62 days of age compared to control rats (approximately 1 mg/100 ml less than in controls) might be explained by a higher consumption of drinking water, which was supplemented with 0.2% and 0.5% NaCl from Day 20 and 35 on, respectively and was available ad libitum. Chloride at higher concentration is known to accelerate the elimination of bromide.

The criterion of errors in the maze learning experiment shows a positive relationship between the number of errors and the strength of the bromide-dosage. The criterion of time shows that the highest dosage group (120 mg/kg bw/day) was significantly slower than each of the other groups but the other groups did not differ among themselves. All groups reached the same level of performance before the 25th day of the test. This fact suggests that in the maze test the deleterious effects of bromide appear in the rate of learning rather than in the performance finally attained.

Previous studies had shown that there is a relationship between the amount of intact cerebral area and the maze learning. Correlation coefficients of 0.3-0.86 were reported most of them from 0.8 to 0.86, between the size of the cerebral lesion and the errors in maze tests.

Another publication reported a higher degree of relationship between the magnitude of the cerebral lesions and the errors in maze learning. This would appear to be provided with the assumption that sodium bromide would interfere with the development of the central nervous system.

5.3 Conclusion The criterion of errors in the maze learning experiment shows a positive relationship between the number of errors and the strength of the bromide-dosage. The criterion of time shows that the highest dosage group (120 mg/kg bw/day) was significantly slower than each of the other groups. Since all groups reached the same level of performance before the 25th day of the test, it can

be suggested that in the maze test the effects of bromide appear in the rate of learning rather than in the performance finally attained. The results of this study demonstrate also a dose-related post-natal pup mortality.

5.3.1 LOAEL 80 mg/kg bw/day (corresponding to 62.4 mg bromide/kg bw/day), since these animals made a significantly greater number of errors than each of the other groups.

5.3.2 NOAEL 40 mg/kg bw/day (corresponding to 31.1 mg bromide/kg bw/day) based on the significantly greater number of errors made by the animals on the next higher dose level

3.9.3.11 [Study 11] Neurotoxicity: Long-lasting microinfusion into the superior cervical ganglion of adult rats

Reference	Joo, F., Dames W., Wolff J. R. (1979). Effect of Prolonged Sodium Bromide Administration on the Fine Structure of Dendrites in the Superior Cervical Ganglion of Adult Rat. Progress in Brain Research, Vol. 51, pp.109-115.
Guideline	No guideline, no GLP
Reliability	2 (reliable with restrictions) according to Registrant(s)
Species / strain	Species:rat Strain:Sprague-Dawley Sex:male/female
Test material	Sodium bromide
Study design	Route of administration:other: Microinfusion Vehicle:other: ethanol (0.2 %) and distilled water Duration of treatment / exposure:Exposure period: 30 minute(s) Frequency of treatment:Single exposure Doses / concentrations: 10 µL of 500 mM solution No. of animals per sex per dose:10 Control animals were given mock cerebrospinal fluid Observation period: 1 month
Findings	Axons and synapsing terminals were frequently seen in the experimental as in control ganglia. After sodium bromide treatment, plastic changes were seen in dendrites which were similar in their main characteristic to those described for GABA. These consisted mostly of the formation of non-inervated post synaptic thickenings, accumulation of microvesicles and changes in shape of dendrites.

3.9.3.12 [Study 12] Neurotoxicity: Sodium bromide treatment of murine neuroblastoma cells in vitro

Reference	Spoerri P. E. and Wolff J. R. (1982). Morphological Changes Induced by Sodium Bromide in Murine Neuroblastoma Cells in vitro. Cell Tissue Res, 222:379-388.
Guideline	No guideline, no GLP
Reliability	2 (reliable with restrictions) according to Registrant(s)
Species / strain	murine C1300 neuroblastoma cells

Test material	Sodium bromide
Study design	Exposure period: 2 h to 10 days Vehicle:Eagle's minimum essential medium supplemented with 10% calf serum Doses / concentrations: 0, 10-4, 10-5 or 10-6 M sodium bromide Control:yes, concurrent vehicle
Findings	Sodium bromide was applied in vitro to mouse neuroblastoma cells of different ages for short and long periods (2 h to 10 days). The changes observed light-and-electron microscopically were similar to those described earlier after GABA treatment. Coated vesicles proliferated and originated by pinching off from the Golgi complex and from the rough endoplasmic reticulum. Numerous coated vesicles were continuous with the plasma membrane, especially near zones in which electron-dense material aggregated at the inner aspect of the plasmalemma. Small invaginations, similar in ultrastructure to coated vesicles, were also formed. It is unclear whether the coated vesicles or the dense plasmalemma invaginations contribute to the "undercoating" by fusing with the adjacent electron-dense plasma membrane. There was a distinct increase in the number and area of specialized contacts (intermediate junctions and zonulae adhaerentes) between cells and their processes. A floccular or filamentous electron-dense substance varying in amount and appearance was occasionally seen between the contacting membranes. Varicosities of terminal swellings of cell processes contained vesicles of variable size, shape and density, and also profiles of the smooth endoplasmic reticulum. Under the influence of sodium bromide, similar to the effect of GABA, mitochondria appeared within the varicosities, and primitive contacts (intermediate junctions) were formed between the terminal swellings and potential postsynaptic elements, which were absent in controls. Additionally, dense-core vesicles proliferated and aggregated at the cell periphery. They were often arranged linearly below the plasma membranes of perikarya and processes, and surrounded by a highly electron-dense substance

3.9.3.13 [Study 13] Neurotoxicity: Sodium bromide treatment of murine neuroblastoma cells in vitro

Reference	Eins S., Spoerri P. E. and Heyder E. (1983). GABA or Sodium-Bromide-Induced Plasticity of Neurites of Mouse Neuroblastoma Cells in Culture. Cell Tissue Res 229:457-460.
Guideline	No guideline, no GLP
Reliability	2 (reliable with restrictions) according to Registrant(s)
Species / strain	C1300 mouse neuroblastoma cells, clone Neuro-2a
Test material	Sodium bromide
Study design	Route of administration:other: in vitro study Vehicle:water Exposure period: 2 day(s) Frequency of treatment:Continuous exposure Doses / concentrations: 0, 10-4, 10-5 and 10-6 M Control animals:yes, concurrent vehicle Observation period: 2 day exposure period
Findings	A concentration of 10-6 M sodium bromide produces no noticeable effect light microscopically even after prolonged application (longer than 2 days). After a 2 day exposure to higher concentrations of sodium bromide and subsequent fixation of the mouse neuroblastoma cells, a pronounced increase in the length and branching of the processes or neurites is revealed. In addition there is an increase in the number of differentiated neuron-like mouse neuroblastoma cells treated with lower concentrations of sodium bromide. The length of processes, the number of branching and the cell number per area is significantly dependent on the concentration of the applied substances. The degree of branching per length of neuronal processes shows a slightly more pronounced effect when higher

concentrations of sodium bromide are used.

3.9.3.14 [Study 14] Neurotoxicity: effects of sodium bromide on the bullfrog sympathetic ganglion

Reference	Montoya G. A. and Riker W. K. (1982). A study of the Actions of Bromide on Frog Sympathetic Ganglion. Neuropharmacology, Vol. 21, pp. 581-585.
Guideline	No guideline, no GLP
Reliability	2 (reliable with restrictions) according to Registrant(s)
Species / strain	Bullfrog, Rana catesbiana Strain:not specified Sex:not specified
Test material	Sodium bromide
Study design	Route of administration:in vitro study. The ganglion was bathed in solution Vehicle:other: Ringer's solution. Doses / concentrations: 112 mM sodium bromide Control animals: no
Findings	A concentration of 10-6 M sodium bromide produces no noticeable effect light microscopically even after prolonged application (longer than 2 days). After a 2 day exposure to higher concentrations of sodium bromide and subsequent fixation of the mouse neuroblastoma cells, a pronounced increase in the length and branching of the processes or neurites is revealed. In addition there is an increase in the number of differentiated neuron-like mouse neuroblastoma cells treated with lower concentrations of sodium bromide. The length of processes, the number of branching and the cell number per area is significantly dependent on the concentration of the applied substances. The degree of branching per length of neuronal processes shows a slightly more pronounced effect when higher concentrations of sodium bromide are used.

3.10 Aspiration hazard

Not assessed in the CLH report.

4 ENVIRONMENTAL HAZARDS

Not assessed in the CLH report.