# CLH report

# Proposal for Harmonised Classification and Labelling

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

# International Chemical Identification:

## Ammonium bromide

**EC Number: 235-183-8**

**CAS Number: 12124-97-9**

**Index Number: -**

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# CONTENTS

# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Name and other identifiers of the substance

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

| | |
|---|---|
| **Name(s) in the IUPAC nomenclature or other international chemical name(s)** | Ammonium bromide |
| **Other names (usual name, trade name, abbreviation)** | n.a. |
| **ISO common name (if available and appropriate)** | n.a. |
| **EC number (if available and appropriate)** | 235-183-8 |
| **EC name (if available and appropriate)** | Ammonium bromide |
| **CAS number (if available)** | 12124-97-9 |
| **Other identity code (if available)** | n.a. |
| **Molecular formula** | $BrH_4N$ |
| **Structural formula** | $NH_4^+Br^-$ |
| **SMILES notation (if available)** | [NH4+].[Br-] |
| **Molecular weight or molecular weight range** | 97.94 g/mol |
| **Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)** | n.a. |
| **Description of the manufacturing process and identity of the source (for UVCB substances only)** | n.a. |
| **Degree of purity (%) (if relevant for the entry in Annex VI)** | Not relevant |

## 1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

| **Constituent (Name and numerical identifier)** | **Concentration range (% w/w minimum and maximum in multi-constituent substances)** | **Current CLH in Annex VI Table 3.1 (CLP)** | **Current self-classification and labelling (CLP)** |
|---|---|---|---|
| Ammonium bromide<br>12124-97-9 | Min. 98.3% (98.3-99.8) | No entry in Annex VI Table 3.1 | Eye Irrit. 2 H319<br>STOT SE 3 H335<br>Skin Irrit. 2 H315<br>Aquatic Chronic 3 H412 |

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

| **Impurity (Name and numerical identifier)** | **Concentration range (% w/w minimum and maximum)** | **Current CLH in Annex VI Table 3.1 (CLP)** | **Current self-classification and labelling (CLP)** | **The impurity contributes to the classification and labelling** |
|---|---|---|---|---|

| Impurity (Name and numerical identifier) | Concentration range (% w/w minimum and maximum) | Current CLH in Annex VI Table 3.1 (CLP) | Current self-classification and labelling (CLP) | The impurity contributes to the classification and labelling |
|---|---|---|---|---|
| No impurities present at ≥1% and none of the impurities present at lower levels are considered relevant for the classification of the substance | | | | |

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

| Additive (Name and numerical identifier) | Function | Concentration range (% w/w minimum and maximum) | Current CLH in Annex VI Table 3.1 (CLP) | Current self-classification and labelling (CLP) | The additive contributes to the classification and labelling |
|---|---|---|---|---|---|
| No additives | | | | | |

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## 2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | | Ammonium bromide | 235-183-8 | 12124-97-9 | Eye Irrit. 2<br>Repr. 1B<br>Lact.<br>STOT SE 3<br>STOT RE 2 | H319<br>H360FD<br>H362<br>H336<br>H373 (nervous system, thyroid) | GHS07<br>GHS08<br>Dgr | H319<br>H360FD<br>H362<br>H336<br>H373 (nervous system, thyroid) | | | |
| Resulting Annex VI entry if agreed by RAC and COM | tbd | Ammonium bromide | 235-183-8 | 12124-97-9 | Eye Irrit. 2<br>Repr. 1B<br>Lact.<br>STOT SE 3<br>STOT RE 2 | H319<br>H360FD<br>H362<br>H336<br>H373 (nervous system, thyroid) | GHS07<br>GHS08<br>Dgr | H319<br>H360FD<br>H362<br>H336<br>H373 (nervous system, thyroid) | | | |

Table 6: Reason for not proposing harmonised classification and status under public consultation

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

# 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no previous classification and labelling for ammonium bromide.

# 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level. Ammonium bromide has CMR properties (reproductive toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under Article 36 of the CLP regulation.

Justification that action is needed at Community level for harmonised classification and labelling of ammonium bromide for STOT SE and STOT RE is based on disagreement by DS with current self-classification.

Justification that action is needed at Community level for harmonised classification and labelling of ammonium bromide for eye irritation is based on ammonium bromide being the precursor to an *in situ* generated active substance, please see details below.

Further detail on need of action at Community level

Ammonium bromide is a substance registered under REACH at 1 000 - 10 000 tonnes per annum, with CMR properties and widespread use and should therefore be prioritized for risk management such as harmonised classification. Ammonium bromide is also one of the precursors of the biocidal active substance bromide activated chloramine (BAC) that is produced in situ when ammonium bromide and sodium hypochlorite are mixed. BAC was incorrectly notified as ammonium bromide under Directive 98/8/EC (Commission Regulation (EC) No 1451/2007) which by its own has no biocidal properties. The SE CA is the Rapporteur Member State for the evaluation of BAC according to Directive 98/8/EC. The toxicological data base of ammonium bromide and BAC will essentially be the same based on the toxicological properties of the bromide ion and therefore the hazard assessment and resulting harmonised classification of ammonium bromide will be highly relevant for the assessment of BAC. Moreover, in the document from the commission 'CA-Nov15-Doc.5.5 – Final_Rev1' it is stated: "*When the eCA considers having appropriate information on the precursor(s), the eCA may also submit a CLH dossier for the precursor(s) to ECHA to establish or amend the harmonised C&L. This can later facilitate the product authorisation stage, and could be important for product authorisation: for instance, where the related classification would prevent the making available on the market of these precursors for use by the general public."*

# 5 IDENTIFIED USES

Identified industrial uses for ammonium bromide are flame retardants and as oxidizing/reducing agents (EPA Chemical Data Report). Ammonium bromide has consumer uses in fabrics; textile and leather products, as well as in photographic supplies; film and photochemicals (EPA Chemical Data Report). Ammonium bromide is also used as a precursor for the in-situ generated biocidal active substance bromide activated chloramine (BAC), which is used as a preservative for liquid-cooling and processing systems.

# 6 DATA SOURCES

The data sources are mainly based on the draft Competent Authority Report of the in-situ generated biocidal active substance bromide activated chloramine (BAC) and its precursors ammonium bromide, sodium hypochlorite, according to Directive 98/8/EC, which is now superseded by Regulation 528/2012/EC. Study summaries of studies included in the CAR are detailed in the confidential annex Document III, Sections A6.1–A6.7 and in Annex I of the CLH report. Additionally, two original study reports (a two-generation reproductive toxicity study and a 90-day repeat dose toxicity study) were made available to the dossier submitter by the applicant and are included in the assessment. The study summaries of these two studies are placed in Annex I of the CLH report.

# 7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 20°C and 101,3 kPa** | Crystalline solid | Innes, Brown and Craig, 1998 (AmBr A3.2/01) | Visual inspection |
| **Melting/freezing point** | Decomposition before melting at ~370°C | Innes, Brown and Craig, 1998 (AmBr A3.1.1/01) | Measured, OECD TG 102 |
| **Boiling point** | Not relevant as decomposition occurs before melting at ~370°C | Innes, Brown and Craig, 1998 (AmBr A3.1.1/01) | |
| **Relative density** | 2.455 g/ml at 20° | Innes, Brown and Craig, 1998 (AmBr A3.1.1/01) | Measured, OECD TG 109 |
| **Vapour pressure** | $1.3x10^{-4}$ Pa at 25°C | Innes, Brown and Craig, 1998 (AmBr A3.2/01) | Extrapolated from the derived vapour pressure curve, OECD TG 104 |
| **Surface tension** | 72.9 mN/m at 20°C for 0.4g/l* | White and Mullee, 2003 | Measured, EU Method A.5 (Surface Tension)<br><br>(*According to REACH registration the tested solution is slightly below the recommended 1g/l but this is not considered an issue given that the result was far above 60 mN/m) |
| **Water solubility** | 783-970 g/l at 25°C, pH not given<br><br>145.6 g/l at 100°C, pH not given | CRC Handbook data, 1991-1992 and 2007-2008 (AmBr A.35/01, and 05) and Cambell and Marsh, 1959 | Measured |
| **Partition coefficient n-octanol/water** | Not relevant | | According to registration, study scientifically not necessary: Ammonium bromide is a highly water soluble inorganic salt. |
| **Flash point** | Not relevant | | Not applicable as the melting point is >40°C |
| **Flammability** | Not relevant | | According to registration, study scientifically not necessary: Ammonium bromide is widely used as a flame retardant. |
| **Explosive properties** | Not explosive | Jackson, 1998 | Measured, EU Method A.14 (Explosive properties) |
| **Self-ignition temperature** | Not relevant | | According to registration, study scientifically not necessary: Ammonium bromide is widely used as a flame retardant. |

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Oxidising properties** | Not an oxidizer | Innes, Brown and Craig, 1998 (AmBr A3.16/01) | Measured, EPA OPPTS 830.6314 (Oxidising or Reducing Action) |
| **Granulometry** | 10% of the substance is within the inhalable fraction (< 100µm).<br>The substance has a low propensity to become airborne with a maximum attainable concentration of 0.1 mg/L.<br>The substance is considered to be not inhalable and has a low fugacity | Other company data, 2012 | Measured, equivalent or similar to OECD Guideline 110 (Particle Size Distribution / Fibre Length and Diameter Distributions) |
| **Stability in organic solvents and identity of relevant degradation products** | 100 g/l in ethanol at 78°C<br>Soluble in acetone and slightly soluble in diethyl ether | CRC Handbook data, 1991-1992 (AmBr A3.7) | Measured |
| **Dissociation constant** | pKa: 9.384 at 25°C | Skibsted LH, 1981 | |
| **Viscosity** | Not relevant | | Ammonium bromide is solid. |

# 8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

# 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| ***Ammonium bromide*** | | | |
| *Human* | | | |
| Biological half-life of bromide ions (no guideline study, prior to GLP-implementation)<br><br>Human<br>6 males and 4 women<br><br>Oral (drinking water)<br>Single administration<br><br>10 µCi of 82Br in the form of $NH_4Br$ was dissolved in 25 ml water; this corresponds to less than 0.05 mg ammonium bromide (0.041 mg bromide)<br><br>Tissues and body fluids sampled: blood<br>Time and frequency of sampling: twice daily for a period of 4 days | Absorption: highest concentration was reached within 1-4 hours after dosing.<br><br>Distribution: The ratio red blood cells to serum of bromide ions was about 0.45.<br><br>Excretion: effective half-life was 32 hours; biological half-life was 12 hours | Ammonium bromide | A6.2/10, Doc. No. 592-010<br><br>Söremark, R., 1960<br><br>Reliability 2 |
| *Mouse* | | | |
| Distribution (no guideline study, prior to GLP-implementation)<br><br>Mouse, albino (strain not specified)<br><br>I.v. in tail vein<br>Single administration<br><br>A 20 mg mouse received 75 µCi, corresponding to 1 mg Br, in the form of a water solution of $NH_4Br^{82}$<br><br>The animals were sacrificed at various intervals after injection: 5 min, 20 min, 1 h, 2 h, 4 h, 24, h, and 48 h. | Bromide is widely distributed, among tissues with highest concentration were: blood, gastric mucosa, retina, cartilage, tendons, bones, lungs and thyroid.<br><br>Autoradiography data also showed transplacental distribution of bromide. Radioactive bromide was found in cartilage of foetuses, but the level was not as high as in dams | Ammonium bromide | A6.2/16. Doc. No. 592-084<br><br>Söremark and Ullberg, 1960 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | | | |
| ***Sodium bromide*** | | | |
| *Human* | | | |
| Pharmacokinetics of oral and intravenous bromide in human volunteers (no guideline), no GLP compliance<br><br>Human<br>7 males<br><br>Oral and i.v.<br>Single administration<br><br>1 ml/kg of 3% sodium bromide, equivalent to 30 mg/kg bromide.<br><br>Blood samples were taken just prior to administration and periodically during the subsequent 24 hours, and again at 3, 7 and 35 days post administration. | Absorption: Oral bioavailability of bromide taken on empty stomach ranged between 75-118% of the single dose given (with a mean of 96%). Absorption completed within a few hours.<br>Distribution: Bromide is distributed into the extracellular water space. The distribution volume of bromide was 408 ±17 ml/kg (the variations in distribution volume between subjects were small).<br>Excretion: Bromide is eliminated by the kidney. The total body clearance was 26 ±1.7 ml/kg/day. Elimination t½ was 11.9 ±1.4 days after oral administration and 9.4 ±1.5 days after intravenous injection. | Sodium bromide | A6.2/09, Doc. No. 592-008<br><br>Vaiseman N., Koren G. and Pencharz P. 1986<br><br>Reliability 2 |
| *Rat* | | | |
| Kinetics and distribution (no guideline study)<br><br>Rat, Wistar<br>5 males per group<br><br>Oral (drinking water), subcutaneous injection or intragastric administration<br><br>A. One group of animals drank distilled water ad libitum that contained 50 µg bromide (NaBr) per gram labeled by the radionuclide $^{82}$Br in the course of 17 d.<br>B. Two groups of animals received approx 1.5 MBq $^{82}$Br in the form of Na $^{82}$Br in saline either by subcutaneous injection or intragastrically; these animals drank ad libitum nonradioactive distilled water containing 50 µg bromide (NaBr) per gram. | The biological half-lives of bromine in different organs and tissues differ significantly from the half-live in whole-blood.<br><br>Half-lifes:<br>Thyroid: 94.3 ± 14.6 h<br>Liver: 235.0 ± 88.9 h<br>Whole body: 197.8 ± 22.2 h | Sodium bromide | A6.2/17, Doc. No. 592-075<br><br>Pavelka S. et al., 2000a<br><br>Reliability 2 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Exposure regimen: 12-396 hours<br><br>Biological half-lives of bromine in 15 different organs and tissues were determined at different time intervals | | | |
| Distribution (no guideline study), no GLP compliance<br><br>Rat, Wistar<br>5 males per group<br><br>s.c. and oral (in drinking water)<br><br>50 µg bromide (NaBr) per gram in drinking water and approximately 20 kBq $^{82}$Br in the form of Na $^{82}$Br in saline by subcutaneous injection.<br>Up to 17 days (396 hours) | The whole stomach of rats was the only organ of those investigated that had a larger uptake of $^{82}$Br than blood. A remarkably high concentration of $^{82}$Br was found in the skin | Sodium bromide | Pavelka S. et al., 2000b |
| 28-day oral repeated dose toxicity study (no guideline study)<br><br>Rat, Wistar<br>4 females per group<br><br>Oral (feed)<br>0, 300, 1200, 4800, 19200 ppm | Dose related increase in bromide concentration in brain, liver, kidney and plasma. Highest bromide concentration found in plasma followed by kidney, liver and brain.<br><br>In the high dose group concentrations were 56 mEq/l, 34 mEq/kg, 21 mEq/kg, and 20 mEq/kg, respectively compared to 0.2 mEq/l, 0.7 mEq/kg, 0.1 mEq/kg and 0.4 mEq/kg, respectively in control. | Sodium bromide | A6.4.1/03, Doc. No. 592-007<br><br>van Logten, M. J. et al., 1973<br><br>Reliability 2 |
| 90-day oral repeated dose toxicity study (no guideline study)<br><br>Rat, Wistar<br>male/female<br>10/sex/group<br><br>Oral (feed)<br>0, 75, 300, 1200, 4800, 19200 ppm | Dose related increase in bromide concentration in brain, kidney and plasma. Highest bromide concentration found in plasma followed by kidney and brain.<br><br>In the high dose group concentrations were 51 mmole/l, 30 mmole/kg and 11 mmole/kg, respectively, compared to 0.2 mmole/l, 0.1 mmole/kg and 0.1 | Sodium bromide | A6.4.1/04, Doc. No. 592-005<br><br>van Logten, M. J., et al., 1974<br><br>Reliability 2 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | mmole/kg, respectively in control. | | |
| *Dog* | | | |
| Intravenous and oral administration to healthy Beagle dogs (no guideline study)<br><br>Dog, Beagle<br>4 females/group<br><br>Oral and i.v.<br>20 mg/kg bw<br>Single treatment. | Absorption of bromide was rapid, reaching peak serum concentrations of 65 to 96 mg/L after 30-45 minutes.<br>The mean bioavailability of bromide was estimated to be approximately 46%.<br>Total body clearance was 9 ± 3.9 mL/day/kg, and the mean apparent volume of distribution was 0.45±0.07 L/kg indicating a preferential distribution of bromide in the extracellular space.<br>Mean apparent elimination half life ($t_{½}$) of bromide was 46 ±9 days (oral) and 37 ±10 days (i.v. administration). | Sodium bromide, 4% solution | A6.2/01, Doc. No. 592-029<br><br>Trepanier L.A. and Babish J.G. 1995.<br><br>Reliability 2 |
| ***Potassium bromide*** | | | |
| *Dog* | | | |
| Bromide pharmacokinetics in vivo (no guideline)<br><br>Dog, Beagle<br>male/female<br>3/sex/group<br><br>Oral (feed)<br>30 mg/kg bw/day (actual ingested) (equivalent to 20 mg Br-/kg bw)<br><br>115 days (daily) | The mean steady-state serum bromide concentration was 245 mg/dL (range from 178 to 269 mg/dL).<br><br>The mean elimination t1/2 using a one-compartment model was 15.2 days.<br><br>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.<br><br>Apparent total body clearance was 16.4 mL/day/kg and volume of distribution was 0.40 L/kg.<br><br>Median renal clearance was 8.2 mL/kg bw/day (range 6.03-12.6 mL/kg bw/day). | Potassium bromide | A6.2/03, Doc. No. 592-032<br><br>March, P.A. Podell, M., Sams, R.A. 2002.<br><br>Reliability 2 |
| ***Bromide*** | | | |
| Dermal penetration (*in vivo* and *in vitro)* (no guideline study) | No significant differences between the penetrations of different ions through the same skin. Excised skin was | Sodium chloride, potassium chloride, sodium bromide, sodiumhydrogenphosphate | A6.2/05, Doc. No. 592-045 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Human<br>Pig<br>Rabbit | as permeable as skin in situ. Low amounts of bromide penetrated human skin (<1%). | | Tregear R.T., 1966<br><br>Reliability 2 |
| Distribution study in vivo (no guideline study)<br><br>Guinea pig, 'Sabra' albino<br>9-11 males per group<br><br>Dermal and inhalation<br><br>Dermal: 30 mins<br>Inhalation: 5 mins<br><br>Dermal: 15% Dead Sea bath salt solution with 10 Ci/L $Na^{82}Br$<br>Inhalation: unknown concentration of $Na^{82}Br$ in the aerosols | Bromide was able to reach almost every internal organ in rates and concentrations higher after inhalation than those rates and concentrations reached after bathing. | Dead Sea bath salt solution with 10 Ci/L Na82Br | Shani J et al., 1982.<br><br>Reliability 2 |
| Dermal penetration in vivo<br><br>Human (Healthy volunteers and psoriatic patients were examined)<br>Guinea pig (males/females)<br><br>Hypertonic salt solutions: 1.5, 5 or 15% of hypertonic salt solution (dead sea bath salt) corresponding to a concentration of 0.0096, 0.032 and 0.096% of bromide, respectively.<br><br>humans: 30 minutes/bath<br>guinea-pigs: 60 minutes/bath | The ions elevated in the sera of the psoriatic patients after one month of daily bathing in the Dead Sea were Br, Rb, Ca and Zn. No elevation of ion concentration in healthy volunteers. Analysis of penetration of radionuclides through the guinea-pig skin demonstrate that the same radionuclide accumulate in the same internal organs in all four groups studies (pregnant, lactating and cycling females as well as mature males).<br>$^{82}Br$ concentrated in the skin, testes and bone but not in the spleen. $^{82}Br$ was also noted in pregnant guinea-pig ovaries. | Dead Sea bath salt solution | A6.2/06, Doc. No. 592-044<br><br>Shani, J. et al., 1985<br><br>Reliability 2 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| ***Studies included in the REACH registration but with unclear relevance beacause of lacking information or low reliability. Listed for completeness.*** | | | |
| Excretion<br><br>Dog<br>10 females<br><br>Oral (feed)<br>10 mmol/kg<br>24 hours | The filtration rates for the two groups were 6.4 ± 1.6 mL/min/kg (group 1 – fasted for 48 h) and 4.0 ± 1.1 mL/min/kg (group 2 – normal feeding + NaCl). | Unknown test material | Wolf R.L. and Eadie G.S. 1951. Reabsorption of Bromide by the Kidney. Am. J. Physiol., Vol. 163, pp. 436-441. |
| Distribution<br><br>Rats and rabbits<br><br>Males: 18-45 mg/kg bw (average 28 mg/kg bw)<br>Females: 18-45 mg/kg bw (average 28 mg/kg bw<br><br>24 hours | The highest concentration of 82Br in all groups of animals was in plasma. | Unknown test material | Mack J.F. and Shipley R.A 1952. Comparative Uptake of 82Br by the Hypophysis and Other Tissues. Proc. Soc. Exp. Biol. Med., 80, 18. |
| Distribution<br><br>Dogs and humans<br><br>Oral (feed)<br>Exposure time for dogs: at least 10 days<br>Dosages for dogs: 3-5 g per day | In cells relative to serum, chloride was shown to have been replaced by bromide to a greater extent, unlike in the urine, spinal fluid, gastric juices or saliva relative to serum | Unknown test material | Mason M.F. 1963. Halide distribution in body fluids in chronic bromide intoxication. J. Biol. Chem., 113, 61. |
| Distribution<br><br>Rabbit<br><br>Infusion into the marginal ear vein<br>Males: 2-15 mmol/kg<br>Females: 2-15 mmol/kg | The steady state between the plasma, brain and CSF bromide levels is reached some 12 hours after injection and maintained over the ensuing 12-hour period | Radiolabelled sodium bromide solution | Pollay M. 1967. The Processes Affecting the Distribution of Bromide in Blood, Brain, and Cerebrospinal Fluid. Experimental Neurology 17, 74-85. |
| ***Studies with reliability 3, included in the REACH registration but not considered in the assessment by the DS. Listed for completeness.*** | | | |
| Metabolism<br><br>Dog<br>2 females<br><br>Oral (gelatin capsules mixed with the diet)<br>One day treatment | 13.36 g ammonium valerianate (equal to 1.08 g nitrogen) caused vomiting and nausea. There were no abnormal symptoms observed after ingestion of the other ammonium salts. Sodium chloride fed under the experimental conditions caused a distinct lowering of the ammonia nitrogen | Ammonium salts (lactate, acetate, butyrate, valerianate, chloride, phosphate, and sulphate) | A6.2/14, Doc. No. 592-019<br><br>Underhill, 1913 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | elimination | | |
| Distribution<br><br>Dog<br><br>Oral (drinking water)<br><br>Single treatment and treatment over several days (the treatment period is not further specified in the report), daily<br><br>Doses/conc.:<br>- dogs for observation: 40 g/750 mL (53.34 g/L, corresponding to 2.65 g NaBr/kg bw/day based on a default water consumption of 500 mL/day and a weight of 10 kg/dog)<br>- diffusion experiment: 60 g/750 mL (80 g/L, corresponding to 4 g NaBr/kg bw/day based on a default water consumption of 500 mL/day and a weight of 10 kg/dog) | Analysis of blood samples from the dog treated with a single dose of 40 g of sodium bromide revealed that the bromide concentration in serum and cells reached a maximum concentration about 90 minutes after administration. The highest bromide distribution ratio occurred 30 minutes after bromide was given.<br><br>Investigation of blood samples from dogs treated repeatedly with sodium bromide showed, that the highest bromide distribution ratio occurred on the morning following the administration of the first dose. Although the blood bromide concentration continued to increase there was a gradual drop in the distribution ratio. | | Van Dyke, H. B. and Baired Hastings, A (1931). Studies of Bromide Distribution in the Blood – the Distribution of Bromides and Chlorides in the Blood of Dogs following the Oral Administration of Sodium Bromide. J. Biol. Chem., 92, 27-32 |
| ***Studies with reliability 4, included in the REACH registration but not considered in the assessment by the DS. Listed for completeness.*** | | | |
| Olver R. E. and Strang L. B. (1974). Ion Fluxes across the Pulmonary Epithelium and the Secretion of Lung Liquid in the Foetal Lamb. Journal of Physiology, (1974) Vol. 241, No. 2, pp. 327-357. | | | |
| Morkeberg J.C., Sheng H.-P. and Huggins R.A. (1991). A Comparison of Chloride, Bromide and Sucrose Dilution Volumes in Neonatal Pigs. Proceedings of the Society for Experimental Biology and Medicine, Vol. 196, No. 3, pp. 344-350. | | | |
| ***Review articles included in the REACH registration, listed for completeness.*** | | | |
| A6.2/08, Doc. No. 592-003<br><br>Rauws A. G. (1983). Pharmacokinetics of bromide ion - an overview. Fd Chem. Toxic., 1983, 21 (4), 379-382 | | | |
| A6.10/19, Doc. No. 592-021<br><br>Dowling, P. M (1994). Management of canine epilepsy with phenobarbital and potassium bromide. Can. Vet. J. 35 (11), 724-725. | | | |
| A6.2/02, Doc. No. 592-033<br><br>Trepanier, L. A. (1995). Use of Bromide as an Anticonvulsant for Dogs with Epilepsy. JAVMA, 207 (2), 163-166. | | | |

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

No test guideline studies of toxicokinetics of ammonium bromide are available. The section on toxicokinetics is based mainly on data available in the open literature (experimental studies and review articles). An overview of the results of these investigations is presented in Table 8 (above).

Based on the read-across justification below the available information on toxicokinetics is mainly focused on the kinetics of the bromide ion since the bromide ion is considered to be the most relevant chemical species from the toxicological point of view.

The read-across information is from studies and investigations performed with inorganic bromide salts on the kinetics and metabolism as well as on the dermal penetration in rodents, dogs and human volunteers. The determination of the distribution pattern of the bromide ion in organs is limited.

Information on metabolism and toxicokinetics of the ammonium moiety of ammonium bromide is limited. However, the fate of ammonium in the organism is considered predictable (incorporation into urea cycle and conversion to urea) due to its physiological role in the supply of nitrogen. Further, the toxicity seems to be triggered by the bromide ion rather than by the ammonium ion as shown in repeated dose toxicity studies (no systemic toxicity seen in an investigation of ammonium sulphate, Takagi et al., 1999; and a comparable toxicity profile demonstrated in the teratogenicity studies with ammonium bromide and sodium bromide).

***Justification for read-across from other bromide salts***

Ammonium bromide is an inorganic salt that dissociates to its corresponding ions in aqueous solutions at environmental pH and temperature. Sodium bromide and potassium bromide are, like ammonium bromide, also bromide salts and highly soluble in water. Comparison of the available data on the various bromide salts has shown that the bromide ion is the relevant ion for determination of the toxicological profile with simple cations such as potassium, sodium or ammonium having little or no influence on the bromide ion properties. In repeated dose toxicity studies of the ammonium ion (ammonium sulphate) no systemic toxicity was reported, and a comparable toxicity profile is demonstrated in teratogenicity studies with ammonium bromide and sodium bromide. Therefore, to read-across data from other inorganic bromide salts to ammonium bromide for systemic effects appears justified. For local toxicity and hazard classes for e.g. skin/eye irritation read-across is not applied. There is sufficient substance specific data to conclude on classification in these hazard classes for ammonium bromide without support from read-across data. Moreover, there is a difference between ammonium bromide versus sodium bromide and potassium bromide in that the ammonium ion, $NH_4^+$, is a weak acid with a pKa of 9.25 that contributes to hydronium ion concentration in solution. In contrast, the sodium ion and the potassium ion do not contribute to the hydronium ion concentration in solution. Thus, read-across from sodium bromide or potassium bromide may not predict the local effects (on skin and other membranes) due to differences in physico-chemical properties.

***Absorption***

Oral

In humans the oral bioavailability of bromide taken on emty stomach is reported to be at least 75% (ranged between 75-118% with a mean of 96%). Absorption is rapid (completed within a few hours).

Dermal

No dermal absorption study is available with ammonium bromide. Although ammonium bromide is a small molecule, it dissociates in water into ions and is not expected to easily penetrate the skin due to its electric charge. Moreover, data from the open literature indicate low dermal absorption of the bromide ion (<1%). Thus, a default value of 10% was proposed to be used for dermal penetration following dermal exposure towards ammonium bromide.

***Distribution***

No guideline study on ammonium bromide or other bromide salts is available. Bromide is mainly distributed in the extracellular fluid and it passes the blood brain barrier. In rats, radioactive bromide was found in red blood cells, cerebrospinal fluid, thyroid gland, blood vessel walls, cartilage, tendons, dentine, kidneys, urinary bladder, stomach and the eye. No significant amounts of bromide was observed in fat or proteins of blood. Bromide can cross the placenta to the foetus more readily than it can be eliminated by the foetus back to the maternal blood. Thus, there is a potential for accumulation in the foetus, and there have been human cases of congenital bromism. In foetuses, most radioactive bromide was found in cartilage. Bromide can also pass into breast milk.

***Metabolism***

No guideline study on ammonium bromide or other bromide salts is available. Bromide is not subjected to hepatic metabolism and is not bound to plasma proteins (this stated in publications from the open literature).

***Excretion***

Excretion of bromide is mainly via the kidneys, where the bromide competes with chloride for tubular reabsorption. Other routes of excretion, such as sweat, saliva and faeces are quite minor. The amount of bromide in excreta is not determined. The plasma half-life is approximately 3 days in rats, 12 days in humans and 15-46 days in dogs. Half-life dependents on chloride intake (decreased half-life with administration of surplus halide ions, e. g. chloride).

# 10 EVALUATION OF HEALTH HAZARDS

## Acute toxicity

## 10.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

| **Method, guideline, deviations if any** | **Species, strain, sex, no/group** | **Test substance,** | **Dose levels, duration of exposure** | **Value $LD_{50}$** | **Reference** |
|---|---|---|---|---|---|
| ***Ammonium bromide*** | | | | | |
| OECD TG 401 (Acute Oral Toxicity)<br><br>EPA OPP 81-1 (Acute Oral Toxicity) | Rat, CD strain (remote Sprague Dawley origin<br><br>male/female<br><br>5/sex/group | Ammonium bromide<br><br>Vehicle: distilled water | Oral (gavage)<br><br>2000, 2714, 3684 and 5000 mg/kg bw | LD50 males: 2868 mg/kg bw<br><br>LD50 females: 2566 mg/kg bw<br><br>LD50 males and females combined: 2714 mg/kg bw | A6.1.1/01, Doc. No. 521-001<br><br>Study report, 1986a<br><br>Reliability 1 |

Table 10: Summary table of human data on acute oral toxicity

| **Type of data/report** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No human studies on acute oral toxicity | | | | |

Table 11: Summary table of other studies relevant for acute oral toxicity

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other studies relevant for acute oral toxicity | | | | |

### 10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

There is one available study of acute oral toxicity of ammonium bromide in rat.

**Ammonium bromide – test guideline studies**

***Acute oral toxicity study of ammonium bromide (Study report, 1986a)***

In an oral acute toxicity study performed according to OECD TG 401 ammonium bromide 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. Clinical signs such as lethargy, decreased motor activity, prone or hunched posture, ataxia, breathing irregularities, unconsciousness and tonic convulsions were observed in rats after administration at a dose level of 2000 mg ammonium bromide/kg bw. Necropsy findings included fur staining, abnormal gastro-intestinal contents, dark areas on the lungs and occasional thymic petechiae. In the surviving animals there were no effects on bodyweight gains and necropsy findings on day 15 were unremarkable. The mortality rate for ammonium bromide 2000 mg/kg bw in both male and females were zero, at a dose of 2714 mg/kg bw 1/5 males and 4/5 female rats died, and all rats died at doses of 3684 and 5000 mg/kg bw.

### 10.1.2 Comparison with the CLP criteria

The acute oral LD50 value (rat) of ammonium bromdie was determined to be 2868 mg/kg bw for males, 2566 mg/kg bw for females and 2714 mg/kg bw for both sexes combined. The LD50 is above the acute toxicity estimates (ATE) for Acute toxicity hazard by the roal route, category 4 ($300 < ATE \leq 2\,000$).

### 10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available information, classification of ammonium bromide for acute oral toxicity is not warranted.

## 10.2 Acute toxicity - dermal route

Table 12: Summary table of animal studies on acute dermal toxicity

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance, | Dose levels, duration of exposure | Value $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| ***Ammonium bromide*** | | | | | |

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance, | Dose levels, duration of exposure | Value $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| OECD Guideline 402 (Acute Dermal Toxicity)<br><br>EPA OPP 81-2 (Acute Dermal Toxicity) (Sub-Division F)<br><br>EEC Directive 92/69/EEC, Annex V, Test B3, July 1992 | Rat (Sprague-Dawley) male/female<br><br>5/sex/group | Ammonium bromide | 2000 mg/kg bw (comparable to 40 mg/cm2)<br><br>Coverage: semiocclusive<br><br>24 hours | LD50: > 2000 mg/kg bw (male/female) | A.6.1.2/01, Doc. No. 522-001<br><br>Study report, 1998a<br><br>Reliability 1 |

Table 13: Summary table of human data on acute dermal toxicity

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No human data on acute dermal toxicity | | | | |

Table 14: Summary table of other studies relevant for acute dermal toxicity

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other data relevant for acute dermal toxicity | | | | |

### 10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

**Ammonium bromide – test guideline studies**

***Acute dermal toxicity study of ammonium bromide (Study report, 1998a)***

Five male and five female rats were exposed to a 24 h dermal application of 2000 mg/kg of ammonium bromide which was applied under occlusive water moistened patches onto skin which was clipped the day before. The rats were observed for 14 days after dosing. The clinical signs of loose faeces, test side slightly red and wet perigenital were the clinical signs observed after dermal application at a dose level of 2000 mg/kg bw. No animals died prematurely during the study.

Under the conditions of the study, the acute dermal LD50 of ammonium bromide was determined to be > 2000 mg/kg in rats.

### 10.2.2 Comparison with the CLP criteria

The acute dermal LD50 (rats) was determined to be >2000 mg/kg bw for ammonium bromide. The LD50 is above the acute toxicity estimates (ATE) for Acute toxicity hazard by the dermal route, category 4 ($1000 < ATE \leq 2\,000$).

### 10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available information, classification of ammonium bromide for acute dermal toxicity is not warranted.

## 10.3 Acute toxicity - inhalation route

Table 15: Summary table of animal studies on acute inhalation toxicity

| **Method, guideline, deviations if any** | **Species, strain, sex, no/group** | **Test substance, form and particle size (MMAD)** | **Dose levels, duration of exposure** | **Value** $LC_{50}$ | **Reference** |
|---|---|---|---|---|---|
| OECD TG 403 (Acute Inhalation Toxicity)<br><br>EU Method B.2 (Acute Toxicity (Inhalation))<br><br>EPA OPP 81-3 (Acute inhalation toxicity) | Rat (Sprague-Dawley) male/female | Ammonium bromide<br><br>Dust aerosol<br><br>MMAD: 2.61 µm | Nominal concentration: 37.96 mg/L<br><br>Measured concentration: 0.1 mg/L (maximum attainable concentration)<br><br>inhalation (nose only)<br><br>4 hours | LC50 (4 h): > 0.1 mg/L air (analytical) (male/female) based on: test mat. (at the maximum attainable concentration of 0.1 mg/L, no mortalities were observed.) | A6.1.3/01, Doc. No. 523-001<br><br>Study report, 1998b<br><br>Reliability 1 |

Table 16: Summary table of human data on acute inhalation toxicity

| **Type of data/report** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No human data on acute inahalation toxicity | | | | |

Table 17: Summary table of other studies relevant for acute inhalation toxicity

| **Type of study/data** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No other studies relevant for acute inhalation toxicity | | | | |

### 10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

**Ammonium bromide – test guideline studies**

***Acute inhalation toxicity study of ammonium bromide (Study report, 1998b)***

The acute inhalation toxicity of aerosolized ammonium bromide was investigated in rats. Five male and five female rats were exposed to a single 4 h snout only exposure of 0.1 mg/L (maximum attainable concentration) of test substance and were observed for clinical signs for a period of 14

days post-exposure. All animals were subjected to necropsy at termination of the study. Lungs were removed and weight taken to allow calculation of lung to body weight ratio.

After inhalation exposure at the maximum attainable concentration of 0.1 mg/L rats were observed to be wet and unkempt and to have staining on the head. These signs were however considered to be related to restraint and not of toxicological importance.

There were no effects on body weights, lung to body weight ratios or necropsy findings considered to be due to treatment with the test material.

The concentration of 0.1 mg/L was found to be the maximum attainable concentration (MAC) and no higher dust aerosol concentrations could be generated. The poor stability of the aerosol at 0.1 mg/L and the 64.4% aerosol mass < 4µm resulted in a low respirable fraction. This dose level was quite low compared to the limit concentration of 5 mg/L proposed in the OECD test guideline 403. The unusually high nominal concentration (37.96 mg/L) in contrast to the measured aerosol concentration (0.1 mg/L) indicates that a substantial fraction of the test material would not readily aerosolise. The losses were attributed in the test as due to sedimentation within the exposure chamber.

### 10.3.2 Comparison with the CLP criteria

Under the conditions of the acute inhalation toxicity study performed with a dust aerosol of ammonium bromide the acute inhalation LC50 (rat, 4 hrs) was considered to be > 0.1 mg/L (maximum attainable concentration). Since no higher dust aerosol concentrations of ammonium bromide than 0.1 mg/L could be generated, and no deaths or clinical signs of toxicological importance were demonstrated at the highest tested dose, the criteria for classification in acute inhalation toxicity is not met.

### 10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on available information, classification of ammonium bromide for acute inhalation toxicity is not warranted.

## 10.4 Skin corrosion/irritation

Table 18: Summary table of animal studies on skin corrosion/irritation

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance, | Dose levels duration of exposure | Results<br>-Observations and time point of onset<br>-Mean scores/animal<br>-Reversibility | Reference |
|---|---|---|---|---|---|
| ***Ammonium bromide*** | | | | | |
| OECD TG 404<br>GLP | Rabbit<br>New Zealand White<br>Female<br>Six animals | Ammonium bromide | 0.5 g/animal<br><br>Duration of exposure: 4 hours<br><br>Post exposure period: 3 days | Average score 24, 48 and 72 h:<br>- Erythema 0.0<br>- Oedema 0.0<br><br>Reversibility: not applicable, there were no erythema or oedema observed.<br><br>Results: Non-irritant to skin | A6.1.4/01, Doc. No. 565-001<br><br>Study report, 1996a |

Table 19: Summary table of human data on skin corrosion/irritation

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No human data on skin corrosion/irritation | | | | |

Table 20: Summary table of other studies relevant for skin corrosion/irritation

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other data relevant for skin corrosion/irritation | | | | |

### 10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

**Ammonium bromide – test guideline studies**

***Acute Dermal Irritation/Corrosion study of ammonium bromide (Study report, 1996a)***

The dorsal area of each rabbit was clipped between the limb girdles (two test sites 6X6 cm) 24 hours before dosing. A quantity of 0.5g of ammonium bromide was applied semi-occluded to six New Zealand White Rabbits for four hours. Dermal reactions were assessed 1, 24, 48 and 72 hours after removal of the dressings. No dermal irritation responses were observed in any animals at any reading time during the observed period.

### 10.4.2 Comparison with the CLP criteria

No dermal reactions were recorded in any animal tested for ammonium bromide at any reading time. Therefore, classification criteria for skin corrosion/irritation are not fulfilled.

### 10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available information, classification of ammonium bromide for skin corrosion/irritation is not warranted.

## 10.5 Serious eye damage/eye irritation

Table 21: Summary table of animal studies on serious eye damage/eye irritation

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance, | Dose levels duration of exposure | Results<br>- Observations and time point of onset<br>- Mean scores/animal<br>- Reversibility | Reference |
|---|---|---|---|---|---|
| ***Ammonium bromide*** | | | | | |
| EPA OPP 81-4 (Acute Eye | Rabbit, New Zealand White | Ammonium bromide | 0.1 g of test substance<br>Reactions of conjunctivae, iris and | Mean scores 24-72 h<br><u>Corneal opacity score:</u><br>0 in all six animals | A6.1.4/03, Doc. No. 566-002<br><br>Study report, 1986b |

| | | | | | |
|---|---|---|---|---|---|
| Irritation)<br>equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion)<br>GLP | 6 females | | cornea were examined 1, 24, 48, and 72 hours after treatment and on day 8. | Iritis score:<br>0, 0, 0.3, 0.3, 0, 0.3 (fully reversible within 48 hours)<br>Conjunctivae redness score:<br>2, 2, 0.6, 2, 2, 1.3 (fully reversible within 8 days)<br>Chemosis score:<br>0, 0.3, 0, 0.6, 0.6, 0 (fully reversible within 72 hours) | Reliability 1 |

Table 22: Summary table of human data on serious eye damage/eye irritation

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No human data on serious eye damage/irritation | | | | |

Table 23: Summary table of other studies relevant for serious eye damage/eye irritation

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other data relevant for serious eye damage/irritation | | | | |

### 10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

**Ammonium bromide – test guideline studies**

***Acute Eye Irritation / Corrosion study of ammonium bromide (Study report, 1986b)***

Six rabbits were subject to single ocular instillation of 0.1g of ammonium bromide into the right eye. The left eye remained untreated and served as control. Reactions of conjunctivae, iris, and cornea were examined 1, 24, 48 and 72 hours after treatment and on day 8. The results from the test showed a well-defined appearance to the conjunctival blood vessels with 4 out of 6 rabbits showing a mean score of 2 for conjunctival redness. Iridial congestion, a moderate or slight discharge and slight chemosis amongst were also observed in the animals one hour after installation of ammonium bromide. No corneal lesions were observed. Gradual resolution was apparent over the following three days. All ocular irritation responses were resolved on day 8.

### 10.5.2 Comparison with the CLP criteria

According to the REACH Registrant(s) the substance is classed as ‘slightly irritating’, and assigned a classification of ammonium bromdide in Eye Irrit. 2 based on the irritation response observed in the conjunctiva. According to the CLP criteria in table 3.2.2 category 2 is warranted if a substance produces, at least in 2 of 3 tested animals, a positive response of conjunctival redness ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days. In the case of using a test with 6 rabbits, as were used in the current two available studies, the Guidance on the application of the CLP criteria states that if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2 for conjunctival

erythema (redness) category 2 is warranted. In the study of ammonium bromide (Study report, 1986b), 4 out of 6 rabbits showed a mean score of 2 for conjunctival redness that were fully reversible within 21 days. Therefore, classification in category 2 for eye irritation is warranted.

### 10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the available information, classification of ammonium bromide for Eye Irrit. 2, H319 is warranted.

## 10.6 Respiratory sensitisation

Table 24: Summary table of animal studies on respiratory sensitisation

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance, | Dose levels, duration of exposure | Results | Reference |
|---|---|---|---|---|---|
| No data | | | | | |

Table 25: Summary table of human data on respiratory sensitisation

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No data | | | | |

Table 26: Summary table of other studies relevant for respiratory sensitisation

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No data | | | | |

### 10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

There is no data for ammonium bromide available on respiratory sensitisation.

### 10.6.2 Comparison with the CLP criteria

Not relevant since no data is available.

### 10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not relevant since no data is available.

## 10.7 Skin sensitisation

Table 27: Summary table of animal studies on skin sensitisation

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance, | Dose levels duration of exposure | Results | Reference |
|---|---|---|---|---|---|
| ***Ammonium bromide*** | | | | | |
| Guinea pig maximisation test<br>OECD Guideline 406 (Skin Sensitisation)<br>EPA OPP 81-6 (Skin Sensitisation)<br>EU Method B.6 (Skin Sensitisation) | Guinea pig, Dunkin-Hartley<br>Female<br>20 in test group<br>10 in control group | Ammonium bromide | Induction:<br>5% test substance for intradermal injection,<br>55% test substance for topical application (maximum practicable concentration)<br>Challenge:<br>55% test substance for topical application (maximum practicable concentration)<br>Evaluation: 24h, 48h after challenge; dermal reactions were also assessed 24h after intradermal and topical induction application | not sensitising<br>No. with positive reactions:<br>1st reading: 0 out of 19 (test group); 24 h after chall.; dose: 5 % / 55 %<br>2nd reading: 0 out of 19 (test group); 48 h after chall.; dose: 5 % / 55 %<br>1st reading: 0 out of 9 (negative control); 24 h after chall.<br>2nd reading: 0 out of 9 (negative control); 48 h after chall.<br>Positive controls: 100% of the test group animals reacted positively following challenge | A6.1.5/01, Doc. No. 567-001<br>Study report, 1998c<br>Reliability 1 |

Table 28: Summary table of human data on skin sensitisation

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No human data on skin sensitisation | | | | |

Table 29: Summary table of other studies relevant for skin sensitisation

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other studies relevant for skin sensitisation | | | | |

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

**Ammonium bromide – test guideline studies**

***Guinea pig maximisation test of ammonium bromide (Study report, 1998c)***

The study was designed to assess skin sensitisation potential of ammonium bromide. In the initial phase of the study, a dose ranging for induction was conducted via intradermal injections and topical application of the test substance. Based on the results of this preliminary investigation concentrations of ammonium bromide were selected at 5% and 55% for the main study due to being the highest non-irritating dose (5%, intradermal) and the maximum practicable concentration (55%, topical. No reactions were noted at concentrations up to 55%).

For the main study in the induction exposure, 20 guinea pigs in the test group were exposed to the test material via two routes, intradermal injection (5% ammonium bromide, day 0) and topical application (55% ammonium bromide; day 7). Animals were also exposed to an adjuvant via intradermal injection. The control group (10 guinea pigs) was exposed to vehicle, sterile distilled water. Thirteen days after the topical induction, each animal was challenged by topical application (55% test substance) on both flanks. Test sites observations were recorded 24 h and 48 h after patch removal. Prior to challenge, two animals (one in test group, one in control group) were humanely killed as the condition of their scapular region had exceeded the severity limit set by the project licence governing the study.

There were no reactions during induction, and zero out of nineteen guinea pigs showed showed positive response after challenge.

### 10.7.2 Comparison with the CLP criteria

Under the conditions of the guinea pig maximisation test of ammonium bromide the sensitisation rate was zero. Thus, the available information does not meet the criteria for classification in skin sensitisation.

### 10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on available information, classification of ammonium bromide for skin sensitisation is not warranted.

## 10.8 Germ cell mutagenicity

Table 30: Summary table of mutagenicity/genotoxicity tests in vitro

| **Test system Method Guideline** | **Organism/ Strain(s)** | **Test substance** | **Concentrations tested (range)** | **Result** | | **Remark** | **Reference** |
|---|---|---|---|---|---|---|---|
| | | | | **+ S9** | **- S9** | | |
| ***Ammonium bromide*** | | | | | | | |
| Bacterial Reverse Mutation Test (Ames test) | *Salmonella Typhimurium* (TA98, 100, 1535, 1537) | Ammonium bromide | 17-5000 µg/plate | negative | negative | No cytotoxicity<br>Positive controls | A6.6.1/01, Doc. No. 557-002 |

| Test system Method Guideline | Organism/ Strain(s) | Test substance | Concentrations tested (range) | Result | | Remark | Reference |
|---|---|---|---|---|---|---|---|
| | | | | + S9 | - S9 | | |
| OECD TG 471 | *Escherichia Coli* (WP2 uvrA) | | | | | demonstrated the sensitivity of the assay | Study report, 1998d |
| Mammalian Cell Gene Mutation Test<br>OECD TG 476 | Mouse lymphoma cells L5278Y | Ammonium bromide | 1000-5000 µg/ml | negative | negative | Cytotoxic at the highest concentration used (5000 µg/mL)<br><br>Positive controls demonstrated the sensitivity of the assay | A6.6.3/01, Doc. No. 557-001<br><br>Study report, 1998e |
| ***Sodium bromide*** | | | | | | | |
| Bacterial Reverse Mutation Test (Ames test)<br>OECD TG 471 | *Salmonella Typhimurium* (TA98, 100, 1535, 1537, 1538) | Sodium bromide | 5-5000 µg/plate | negative | negative | No cytotoxicity<br><br>Positive controls demonstrated the sensitivity of the assay | A6.6.1/02, Doc. No. 557-004<br><br>Study report, 1988f |
| Mammalian Chromosome Aberration Test<br>OECD TG 473 | Cultured human lymphocytes | Sodium bromide | Without metabolic activation: 500-5000 µg/mL<br>With metabolic activation: 500.2-5002 µg/mL | negative | negative | No cytotoxicity<br><br>Positive controls demonstrated the sensitivity of the assay | A6.6.2/01, Doc. No. 557-005<br><br>Study report, 1988g |
| Unscheduled DNA Repair Synthesis<br>(deleted OECD TG 482) | HeLa S3 epitheloid cells, human cervical lymphoma | Sodium bromide | 12.5-25600 µg/ml | negative | negative | Cytotoxic at the highest dose level used (25600 µg/mL)<br><br>Positive controls demonstrated the sensitivity of the assay | A.6.6.3/02, Doc. No. 557-006<br><br>Study report, 1988h |

Table 31: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

| **Test system**<br>**Method**<br>**Guideline** | **Species**<br>**Strain**<br>**Sex**<br>**No/group**<br>**Vehicle** | **Test substance** | **Frequency of application** | **Sampling times** | **Dose levels** | **Results** | **Remarks** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| Mammalian erythrocytes micronucleus test<br>OECD TG 474 | Mouse CD1<br>M&F<br>5/sex (negative control)<br>5 M (low and mid dose group)<br>5 M&F (high dose group)<br>5 M (positive control)<br>Vehicel.: water | Ammonium bromide | 2 | 48 h after first application | 400-1600 mg/kg bw/day | negative | - | A6.6.4/01, Doc. No. 557-003<br><br>Study report, 1998f |

Table 32: Summary table of human data relevant for germ cell mutagenicity

| **Type of data/report** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No human data relevant for germ cell mutagenicity | | | | |

### 10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

**Ammonuim bromide - test guideline studies**

***Bacterial Reverse Mutation Test (Ames test; OECD TG 471) of ammonium bromide (Study report, 1998d)***

Ammonium bromide was tested for mutagenic activity in Salmonella typhimurium strains TA 1535, TA 1537, TA98 and TA 100 and Escherichia coli WP2uvrA.

Bacteria were exposed at concentrations ranging from 17 to 5000 µg ammonium bromide per plate in presence and absence of S9 mix in triplicate plates each. No cytotoxicity (clearing of background lawn) or precipitation was observed up to and including the maximum concentration of 5 mg/plate.

A test substance was considered mutagenic if:
- a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 1535, TA 1537 and WP2 uvrA) or 1.5-times (strains TA 100) the colony count of the corresponding vehicle control was observed
- a related dose response where mutagens require metabolic activation was seen
- a reproducible effect in independent tests was observed

No mutagenic activity was observed in any of the 5 bacterial strains tested both in the absence and presence of S9 mix. Positive controls were shown to have significantly increased the number of revertants per plate and results obtained were within the ranges expected for each bacterial strain and activation condition.

***Mammalian Cell Gene Mutation Test (OECD TG 476) of ammonium bromide in mouse lymphoma cells L5278Y (Study report, 1998e)***

Ammonium bromide was examined for its ability to induce mutations at the thymidine kinase locus: tk+tk-to tk-tk-of mouse lymphoma L5178Y cells. The test substance was dissolved in water and cells exposed to 1000-5000 µg/mL final concentration both in the presence and absence of metabolic activation (S9 mix). Cell sample cultures were incubated either with test solution, vehicle or positive control for four hours at 37°C.

An initial toxicity test was performed in the absence and presence of S9 mix to find the maximum usable limit dose allowing cell growth and subsequent cloning efficiency giving at least 10% of the concurrent vehicle control values. Concentrations of ammonium bromide used were in the range of 0.5 – 5000 µg/ml.

The preliminary toxicity test showed that ammonium bromide caused a significant reduction in the relative suspension growth only at the pre-set maximum concentration of 5000 µg/ml (56 % and 35 % in the absence and presence of S9 mix respectively).

No evidence of mutagenic activity was obtained from cultures treated with ammonium bromide in any of the 4 assays with and without metabolic activation. The solvent control values were within the normal ranges experienced in the performing laboratory and reported in the literature with the L5178Y cell line. The high mutant fractions obtained with EMS, MMS and 3-MC were within the normal ranges. 3-MC (which is not mutagenic in the absence of S9 mix) proved the efficacy of the S9.

***In vivo micronucleus study (OECD TG 474) of ammonium bromide in mice (Study report, 1998f)***

In an OECD TG 474 study (Mammalian Erythrocyte Micronucleus Test) CD-1 mice were orally exposed at concentrations of 400, 800 and 1600 mg/kg/day of test substance at 0 and 24 hours. Bone marrow samples were taken 48 hours after the initial dose. Suitable dose levels for the main test were selected in a dose range finding and limit toxicity test. A group of 5 male mice received the positive control cyclophosphamide at 0 and 24 hours at 50 mg/kg bw. Two thousand PCEs per animal were scored for micronuclei and the frequency of micronucleated PCEs determined. The PCE/NCE ratio as a measure of systemic toxicity was determined by using a minimum of 1000 erythrocytes (PCE + NCE) per marrow preparation.

During the dose range finding study with oral doses of ammonium bromide ranging from 50 to 2000 mg/kg/day clinical signs of subdued behaviour, hunched appearance, piloerection and rolling gait were observed. But no animal deaths occurred following dosing.

The non-toxicity in the dose range finding study was further investigated in a limit test using 2000 mg ammonium bromide/kg/day as oral doses for three male and three female mice as 2 daily doses. One female died following dosing. Clinical signs were subdued behaviour, hunched appearance, piloerection, rolling gait, tremors, unwilling to move, unable to use hindlimbs properly, cold, discharge, eyes half closed and pale, wet and stained perigenital region and ventral surfaces. Based on these investigations, the maximum tolerated dose of ammonium bromide was judged by the study author(s) to be in the region of 1600 mg/kg/day. Dose levels of 400 and 800 mg/kg bw were selected as the low and mid dose levels, respectively.

No micronucleus induction was detected in bone marrow erythrocytes of mice dosed with ammonium bromide concentrations of 400, 800 and 1600 mg/kg/day. However, it is also noted that

no effect on the PCE/NCE ratio was recorded and it is thus unclear if the bone marrow was exposed to the test substance. The positive control cyclphosphamide induced a significant increase in the number of micronucleated polychromatic erythrocytes and a suppression of the PCE/NCE ratio indicative for bone marrow toxicity was observed as well.

**Sodium bromide - test guideline studies**

***Bacterial Reverse Mutation Test (Ames test; OECD TG 471) of sodium bromide (Study report, 1988f)***

The mutagenic potential of sodium bromide (98 %) was examined in Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100, TA 1538 (EPA FIFRA 84-2). Tester strains were treated with 50, 150, 500, 1500 and 5000 µg/plate sodium bromide in the presence and absence of a metabolic activation system (S9 mix) in triplicate each and revertant colonies were counted after 72-hour incubation time. Sodium bromide was not cytotoxic in a dose range finding study up to and including a concentration of 5000 µg/plate. Therefore, 5000 µg/plate was chosen as the top dose level in the mutation test. A compound was deemed to provide evidence of mutagenic potential if a statistically significant dose-related increase in the number of revertant colonies of at least twice the concurrent solvent control was obtained in two separate experiments

No substantial increase in revertant colony numbers of any of the five tester strains when compared with revertants in solvent controls were observed following treatment with sodium bromide at any dose level, either in the presence or in the absence of metabolic activation (S9 mix). No cytotoxicity or precipitation was observed up to and including the maxium concentration of 5 mg/plate.

Positive controls were shown to have significantly increased the number of revertants per plate and results obtained were within the ranges expected for each bacterial strain and activation condition.

***Mammalian Chromosome Aberration Test (OECD TG 473) of sodium bromide in cultured human lymphocytes (Study report, 1988g)***

Sodium bromide, technical grade was tested for its ability to induce chromosomal aberrations in human lymphocytes cultured in vitro. Cultured human lymphocytes were exposed to the test substance both in the presence and in the absence of a metabolic activation system (S9 mix). Cell division was arrested using colchicine, chromosomes were prepared and examined for structural and numerical aberrations.

A preliminary toxicity test indicated that with and without metabolic activation, no decrease of the mitotic index below 50% of the solvent control was observed at any dose level. The highest dose level was the maximum achievable concentration of 5002 µg/mL was therefore chosen as the highest dose level for the metaphase analysis in addition of lower dose levels of 2501 and 500.2 µg/mL. In both the presence and absence of metabolic activation, sodium bromide caused no statistically significant increase in the proportion of metaphase figures containing chromosomal aberrations when compared with the concurrent control. The respective positive controls caused statistically significant increases in the number of chromosomal aberrations thereby proving the sensitivity of the test system.

***Unscheduled DNA Repair Synthesis study of sodium bromide in HeLa S3 epitheloid cells, human cervical lymphoma (Study report, 1988h)***

The study was designed to test sodium bromide for mutagenic potential by measuring its ability to induce DNA repair in cultured human epitheloid cells (HeLa S3 cells). 100 µL aliquots of sodium bromide solution was added to cells at twelve concentrations ranging from 12.5 to 25600 µg/mL. 4-nitroquinoline-1-oxide and 2-aminoanthracene, both dissolved in dimethylsulfoxide, served as control compounds in absence and presence of S9 mix respectively. After incubation for 180 minutes the cultures were harvested, washed, fixed, stained and processed for autoradiography. One-hundred (100) non-S-phase nuclei were examined from each culture and the number of silver grains overlying these nuclei and a corresponding adjacent area of cytoplasm was counted.

The highest concentration of sodium bromide used (25600 µg/mL) was toxic to the cells. The test substance did not show any statistically significant increase in the nuclear grain count in either test in the absence of S9 mix. In the first test in the presence of S9 mix Sodium Bromide did not cause statistically significant increases in nuclear grain count. In the second test in the presence of S9 mix a small but statistically significant increase (P less than 0.05) in the gross nuclear grain count was obtained at a single concentration of sodium bromide (1600 µg/mL). No increase in the grain count was apparent, however, when the corresponding cytoplasmic labelling levels were taken into account, i.e. there was no increase in the net nuclear grain count. There was also a small increase in the net nuclear grain count at 400 µg/ml of test substance, but no increase in the gross nuclear grain count at the same concentration. These two increases are thought to be due to chance variation rather than a treatment-related effect since the two increases were small, not reproducible between tests and unrelated to dose.

### 10.8.2 Comparison with the CLP criteria

In the available in vitro studies of ammonium bromide and sodium bromide there was no increase in mutant frequencies in bacterial cells and no increase in chromosomal aberrations or in mutant frequencies in mammalian cells. Moreover, there was no micronucleus induction in vivo in mice administered ammonium bromide, although it is noted that exposure of the bone marrow was not demonstrated. In comparison with criteria for germ cell mutagenicity, classification of ammonium bromide is therefore not possible.

### 10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on available data no classification of ammonium bromide for germ cell mutagenicity is warranted.

## 10.9 Carcinogenicity

Table 33: Summary table of animal studies on carcinogenicity

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| ***Potassium bromide*** | | | |
| Equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)<br><br>Rat, Fischer (F344) | Potassium bromide or bromine by methyl bromide fumigation<br><br>Oral (feed)<br><br>104 weeks<br><br>(interim sacrifice at 52 weeks) | Potassium bromide:<br>The incidence of prostatitis (20/60) was statistically significantly increased in males treated at 500 ppm potassium bromide in comparison with the control group (10/60)<br><br>The incidence of mononuclear cell leukaemia (11/60) was statistically significantly increased in females treated at 500 ppm potassium bromide, compared to control group (4/60)<br><br>LOAEL:>500 ppm (>16.5 and >20 mg bromine/kg bw/day for males and females, respectively)<br><br>Methyl bromide: | Mitsumori et al., 1990 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| ***Potassium bromide*** | | | |
| 60/sex/group | Potassium bromide:<br>500 ppm<br>(16.5 mg bromine/kg bw/day in males; 20 mg bromine/kg bw/day in females)<br><br>Methyl bromide:<br>80, 200 or 500 ppm (2.67, 6.77, 16.9 mg bromine/kg bw/day for males; 3.23, 8.29, 20.2 mg bromine/kg bw/day for females) | Slight depression of body weight gain from wk 60 onwards in males of the 500 ppm group (3-6%)<br><br>Tumour incidence rate was unaffected.<br><br>LOAEL:>500 ppm (16.9 and 20.2 mg bromine/kg bw/day, respectively) | |

Table 34: Summary table of human data on carcinogenicity

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No relevant human data on carcinogenicity | | | | |

Table 35: Summary table of other studies relevant for carcinogenicity

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other studies relevant for carcinogenicity | | | | |

### 10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

There are no chronic toxicity/carcinogenicity studies on ammonium bromide. A chronic toxicity and carcinogenicity study with potassium bromide and methyl bromide fumigated diet is however, available (presented in Appendix to Document III (PT12), Sections A6 (Toxicological and Metabolism Studies)). The

study was evaluated by the Netherlands (NL) in the draft CAR on sodium bromide PT 11/12. The accompanying text is copied from this report (Doc IIA):

"In a 104 week dietary toxicity study (Mitsumori et al, 1990) rats were exposed to 500 ppm bromide as potassium bromide (KBr) (equal to 16.5 and 20 mg total bromine/kg bw/day for males and females resp.) or 0, 80, 200 or 500 ppm bromide by methyl bromide fumigation (equal to 2.67, 6.77, 16.9 mg total bromine/kg bw/day respectively for males and 3.23, 8.29, and 20.2 mg total bromine/kg bw/day respectively for females). Despite some slight deviations from OECD Guidelines, the study was considered acceptable. Except for some slight changes (3-6%) in body weight gain in males of the highest dose of the methylbromide fumigated diet group, no detrimental changes were observed in the clinical examinations, mortality, food and water consumption, ophthalmoscopic examination, haematology, clinical chemistry, urinalysis or organ weights of the treated animals. It was concluded that a chronic NOAEL of 16.9 mg total bromine/kg bw/day can be derived."

In the disseminated REACH registration and in Appendix IIIA to the CAR of BAC and ammonium bromide it is also noted in the study summary of Mitsumori et al (1990) that the overall incidence of prostatitis (20/60) was significantly increased in males treated at 500 ppm potassium bromide in comparison with the control group (10/60) and that this lesion was described by the study author as a focal inflammatory lesion characterised by neutrophilic infiltration into the glandular space. However, there was no aggravation in the severity of prostatitis in the treated group: the severity of the prostatitis was very similar to that in the control group. From these findings, the study author considered that the increased incidence of prostatitis was of no toxicological significance.

In females treated at 500 ppm potassium bromide, mononuclear cell leukaemia (11/60) was statistically significantly increased compared to control group (4/60). In contrast, there was no increase in the incidence of mononuclear cell leukaemia observed in the females of the 500 ppm fumigated diet group. The authors of the study therefore reasoned that the increased incidence in mononuclear cell leukaemia in females of the 500 ppm potassium bromide group probably was not attributable to bromide.

Furthermore, the study authors concluded that histologically the non-neoplastic lesions as well as the tumours observed in the study were similar to those that are known to occur spontaneously in this strain (F344) of rats. Historical control data was stated to indicate that the increase of mononuclear cell leukaemia was not treatment related. The study authors reported the historical accumulated incidence of mononuclear cell leukemia among rats in their institute to be 91/389 (10.89%) and the range was 3.8 to 16.3%. The incidence (11/60, 18.3%) of mononuclear cell leukemia in females treated with potassium bromide was not statistically significant different compared with the historical accumulated control data and the incidence was slightly above the upper the range of the historical control incidences. However, it is unclear from the publication if the historical control data relates to animals of the same age and strain, and if the study period for the generated data was five years preceding the study, as recommend in OECD TG 453. Thus, the results of the study by Mitsumori et al show no evidence for a carcinogenic potential of bromide. However, it could be noted that effect levels in repeated dose toxicity studies of ammonium bromide and sodium bromide are approximately in the range of 100 – 750 mg/kg bw/day, and the acute toxicity of ammonium bromide and sodium bromide is low. Therefore, the doses in the carcinogenicity study of potassium bromide and fumigated methyl bromide appears to have been set very low in comparison, and possibly too low to be able to detect a carcinogenic potential of bromide.

In several reports from the open literature the bromide ion was shown to interfere with the morphology and function of the thyroid and thyroid hormones in rodents. The activation of the thyroid gland was characterised by an increase in weight of the organ and a histopathologically observed reduction in follicle size and increase in height of the follicular epithelium. These were accompanied by a decrease in serum thyroxine (T4), resulting in a sustained increase in the synthesis and secretion of TSH (Loeber et al., 1983; Velický et al., 1997a; Velický et al., 1997b; Velický et al., 1998; Velický et al., 2004). Sustained excessive level of TSH is considered to be the pathogenic factor responsible for thyroid tumour formation. However, in available test guideline repeated dose toxicity studies of ammonium bromide and sodium bromide there was no evidence of cellular changes (e.g. hyperplasia or metaplasia) that could be considered as preneoplastic changes. Of note for human relevance, is that there is a species difference in thyroid gland neoplasia

secondary to hormone imbalance. The rodent will exhibit an increase in thyroid gland neoplasia in the presence of mild to moderate increase in TSH (Van Leeuwen and Sangster, 1987). In contrast, no clear etiologic role for hypothyroidism in human thyroid cancer has been established even though chronic hypothyroidism, in the moderate to severe range, has occurred in humans in areas of endemic goiter (McClain, 1995, Capen, 1997).

Table 36: Compilation of factors to be taken into consideration in the hazard assessment

| Species and strain | Tumour type and background incidence | Multi-site responses | Progression of lesions to malignancy | Reduced tumour latency | Responses in single or both sexes | Confounding effect by excessive toxicity? | Route of exposure | MoA and relevance to humans |
|---|---|---|---|---|---|---|---|---|
| Rat, F344 | Mononuclear cell leukemia<br>HCD mean: 91/389 (10.89%)<br>HCD range: 3.8% to 16.3%.<br>(unclear if the HCD relates to animals of the same age and strain, and if the study period for the generated data was five years preceding the study, as recommend in OECD TG 453) | No | No information | No information | Single (females) | No | Oral, via diet | MoA not known |

### 10.9.2 Comparison with the CLP criteria

There is no evidence relevant to carcinogenicity from studies in humans of ammonium bromide or any other bromide salt. There is no animal carcinogenicity study of ammonium bromide available; the only available and relevant study is a chronic toxicity and carcinogenicity study of potassium bromide and methyl bromide fumigated diet in F344 rats. In this study, statistically significant increased incidence of mononuclear cell leukemia was observed only in females, not in males, administered the only tested dose of potassium bromide (500 ppm, equal to 16.5 and 20 mg total bromine/kg bw/day for males and females resp.) However, an increased incidence of tumors was not seen in either males or females administered methyl bromide fumigation of equivalent bromine dose (500 ppm, 16.9 and 20.2 mg total bromine/kg bw/day for males and females resp.). Comparison of the tumour incidence with historical control tumour data is particurlarly important in this case since F344 rats is a known to have a high spontaneous tumour incidence of mononuclear cell leukaemia. According to the study authors the incidence of mononuclear cell leukaemia was not statistically

significantly higher than the historical background incidence. However, the appropriatness of the historical control data is not clear based on the available information.

Therefore, available data do not fulfil the criteria for classification due to insufficient data.

### 10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification of ammonium bromide for caracinogenicity is warranted due to insufficient data.

## 10.10 Reproductive toxicity

### 10.10.1 Adverse effects on sexual function and fertility

Table 37: Summary table of animal studies on adverse effects on sexual function and fertility

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| ***Ammonium bromide – non-guideline reproductive toxicity studies*** | | | |

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| Dose range finding study of a reproduction toxicity study<br><br>GLP<br><br>Rat, Sprague Dawley<br><br>10/sex/group | Ammonium bromide<br><br>Purity 99.94%<br><br>Oral (feed)<br><br>0, 1600, 3200, 6400 ppm<br><br>Group mean achieved dosages of test material during pre-mating, mating, gestation and lactation:<br><br>0, 127, 242 and 503 mg/kg bw/day for males and 0, 228, 454 and 651 mg/kg bw/day for females<br><br>Duration: 2 weeks prior mating until the first generation had been weaned | *Note: no statistical analysis conducted due to the small group size. No histopathological examination.*<br><br>**Parental generation – general toxicity**<br><br><u>Mortalities and clinical observations</u><br><br>*Males and females:*<br><br>There were no premature deaths during the study.<br><br>↑ incidences of transient piloerection starting at 127/228 mg/kg bw/day<br><br>↑ incidences of rolling gait at 242/454 mg/kg bw/day: 9/10 males and 6/10 females<br><br>↑ incidences of neurotoxic effects including rolling gait, piloerection, hunched posture, generally ill condition, staining on the body and an unkempt appearance of the coat at 503/651 mg/kg bw/day in all animals displayed during the the whole study period<br><br>↑ incidences of hyperactivity in females (approx. 50%) at 503/651 mg/kg bw/day<br><br><u>Body weight and food consumption</u><br><br>*Males*<br><br>↓ bodyweight gain during weeks 0-8 (pre-mating) at 242 mg/kg bw/day (87% of control) and at 503 mg/kg bw/day (84% of control)<br><br>↓ food consumption during week 8 at 242 mg/kg bw/day and 503 mg/kg bw/day (88% of control in both groups)<br><br>*Females*<br><br>No obvious treatment related effect on bodyweight prior to mating at any dose level.<br><br>↓ bodyweight gain gestation days 0-20 (67% of control) at 651 mg/kg bw/day<br><br>↓ food consumption during lactation days 0-7 at 454 mg/kg bw/day (89% of control) (note: only animals rearing young to day 21 included, and there were no animals rearing young at 651 mg/kg).<br><br>Food consumption during the pre-mating and gestation periods was similar in all dose groups.<br><br>**Fertility, parturition and sexual function**<br><br>↓ fertility at 242/454 mg/kg bw/day (male fertility index[a]: 80%, female fertility index: 90%) and at 503/651 mg/kg bw/day (male | A6.8.2/01, Doc. No.553-001<br><br>Study report, 2001<br><br>Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | fertility index: 10%, female fertility index: 10%) compared to control (100% in both males and females)<br>([a]) *fertility index calculated as no.of males siring a litter/total no. of males used for mating, or no. of females with confirmed pregnancy/total no. of females used for mating)*<br>↑ mean duration of gestation at 454 mg/kg bw/day (22.1 days compared to 21.6 days in controls) and at 651 mg/kg bw/day (22.0 days compared to 21.6 days in controls). | |
| ***Ammonium bromide – test guideline repeated dose toxicity studies*** | | | |
| OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)<br>EPA OPPTS 870.3100 (90-Day Oral Toxicity in Rodents)<br>GLP compliant | Ammonium bromide<br>Purity: 99.91%<br>Oral (feed)<br>0, 100, 225, 500 (males), 750 mg/kg bw/day (nominal in diet)<br>13 weeks (90-days)<br>Post-exposure period: 4 weeks for the control and high | **Mortality and clinical observations**<br>3 premature terminations among males at 500 mg/kg bw/day<br>↑ clinical signs of subdued behaviour at 225 mg/kg bw/day (11/15 males) and 500/750 mg/kg bw/day (14/15 males and 9/15 females), rolling gait at 225 mg/kg bw/day (4/15 males) and 500/750 mg/kg bw/day (15/15 males and 13/15 females), staggering at 225 mg/kg bw/day (2/15 males) and 500/750 mg/kg bw/day (12/15 males and 11/15 females) and nasal bleeding at 500/750 mg/kg bw/day (5/15 males and 8/15 females)<br>**Body weight** | A6.4.1/01, Doc. No. 533-001<br>Study report, 2000a<br>Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| Rat, Sprague-Dawley male/female<br><br>25/sex/group (control and high doses), 15/sex/group (low and intermediate dose) | 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 | ↓ body weight gain at 225 mg/kg bw/day (10% males) and at 500/750 mg/kg bw/day (males 23% and females 22%)<br><br>**Food consumption**<br><br>↓ food consumption 500 mg/kg bw/day (males 7%)<br><br>**Reproductive organ weights**<br><br>↓ absolute weight of epididymides at 100 (10%, p<0.05), 225 (12%, p<0.01) and 500 mg/kg bw/day (22%, p< 0.001)<br><br>↓ absolute weight of testes at 225 (10%, p<0.05) and 500 mg/kg bw/day (16%, p< 0.001)<br><br>**Histopathology**<br><br>No effects reported. | |
| ***Ammonium bromide – non-guideline repeated dose toxicity studies*** | | | |
| Repeated dose toxicity: 4 week dose range-finding study<br><br>GLP compliant<br><br>rat (Sprague-Dawley) male/female<br><br>5/sex/group | Ammonium bromide<br><br>Purity: 99.94%<br><br>Oral (feed)<br><br>0, 100, 500, 1000 mg/kg bw/day (nominal in diet)<br><br>4 weeks (28 days) | ***Mortality and clinical observations***<br><br>↑ clinical signs of neurotoxicity and subdued behaviour at 500 (all males) and 1000 mg/kg bw/day (all males and 4/5 females)<br><br>***Body weight***<br><br>↓ body weight gain (males 49% and females 31%) at 1000 mg/kg bw/day<br><br>***Food consumption***<br><br>↓ food consumption (males 29%) at 1000 mg/kg bw/day<br><br>**Reproductive organ weights**<br><br>↓ mean absolute testes weight at 100, 500 and 1000 mg/kg bw/day (-11% (p<0.05), -11% (p<0.05), -16% (p<0.01) respectively)<br><br>↓ mean absolute epididymis weight at 500 and 1000 mg/kg bw/day (-14% (p<0.05) and -16% (p<0.01), respectively)<br><br>**Histopathology**<br><br>Not performed | A6.3.1/01, Doc. No. 532-001<br><br>Study report, 1999<br><br>Reliability 1 |
| ***Sodium bromide – test guideline reproductive toxicity studies*** | | | |
| Two-generation reproductive | Sodium bromide<br><br>Purity: 99.5% | **P generation – general toxicity**<br><br><u>Mortalities and clinical observations</u> | Study report, 2016a |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| toxicity study<br><br>OECD TG 416<br><br>GLP compliant<br><br>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, selected for post-weaning assessments (including reproductive assessments and production of the F2a litters).<br><br>The offspring from the second pairing of the P generation rats formed the F1b generation, terminated at day 40 postpartum.<br><br>Rat, Crl:CD(SD) | Oral (gavage)<br><br>0, 50, 175, 350/500 (M/F) mg/kg bw/day<br><br>P generation: once daily beginning 10 weeks before the first cohabitation period, during cohabitation(s) and, gestation, littering and postpartum periods until all F1a and F1b generation pups were weaned and continuing through to the day before euthanasia (>180 dosing days).<br><br>F1a generation (Subset A, rearing and mating): 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, and continuing through to the day before euthanasia.<br><br>Any dam in the process of parturition was not given the test or control substance formulations until the following work day. | *Males*:<br><br>↑ mortality: 4/24 in high dose group, 1/24 in intermediate, and 1/24 in low dose group were euthanized prior to study termination or found dead.<br><br>↑ incidences of adverse clinical signs ($p \leq 0.01$) in all 24 males in high dose at some time point during the study, including signs of neurotoxicity (decreased motor activity, ataxia, ptosis).<br><br>*Females:*<br><br>↑ mortality: 9/24 females (during pre-mating, mating and lactation) in high dose group and 2/24 females (during gestation and lactation) in intermediate dose group, 1/24 in low dose group (during gestation), 1/24 in control group (during lactation) were euthanized prior to study termination or found dead.<br><br>↑ incidences of adverse clinical signs, including signs of neurotoxicity (decreased motoractivity, ptosis and/or ataxia) in high dose group during the premating period (3 to 6 females, $p \leq 0.01$), during gestation (2 to 9, $p \leq 0.01$) and lactation (1 to 5 females, $p \leq 0.01$) compared to control group females.<br><br><u>Body weights</u><br><br>*Males:*<br><br>↓ body weight at high dose level: stat. sign. from study day 50 and throughout the study, > 10% lower than control from study day 78, >20% lower from study day 155 and up to 25% lower than control from study day 169.<br><br>↓ body weight at intermediate dose level: >10% lower than control ($p \leq 0.01$) from study day 134 (day 8 of second mating).<br><br>↓ body weight gain at high dose level: stat. sign. from study days 15-22, 14-55% lower than control until study day 71. Weight loss during study days 71-78 (first mating). Stat. sign. lower at most time points study days 113-169. Overall the body weight gain was 37% lower than control ($p \leq 0.01$) study days 1-183.<br><br>↓ body weight gain at intermediate dose level: stat. sign. decreased body weight gain at most time intervals. During the first week of first mating (study days 71-78) and during the first week of the second mating body weight loss was recorded ($p \leq 0.01$). During study days 155-176 the body weight gain was 19-35% of control gain.<br><br>*Females:*<br><br>No stat. sign effects on body weight or body weigh gain during premating phase and mating phase of first paring and no stat. sign. effects on body weight or body weigh gain during second pairing.<br><br>Gestational phase, first pairing:<br><br>↓ body weight at high dose level: 89.2% of control weights ($p \leq$ | Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| 24/sex/group in P generation mating to produce F1a.<br>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.<br>23/sex in control group, 22/sex in low dose group, 15/sex in intermediate dose group in F1a generation mating to produce F2. | | 0.05) at GD 20.<br>↓ body weight gain at high dose level: 72.3% of control ($p \leq 0.01$) during GD 14-20.<br>Lactational phase, first pairing:<br>↓ body weight at high dose level: 89.1% and 86.7% of control weights ($p \leq 0.05$, $p \leq 0.01$) at lactation day 7 and 21, respectively.<br>↓ overall body weight gain lactation day 0-21 at high dose level: only 1% of control gains ($p \leq 0.05$).<br><br><u>Food consumption</u><br>*Males:*<br>↓ food intake from week 4 (from 6%, p≤0.05 to 24%, p≤0.01) and onwards in high dose group and from week 6 (from 8%, p≤0.01 to 13%, p≤0.01) onwards in intermediate dose group.<br>*Females:*<br>↓ food consumption in first pairing during late gestation (14%, p≤0.01) and during lactation (from 43%, p≤0.01 to 73%, p≤0.01) at high dose, and in during lactation at intermediate dose (from 18%, p≤0.05 to 14%, p≤0.01).<br><br>**P generation - Organ weights and Histopathology**<br>No gross lesions related to treatment with sodium bromide occurred in males or females in any dose group<br>*Males:*<br>↓ absolute weight of left (8% and 19%) and right (6% and 21%) epididymis, left cauda epididymis 9% and 25%), left (8% and 14%) and right (7% and 13%) testis, seminal vesicles with (13% and 23%) and without ((12% and 16%) fluid, prostate 18%) and pituitary (12% and 19%) at 175 and 350 mg/kg bw/day compared to the control group (p≤0.05 and p≤ 0.01). But organ weights adjusted to body weights were all either not different or higher compared to control.<br>↑ debris in the epididymis at 350 mg/kg bw/day (19/20 males, severity ranging from mild to moderate) and at 175 mg/kg bw/day (4/23 males, minimal changes).<br>↑ spermatid retention in Sertoli cells at 175 mg/kg bw/day (11/23) and 350 mg/kg bw/day (all males)<br>↑ tubular spermatid retention at 175 mg/kg bw/day (9/23) and 350 mg/kg bw/day (17/20)<br><br>*Females:* | |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | ↓ absolute and relative (to body weight) ovary weight in high dose group (33% and 37%, respectively, p≤ 0.01).<br><br>↑ pituitary weight, absolute (30%, p≤ 0.05) and relative to body and brain weight (33%, p≤ 0.01) in high dose group.<br><br>↑ incidence of depletion of corpora lutea in intermediate dose group (3/24) and in high dose group (10/24) (including animals found dead or terminated prior to scheduled euthanasia) compared to no depletion in control group. One animal in low dose group had no corpora lutea present.<br><br>**P to produce F1a - Fertility, parturition and sexual function**<br><br>↓ Mating index[b] in high dose group, in males 42.1% compared to 95.8% in control and females 45.5% compared to 100% in control. In the 175 mg/kg/day dose group, in males 91.7% compared to 95.8% in control<br><br>↓ Fertility index[c] in intermediate and high dose group, in males 72.7 and 62.5%, respectively compared to 100% in control (p≤ 0.05) and females 72.7 and 60%, respectively compared to 100% in control (p≤ 0.05 and p≤ 0.01).<br><br>([b]) *percent of pairing that resulted in matings)*<br>([c]) *percent of matings that resulted in pregnancies)*<br><br>**P to produce F1b - Fertility, parturition and sexual function**<br><br>↓ Mating index in the 175 mg/kg/day dose group, in males 86.4% compared to 100% in control and females 86.4% compared to 100%<br><br>↓ Fertility index in the 175 mg/kg/day dose group, in males 73.7% compared to 100% in control (p≤ 0.01) and females 73.7% compared to 100% in control (p≤ 0.01).<br><br>**F1a generation (post weaning) – general toxicity**<br><br><u>Mortalities and clinical observations</u><br><br>There were no mortalitites in males. One female in control was euthanized due to a fractured limb. One female in the 50 mg/kg/day dose group was euthanized due to apparent complications with delivery.<br><br><u>Body weights</u><br><br>*Males:*<br><br>↓ body weight (p≤ 0.01) in the 175 mg/kg/day dose group 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 | |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | values, respectively.<br>↓ body weight gain (p≤ 0.01) in the 175 mg/kg/day dose group over the entire dosing interval (PND 22 to 148) and for specific intervals between PNDs 57 to 78 and 92 to 99 (p≤ 0.05 to p≤ 0.01).<br><br>*Females:*<br>No stat. sign effects on body weight or body weigh gain during premating phase and mating phase.<br>Gestational phase:<br>↓ body weight (p≤ 0.05) on GD 7 (-7%) and 10 (-6%) in the 175 mg/kg/day dose group<br>↓ body weight gain (-20% compared to control group, p≤ 0.05) GDs 0 to 7<br>Lactational phase:<br>↓ body weight (p≤ 0.01) on LDs 0 (-8%), 4 (-8%), 7 (-8%) and 14 (-6%) in the 175 mg/kg/day dose group<br><br><u>Food consumption</u><br>*Males:*<br>↓ food consumption ( -11%) p≤ 0.01) in the 175 mg/kg/day dose group over the entire dosing period (PNDs 22 to 147).<br>*Females:*<br>↓ food consumption in the 175 mg/kg/day dose group during late gestation and lactation reported for GDs 14 to 20 (-10%, p≤ 0.01) and 0 to 20 (-9%, p≤ 0.05), and LDs 0 to 14 (-11%, ≤ 0.01).<br><br>**F1a generation (post weaning) - Organ weights and Histopathology**<br>*Males:*<br>↓ absolute weight of the left cauda epididymis (12%), left testis (9%), seminal vesicles with fluid (15%), and prostate (18%) at 175 mg/kg bw/day compared to the control group (p≤ 0.01). But organ weights adjusted to body weight were stat. sign. different.<br>↑ weight (adjusted to body weight) of the left epididymis (7%, (p≤ 0.05 compared to control)<br>↓ number of motile sperm (75% of control, p≤ 0.01) and the total count of sperm (76% of control, p≤ 0.05) in the vas deferens at 175 mg/kg/day compared to the control value.<br>*Females:*<br>There were no adverse effects on ovary, uterus or pituitary | |

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>weights, absolute or relative (to brain or to body weight), in the 50 and 175 mg/kg/day dose groups<br><br>F1a generation to produce F2 - Fertility, parturition and sexual function<br>No effect on mating or pregnancy rate in any dose group.<br>↓ average number of estrous stages in the 14 day assessment in the 175 mg/kg/day dose group (2.7, p≤ 0.05) compared to the control group value (3.3)</td><td></td></tr>
<tr><td colspan="4">Sodium bromide – non-guideline reproductive toxicity studies</td></tr>
<tr><td>Three-generation reproduction study<br>Not GLP-compliant<br>Deviations including no food consumption, pup body weights, litter size determination.<br>Rat, strain not specified<br>Males and females<br>7-19/sex/ group</td><td>Sodium bromide<br>Purity: not reported<br><br>Oral (feed)<br><u>Breeding study and thyroid hormone investigation:</u><br>0, 75, 300, 1200, 4800, 19200 ppm (corresponding to 0, 6.75, 27, 108, 432 and 1728 mg/kg bw/day based on a default conversion of 1 ppm=0.09 mg/kg bw/day)<br><u>Cross-mating experiment:</u><br>Untreated males and females were mated with females and males of the 19200 ppm group.</td><td><u>Reproduction study</u><br><u>No adverse effects on body weights recorded. No information on clinical condition available.</u><br>↓ fertility index in F0: 25% at 4800 ppm and 0% at 19200 ppm compared to 70% in control (unclear if statistical analysis has been performed).<br>Fertility (%)<table><tr><td></td><td>0</td><td>75 ppm</td><td>300 ppm</td><td>1200 ppm</td><td>4800 ppm</td><td>19200 ppm</td></tr><tr><td>F1</td><td>70</td><td>70</td><td>72</td><td>65</td><td>25</td><td>0</td></tr><tr><td>F2</td><td>62</td><td>54</td><td>44</td><td>53</td><td>-</td><td>-</td></tr><tr><td>F3</td><td>52</td><td>67</td><td>80</td><td>45</td><td>-</td><td>-</td></tr></table>- = no breeding<br><br><u>Cross-mating study</u><br>↓ fertility index for females treated at 19200 ppm sodium bromide and mated with untreated males (20%), and for the untreated females mated with males treated at 19200 ppm (0%).<br><br><u>Thyroid hormone investigation:</u><br>Results are presented in section 10.12</td><td>A6.8.2/02, Doc. No. 592-002<br>Van Leeuwen, F. X. R. et al, 1983<br><br>Reliability 2</td></tr>
<tr><td colspan="4">Sodium bromide – test guideline studies repeated dose toxicity studies</td></tr>
<tr><td>OECD TG 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents) with</td><td>Sodium Bromide<br>Purity: 100.0%</td><td>Mortality and clinical observations<br>Males<br>↑ mortality at 500 mg/kg bw/day (4 animals euthanasied)<br>↓ motor activity postdose in 6/10 males in week 2 at 175</td><td>Study report, 2016b</td></tr>
</table>

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| the relevant sections relating to oestrous cycles, sperm evaluation and histopathology<br>OECD TG 416 (Two-Generation Reproductive Toxicity Study)<br><br>Rat, Crl:CD(SD)<br><br>Male/female<br>10/sex/group and an additional 10/sex/group for control and high dose groups were included for the recovery assessment. | Oral (gavage)<br><br>0, 60, 175, 500 mg/kg/day<br><br>90 days | mg/kg/day, but all animals recovered before the end of the working day.<br><br>↑ incidences (p≤ 0.01) 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) at 500 mg/kg/day.<br><br>*Females*<br><br>↓ motor activity postdose in 8/10 animals, only observed on study days 11-13 at 175 mg/kg/day, and all animals recovered before the end of the working day.<br><br>↑ incidences (p≤ 0.01) of 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) at 500 mg/kg/day.<br><br>**Body weight**<br><br>*Males*<br><br>↓ body weight at the end of dosing period, 81.2 % of control, (p≤ 0.01) at 500 mg/kg/day<br><br>↓ body weight gain for most weekly intervals after week 2, and at the end of the dosing period, 68.8% of control, (p≤ 0.01) at 500 mg/kg/day<br><br>*Females*<br><br>↓ body weight gain at the end of the recovery period (66.5% of control values) at 500 mg/kg/day (but body weight and weight gain were unaffected during the treatment period)<br><br>**Food consumption**<br><br>*Males*<br><br>↓ absolute intake over the treatment period, 91.4% of controls (p≤ 0.05) at 175 mg/kg/day and at 500 mg/kg/day average and relative food consumption were significantly reduced (p≤ 0.01) over the dosing period (study days 1 to 90) and for each weekly interval after week 1.<br><br>↓ average absolute food consumption values (p≤ 0.01) in first 2 weeks during the recovery period and relative food consumption values were significantly lower (p≤ 0.05) at the start of the recovery period (study days 90 to 92).<br><br>↑ overall intake during the recovery period (study days 90 to 147, p≤ 0.01).<br><br>*Females*<br><br>↓ absolute food consumption (study days 43 to 50, p≤ 0.01) and relative food consumption (study days 8 to 15, p≤ 0.01 and study | Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | days 43 to 50, p≤ 0.05) at 500 mg/kg/day<br><br>**Organ weights**<br>↓ absolute weights of left and right epididymis, left caudal epididymis, left and right testes, seminal vesicles with/without fluid and prostate at 500 mg/kg bw/day (p≤ 0.01) compared to control. But organ weights adjusted to body weights were all either not different or higher compared to control.<br><br>**Histopathology**<br>↓ number of normal sperm (88.6% of control, p≤ 0.01) at 500 mg/kg bw/day (% abnormal sperm 0.6, 0.6, 3.0**, 11.9** in control, 60, 175 and 500 mg/kg bw/day respectively)<br>↓ percent motile sperm from the vas deferens (75.3% of control, p≤ 0.05) at 500 mg/kg bw/day. (% motile sperm 91.2, 91.7, 87.1, 68.7* in control, 60, 175 and 500 mg/kg bw/day respectively)<br>↑ mean number of sperm with detached head or no head both at 175 mg/kg bw/day (deatched head: 5%, p≤ 0.01) and 500 mg/kg bw/day (detached head: 20.6%, p≤ 0.01, no head: 3.2%, p≤ 0.01) compared to the control group (detached head: 0.8% and no head 0.83).<br>↑ incidences of retained spermatids at the luminal surface or in basal Sertoli cell cytoplasm in the 175 and 500 mg/kg terminal euthanasia animals (2/10 and 9/9, respectively).<br>↑ incidences of females with no corpora lutea in the ovary (3/10) in the 500 mg/kg/day dose group (but overall follicle counts were not affected) | |
| ***Sodium bromide – non-guideline repeated dose toxicity studies*** | | | |
| No guideline study: 90-day oral repeated dose toxicity study<br><br>Rat, Wistar<br><br>male/female<br>10/sex/group | Sodium bromide<br><br>Oral (feed)<br><br>0, 75, 300, 1200, 4800, 19200 ppm (corresponding to 0, 6.75, 27, 108, 432, 1728 mg/kg bw/day)<br><br>90 days | **Mortality and clinical observations**<br>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.<br>↑ clinical signs of neurotoxic effects (motor incoordination of legs) at 19200 ppm (unclear if both males and females)<br><br>**Body weight and food consumption**<br>↓ body weight gain (23% in males) at 19200 ppm<br><br>**Organ weights**<br>↓ relative prostate weight at 4800 (33%, p≤0.01) and 19200 ppm (50%, p≤0.001) | A6.4.1/04, Doc. No. 592-005<br><br>Van Logten, M. J. et al., 1974<br><br>Reliability 2 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | ↑ relative adrenal weight (males 31%, p≤0.001) at 19200 ppm<br><br>**Histopathology**<br>*(Note: numbers, severity, or incidences not reported in the publication)*<br>↓ number of corpora lutea at 19200 ppm<br>↓ size of tubuli at 19200 ppm<br>↑ pituitary cysts (males) at 19200 ppm<br>↓ spermatogenesis (tendency) at 19200 ppm<br>↓ secretory activity of prostate (tendency) at 4800 and 19200 ppm | |
| No guideline study: 90-day oral repeated dose toxicity study on a low chloride diet<br><br>Rat , Wistar<br><br>male/female<br>10/sex/group | Sodium bromide<br><br>Oral (feed)<br><br>0, 8, 31, 125, 500 and 2000 ppm (corresponding to 0, 0.72, 2.8, 11, 45, 180 mg/kg bw/day)<br><br>90 days | *Note: the reults from the 90-day repeated dose toxicity on normal chloride diet is given in the study van Logten, M. J., et al., 1974. Here only results from the low chloride study is presented.*<br>**Mortality and clinical observations**<br>3 males and 3 females at 2000 ppm (low chloride diet) died during the study period<br>↑ motor incoordination of hind legs (no incidences given and unclear if both males and females) at 2000 ppm (low chlrodie intake)<br>**Body weight and food consumption**<br>↓ body weight gain after 12 weeks at 2000 ppm (males 31% and females 35%)<br><br>**Organ weights**<br>↓ relative (to heart weight) pituitary weight in females at 2000 ppm<br>↑ relative (to heart weight) adrenal weight in males at 2000 ppm<br><br>**Histopathology**<br>*(Note: numbers, severity, or incidences not reported in the publication)*<br>↓ spermatogenesis at 2000 ppm<br>↓ corpora lutea at 2000 ppm<br>↓ maturation of uterus at 2000 ppm | A6.4.1/05, Doc. No. 592-006<br><br>Van Logten M.J. et al., 1976<br><br>Reliability 2 |

Table 38: Summary table of human data on adverse effects on sexual function and fertility

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No relevant human data on adverse effects on sexual function and fertility | | | | |

Table 39: Summary table of other studies relevant for toxicity on sexual function and fertility

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| Endocrinology<br>4 or 12 weeks feeding study, alterations in the endocrine system were investigated<br>The study was initiated to ascertain whether alterations in the endocrine system in the rat detected during a semi chronic feeding study could be detected in male rats after exposure to high dietary concentrations of sodium bromide and moreover whether histopathological and immunocytochemical findings could be | Sodium bromide | Rat, Wistar<br>males<br>10/group<br>Oral (diet)<br>Duration: 4 or 12 weeks<br>0, 20, 75, 300, 1200 and 19200 mg/kg diet (corresponding to 0, 1.8, 6.75, 27, 108 and 1728 mg/kg bw/day) | 20, 75, 300 ppm:<br>No treatment related effects.<br><br>1200 ppm:<br>↑ thyroid weight (at 4 weeks)<br>↓ T4 level (at 4 weeks)<br>↑ FSH level (at 12 weeks)<br><br>19200 ppm:<br>↓ body weight (9% at 4 weeks; 21% at 12 weeks)<br>↑ thyroid weight (at 4 and 12 weeks)<br>↑ incidences of histopathological changes in thyroidea at 4 and 12 weeks (increase of follicles and a decrease in their size)<br>↑ incidences of histopathological changes in testes at 12 weeks (reduction of tubule diameter)<br>↓ spermatogenesis (at 12 weeks)<br>Changes in immunoreactivity in pituitary gland:<br>↓ GH at 4 and 12 weeks,<br>↑ TSH at 12 weeks,<br>↑ ACTH at 12 weeks<br><br>Changes in hormone levels in serum:<br>↑TSH at 4 and 12 weeks,<br>↑ FSH at 4 and 12 weeks,<br>↑ insulin,<br>↓ T4 level at 4 and 12 weeks,<br>↓ testosteron at 4 and 12 weeks,<br>↓ LH at 4 weeks,<br>↓ corticosterone at 4 and 12 weeks | A6.10/09, Doc. No. 592-036<br><br>Loeber et al., 1983<br><br>Reliability 2 |

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| correlated with serum-hormone levels | | | | |

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

The studies available to assess reproductive toxicity of ammonium bromide include one dose-range finding reproductive toxicity study, one oral 90-day repeated dose toxicity study and one four-week dose-range finding study for oral repeated dose toxicity. It is unclear why the the dose-range finding study for reproductive toxicity of ammonium bromide was not followed up by a main study.

Available and relevant studies with sodium bromide included in the assessment are a recent two-generation reproductive toxicity study, a three-generation reproductive toxicity study, a recent 90-day repeated dose toxicity study and two older (non-guideline) 90-day repeated dose toxicity studies.

**Ammonium bromide - non-guideline reproductive toxicity studies**

***Dose-range finding study for reproductive toxicity of ammonium bromide in rat (Study report, 2001)***

Ammonium bromide was administered to rats (10/sex/group) via food at concentrations of 0, 1600, 3200 and 6400 ppm (corresponding to 0, 127, 242 and 503 mg/kg bw/day in males; 0, 228, 454, 651 mg/kg bw/day in females). Animals were treated from two weeks prior to mating until the first generation had been weaned. No statistical analysis was performed due to the small group size.

Fertility, parturition and sexual function

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 was reduced at 3200 ppm at 6400 ppm and fertility index at was 80% at 3200 ppm in males and 90% in females, and at 6400 ppm fertility index was 10% in both sexes. Only one female out of seven with clear indication of mating became pregnant at 6400 ppm and the litter produced was dead before day 4 of lactation.

General toxicity

The clinical observations at 6400 ppm included rolling gait which was noted in all animals and was generally noted following the first few days of treatment and persisted throughout the treatment period (This is further discussed in section 10.12.). In addition, piloerection and hunched posture accompanied this finding. In females, approximately half of the animals showed hyperactivity. Most of the animals (both males and females) in this dose group also had an unkempt coat. The clinical effects observed in the group treated at 3200 ppm were the same, however no unkempt coat was noted and the effects were less severe. 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. At 1600 ppm, animals (3) showed transient piloerection only. Reduced overall (week 0-8) bodyweight gain was noted in males at 3200 ppm (13%) and 6400 ppm (16%). The reduction in body weight gain in the high dose group was

evident throughout the first week of treatment, but was comparable with the control group after that for the remainder of the study. Reduced food consumption was also noted in males at ≥3200. For females there was no obvious treatment related effect on bodyweight prior to mating at any dose level. In the 1600 and 3200 ppm dose groups there were no differences in body weight gain during gestation when compared with control. At 6400 ppm, the body weight gain during gestation were 33% less than control. However, there was only one animal in this group due to the poor pregnancy rate at this dose level. For this animal, the bodyweight gain throughout the gestation period was less than any individual animal in the control group. At the start of lactation body weights at 3200 and 1600 ppm were greater than control, however, by day 14 of lactation, the absolute weights were essentially similar in all dose groups.

Conclusion

The effects on fertility are considered as being treatment related. The slightly reduced fertility index in the mid dose group and the markedly decreased fertility index in high dose groups (only one dam became pregnant in the high dose group) are not considered as being secondary to adverse general toxicity. The general toxicity was not significant in terms of effects on body weight and body weight gains, but there were clinical observations (including rolling gait) in high dose group throughout the treatment period. However, the observed neurotoxicity is not expected to impact on fertility, but probably impacted the mating performance in the high dose group.

**Ammonium bromide – test guideline repeated dose toxicity studies of relevance for reproductive toxicity**

***Oral 90-day repeated dose toxicity study of ammonium bromide in rat (Study report, 2000a)***

In a 90-day repeated dose toxicity study of ammonium bromide rats were adminstered doses of 100, 225 mg/kg bw/day to both sexes, 500 mg/kg bw/day to males and 750 mg/kg bw/day to females.

Effects on reproductive organ weights after 13 weeks were only seen in males: decreased weight of epididymides at 100 (absolute: 10%, $p<0.05$), 225 (absolute: 12%, $p<0.01$) and 500 mg/kg bw/day (absolute: 22%, $p< 0.001$) and of testes at 225 (absolute: 10%, $p<0.05$) and 500 mg/kg bw/day (absolute: 16%, $p< 0.001$). The changes in organ weight following adjustment to body weight were not statistically significant different from control.

At 500 mg/kg bw/day following the 4 week recovery period epididymides weights (absolute 15% and adjusted 14%) were significantly lower than control. Also absolute prostate (24%, $p<0.05$) and absolute testes (9%, $p<0.01$) weights were significantly reduced, although the statistical significance had disappeared after adjustment to body weight. No correlation with histopathological findings was demonstrated.

The body weight after 13 weeks was reduced in males at 225 mg/kg bw/day (10%, $p <0.01$) and at 500 mg/kg bw/day (22%, $p<0.001$) and after the the 4 week recovery period at 500 mg/kg bw/day (9%, $p<0.05$). Reduced body weight gain in males were noted at ≥ 225 mg/kg bw/day and reduced food consumption at 500 mg/kg bw/day.

There were three premature terminations among males at 500 mg/kg bw/day, which were considered not treatment related according to the study author. Clinical signs of subdued behaviour and neurotoxic effects (abnormalities of gait) 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. The signs generally became apparent after approximately 8 weeks of treatment, and persisted until necropsy (main study animals) or at least the third week of the recovery period.

Conclusion

Dose-dependent decrease in absolute weights of epididymis and testes starting from doses at 100 mg/kg bw/day were recorded without association to any histopathological effects during the 13 week treatment period. Reduced organ weights of epididymis (both absolute and adjusted to body weight) and testes (absolute) also persisted through the 4-week recovery period at the highest dose tested in males (500 mg/kg bw/day). The reductions of weights of epididymis are thus considered as a direct effect of treatment in high dose group and not a consequence of lower body weight.

**Ammonium bromide – non-guideline repeated dose toxicity studies of relevance for reproductive toxicity**

***Dose-range finding study for a 90-day oral repeated dose toxicity of ammonium bromide in rat (Study report, 1999)***

In the 4-week dose-range finding study performed in rats (5/sex/group) with ammonium bromide concentrations of 100, 500 and 1000 mg/kg bw/day mixed with the diet, effects on reproductive organ weights were also noted.

High dose males showed marked decreased mean epididymides (16%, $p<0.01$), and testes (16%, $p<0.01$) absolute weights compared to control group. Intermediate dose group males also showed decreases in mean epididymides (11%, $p<0.05$), and testes (11%, $p<0.05$) absolute weights when compared to the control group. In low dose testes weight was 11% ($p<0.05$) lower compared to control. These apparent effects disappeared after covariance analysis.

Statistically significant decreased mean body weight (26%, $p<0.001$ compared to control) and body weight gain (49%, $p<0.001$ compared to control) were seen in high dose males.

No histophatological examination was done and therefore no association between changes in organ weight and histopathological effects can be done.

Conclusion

Statistically significant dereased mean absolute weights of male reproductive organs were observed at 100 and 500 mg/kg bw/day, without any statistical significant effects on body weight changes, and at 1000 mg/kg bw/day where body weight and body weight changes were markedly and statistically significantly reduced. Since only the absolute organ weight changes were affected and not the relative (to body weight) organ weights, and a correlation of the organ weight changes with histopathological effects was not possible due to the lack of histopathological data, the findings on male reproductive organ weights are of unclear toxicological significance.

**Sodium bromide – test guideline reproductive toxicity studies**

***Two generation reproductive toxicity study of sodium bromide (Study report 2016)***

In a two-generation reproduction study (performed according to a protocol similar to OECD TG 416) sodium bromide was administered via oral gavage to Crl:CD(SD) rats at dose levels of 0, 50, 150, 350/500 (male/female) mg/kg bw/day. Male and female P generation rats in control, low and intermediate dose groups were paired twice, owing to reduced pregnancy rate in intermediate and high dose groups. The first cohabitation period (F1a) was scheduled after 10 weeks of treatment and a second cohabitation period (F1b) was conducted for all but the high dose group. The first litter formed the F1 generation, dosed from day 21 postpartum and selected for post-weaning assessments (including reproductive assessments and production of the F2a litters). The litter (F1b) from the second cohabitation of the P generation rats was terminated at day 40 postpartum. The high dose groups (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. Consequently, P-generation male and female rats were administered sodium bromide for approximately 183 days and the F1-generation male and female rats, selected from the F1a litters, were exposed in utero, via lactation, and via oral gavage after weaning to dose levels of 0, 50 or 175 mg/kg/day for approximately 131 days.

P generation – fertility, parturition and sexual function

In the first cohabitation period (P generation to produce F1a) there was no effect of treatment on male or female mating performance or fertility at 50 mg/kg bw/day. At intermediate dose the mating index was 95.8% with all (treated + untreated) 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 ($p \leq 0.05$).

At high dose, eight out of 19 males (three males died prior to cohabitation, two males were not paired) paired with treated females were confirmed mated, and there were six pregnant females. Two females were mated by the same male so the total number of males siring a pregnancy was five. 12 males were re-paired with untreated females, of which 10 females were confirmed mated (83.3%) and seven achieved a pregnancy (70%). Two males did not mate either allocated (treated/untreated) female. Of the 17 mated males, 11 impregnated at least one female. 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 ($p \leq 0.05$ to $p \leq 0.01$).

Table 40: Summary of adverse effects on fertility and sexual function – P generation males

| | **Control** | **50 mg/kg bw/d** | **175 mg/kg bw/d** | **350 mg/kg bw/d** |
|---|---|---|---|---|
| **First cohabitation (generation of F1a)** | | | | |
| Number of males in cohabitation [N] | 24 | 23[a] | 24 | 19 [b,c] |
| Number of males that mated [d] [N (%)] | 24 (100.0) | 23 (100.0) | 23 (95.8) | 17 (89.5) |
| Fertility index [e,f] [N/N (%)] | 24/24 (100.0) | 21/23 (91.3) | 17/23 (73.9)** | 11/17 (64.7)** |
| **Second cohabitation (generation of F1b)** | | | | |
| Number of males in cohabitation [N] | 23 [g] | 23[b] | 23 [g,h] | - |
| Number of males that mated [e] [N (%)] | 23 (100.0) | 22 (95.6) | 19 (86.4) | - |
| Fertility index [f,g] [N/N (%)] | 23/23 (100.0) | 22/22 (100.0) | 14/19 (73.7)** | - |

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

a. Excludes values for rat 2248, which was found dead (accidental death) on Day 8 of study.
b. Excludes values for rats that were found dead or euthanized due to adverse clinical observations during cohabitation.
c. Excludes values for rats that were not assigned to cohabitation because there were no available female rats or the female rat was euthanized due to adverse clinical observations during cohabitation.
d. Includes only one mating for each male rat
e. Number of pregnancies/number of rats mated
f. Includes only one pregnancy for each rat that impregnated more than one female rat
g. Excludes values for rats that were not assigned to cohabitation because there were no available female rats.
h. Excludes values for rat 2252, which was euthanized on Day 114 of study due to adverse clinical observations.

In high dose females, 10 out of the 22 females (two females died before or during cohabitation) paired with treated males were confirmed mated and six females were pregnant. The 12 females which had not mated were re-paired with untreated males. In total, 20 of 22 females mated with treated/untreated males, and 15 were pregnant. The mating index was 45.5% for females mated with treated males (10/22), 83.3% (10/12) for females mated with untreated males, and 90.9% (20/22) including both treated and untreated males. The fertility index was 60% (6/10) for females mated with treated males ($p \leq 0.01$), 90% (9/10) for females mated with untreated males and 75% (15/29) including both treated and untreated males, compared to 100% in control females.

It appears from the above results that mating and fertility are decreased irrespective if males or females are treated. In high dose group, when treated females are paired with untreated males the fertility index is slightly higher compared to when treated males are paired with untreated females (90% versus 70%), pointing to that males may be more severely affected. The decreased mating index is probably due to the neurotoxic effects observed in high dose animals, since this is not seen in the intermediate dose group where clinical signs were less marked and at a lower incidence. Thus, the effect on mating index seen in high dose is likely treatment related but secondary to the the observed clinical signs of neurotoxicity.

However, the decreased fertility index may be concluded to be treatment related and not secondary to general toxicity since in the intermediate dose group this effect was statistically significantly lower than control and observed in absence of any marked general toxicity.

The number of estrous stages per 14 day assessment period was significantly reduced (2.3 versus 3.2 in control, $p \leq 0.01$) in the 500 mg/kg/day dose group compared to the control group, and there were more females with ≥ 6 consecutive days of diestrus (not statistically significant different from control). There was no effect on estrous cycles at 50 or 175 mg/kg/day.

In the second cohabitation period (P generation to produce F1b), there was no effect of treatment on male or female mating performance or fertility at 50 mg/kg bw/day. 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. At 175 mg/kg bw/day 22 males paired (one male died prior to cohabitation and one was not paired) were mated and 14 females were pregnant. There was no statistically significant difference in mating index (86.4% compared to 100% in control), however, the fertility index was significantly lower (73.7%) than controls (100%, $p \leq 0.01$). Both indices were within the historical control range according to the study authors. However, the dossier submitter notes that there is no information available for the historical control data (e.g. the number of studies, from which time period the HCD values are and if they are from the same laboratory, strain etc) and therefore considers the validity if this statement as low. The observed decrease is in fertility is in line with the observed decrease in fertility index at the same dose level in the first pairing.

There was a difference in the timing of matings in the high dose animals in the first pairing: significantly fewer matings (6/19) occurred in the first 5 days of cohabitation, when compared to controls (20/22). In the second cohabitation period, however, there was no effect of treatment on the timing of mating: all matings were within the first 5 days of the cohabitation period.

There was no difference in gestation index or duration of gestation at any dose level compared to control in either the first or the second cohabitation.

In the first cohabitation the number of P generation dams with stillborn pups and number of dams with all pups dying before day 4 postpartum were significantly increased ($p \leq 0.01$) at 500 mg/kg bw/day. In the second cohabitation the number of dams with stillborn pups was increased at 50 mg/kg bw/day (4/24, $p \leq 0.01$) compared to control (0/23) but not at 175 mg/kg bw/day. In the absence of effects at the higher dose the relevance of this finding is unclear. Moreover, there was no adverse effect on litter size.

Table 41: Summary of adverse effects on fertility and sexual function – P generation females

| **First cohabitation (P generation to produce F1)** | | | | |
|---|---|---|---|---|
| | **Control** | **50 mg/kg bw/d** | **175 mg/kg bw/d** | **500 mg/kg bw/d** |
| Number of females in cohabitation [a]<br>N | 24 | 24 | 24 | 22 |
| Number of mated females[b]<br>N/N (%) | 24/24 (100.0) | 24/24 (100.0) | 22/24 (91.7) | 10/22 (45.5) |
| Fertility index [c]<br>N/N (%) | 24/24 (100.0) | 22/24 (91.7) | 16/22 (72.7)** | 6/10 (60)** |
| Number of dams that delivered a litter<br>N (%) | 24 (100.0) | 22 (100.0) | 16 (100.0) | 6 (100.0) |
| Duration of gestation<br>mean±SD | 22.8 ± 0.4 | 22.6 ± 0.5 | 22.9 ± 0.4 | 22.8 ± 0.4 |
| Number of dams with stillborn pups<br>N (%) | 2 (8.3) | 4 (18.2) | 0 (0.0) | 3 (50.0)** |
| Number of dams with no liveborn pups<br>N (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Gestation index [d]<br>N/N (%) | 24/24 (100.0) | 22/22 (100.0) | 16/16 (100.0) | 6/6 (100.0) |
| Dams with all pups dying<br>Days 0-4 post partum<br>N (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 5 (83.3)** |
| **Second cohabitation (P generation to produce F1b)** | | | | |
| | **Control** | **50 mg/kg bw/d** | **175 mg/kg bw/d** | **-** |
| Number of females in cohabitation<br>N | 23 | 24 | 22 | - |
| Number of mated females<br>N (%) | 23 (100.0) | 23 (95.8) | 19 (86.4) | - |
| Fertility index<br>N/N (%) | 23/23 (100.0) | 23/23 (100.0) | 14/19 (73.7)** | - |
| Number of dams that delivered a litter<br>N (%) | 22 (100.0)<br>(excludes values for rats that were found dead during gestation) | 22 (100.0)<br>(excludes values for rats that were found dead during gestation) | 14 (100.0) | - |
| Duration of gestation<br>mean±SD | 22.7 ± 0.6 | 22.7 ± 0.5 | 22.8 ± 0.4 | - |
| Number of dams with stillborn pups<br>N (%) | 0 (0.0) [e] | 4 (18.2)** | 0 (0.0) | - |
| Number of dams with no liveborn pups<br>N (%) | 0 (0.0) [e] | 0 (0.0) | 0 (0.0) | - |
| Gestation index<br>N/N (%) | 21/21 (100) [e] | 22/22 (100.0) | 14/14 (100.0) | - |
| Dams with all pups dying<br>Days 0-4 post partum<br>N (%) | 0 (0.0) [e] | 0 (0.0) | 0 (0.0) | - |

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).

P generation – reproductive organ weights and histopathology

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

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 bw/day sodium bromide compared to the control group. Corpora lutea were present for all control females at the terminal kill, and one animal in each of 50 mg/kg bw/day and 175 mg/kg bw/day dose groups had no corpora lutea present. Two further females from the 175 mg/kg/day group (mated but not pregnant at the second cohabitation and killed on gestation day 25) had no corpora lutea present and another female (killed gestation day 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. In the high dose group, 10 females had depleted corpora lutea (although there was no effect on ovarian follicle counts and six of these females became pregnant): 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. This finding is considered to be likely related to the test substance since 3/20 females treated at 500 mg/kg bw/day in the recent 90-day dose toxicity study of sodium bromide (Study report 2016b) had no corpora lutea present, and in addition, the number of corpora lutea per female was reduced at the high dose level of 19200 ppm/kg diet (in excess of 1000 mg/kg body weight/day) in the study by van Logten et al (1974).

Table 42: Summary of ovarian changes in P-generation females (terminal euthanasia + animals euthanized earlier or found dead)

| | **Dose Level (mg/kg/day)** | | | |
|---|---|---|---|---|
| | **0** | **50** | **175** | **500** |
| **No. Rats per Group** | 24 | 24 | 24 | 15 |
| **Ovary (No. Examined)** | 22 | 23 | 22 | 15 |
| Depletion, luteal | (0) | (0) | (3) | (10) |
| Mild | 0 | 0 | 0 | 1 |
| Marked | 0 | 0 | 3 | 9 |

In the males dose groups 175 and 350 mg/kg/day, there were reductions (p≤ 0.05 to p≤ 0.01) in absolute weight occurring in all reproductive organs evaluated. According to the study author these changes reflected the reduced terminal body weights (86.3% and 75% of control body weight) 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 also significantly reduced (p≤ 0.05 to p≤ 0.01) compared to control values.

Test substance-related microscopic findings were also noted in the reproductive tract of males that had received 175 or 350 mg/kg bw/day sodium bromide, with a dose-related trend in incidence and severity.

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, showed a test substance-related trend in males. All males (20) at 350 mg/kg bw/day showed retained spermatid heads of minimal to moderate severity. 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. At 175 mg/kg/day 11/23 males were affected, with the majority showing only minimal changes and only 4 showing epididymal debris.

At 350 mg/kg/day, the percentage of motile sperm in the vas deferens was significantly reduced (80.7% compared to 92.3% in control, $p \leq 0.01$) and static count was statistically significantly increased (111.2 compared to 43.4, $p \leq 0.01$). The percentage of motile sperm in the vas deferens at 175 mg/kg/day was also significantly reduced (89.2% $p \leq 0.05$ compared to control) and static count was increased although not statistically significant different from control values.

Table 43: Selected sperm evaluation parameters - P-generation males

| | **Dose Level (mg/kg/day)** | | | | **HCD[a]** |
|---|---|---|---|---|---|
| | **0** | **50** | **175** | **350** | **Mean<br>Min-Max** |
| **No. Rats per Group** | 24 | 23 | 23 | 20 | |
| **Vas Deferens** | | | | | |
| Number Motile | 458.8 | 470.9 | 482.3 | 448.0 | 481.1<br>308.4 - 821.1 |
| % | 92.3 | 91.7 | 89.2* | 80.7** | 95.6<br>80.3 - 96.0 |
| Static Count | 43.4 | 45.3 | 63.0 | 111.2** | 50.5<br>15.6 - 101.8 |
| Total Count | 502.2 | 516.2 | 545.4 | 599.2 | 523.5<br>348.4 – 852.6 |
| **Caudal Epididymal** | | | | | |
| Sperm Count | 113.8 | 113.6 | 143.5 | 99.8 | 281.8<br>64-411 |
| Sperm Density[b] | 465.47 | 463.90 | 632.64 | 529.31 | 1142.5<br>599.6-1713 |
| **Testicular** | | | | | |
| Spermatid Density[c] | 175.8 | 143.23 | 152.55 | 187.00 | 122.5<br>27-227 |
| **Morphology (epididymal)** | | | | | |
| Normal | 192.0 | 189.9 | 184.7** | 157.4** | 190.4<br>168.8 – 202.1 |
| % Abnormal | 4.0 | 5.1 | 7.6** | 21.3* | 4.9<br>0.5 - 15.8 |
| Detached Head | 4.4 | 6.0 | 7.3* | 23.7** | 6.0<br>1.0 - 19.4 |

| | | | | | |
|---|---|---|---|---|---|
| No Head | 3.2 | 3.0 | 5.4 | 17.8** | 3.2<br>0.1 - 12.3 |
| Flagellum | 0.3 | 1.1** | 1.2 | 0.7 | 0.4<br>0 - 2.5 |

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).
(a) Historical control data generated from 65 studies from 1998 to 2013.
(b) million sperm/mL.
(c) million spermatid/mL.

P generation – general toxicity

In the high dose groups of males and females (350/500 mg/kg bw/day) severe toxicity was reported, characterized by increased mortality (4 males and 9 females died or were terminated early) and adverse clinical observations ($p \leq 0.01$), including 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. Reduced body weight gain was observed in males from week 6 onwards and body weight at the end of the dosing period was 74% ($p \leq 0.01$) of control values: food intake was also lower from week 4 onwards. In females, reduced body weight gain and food intake was observed only during late gestation (-10.8%) and lactation (-10.9% to -13.3%). Since the mortality rate of males and females at 350/500 mg/kg bw/day in the P generation were both > 10%, the general toxicity is considered as excessive and results from this dose group would normally not be acceptable for further evaluation according to the OECD TG 416 and CLP Annex I: 3.7.2.4.4.

In the 175 mg/kg bw/day dose group, similar clinical signs (dehydration, ungroomed coat, chromodacryorrhea, hunched posture, ptosis, urine-stained abdominal fur, chromorhinorrhea) occurred but they were less marked and at a lower incidence (not statistically significant different from control), especially in females. 2 out of 24 females died during gestation and lactation. Effects on body weight were only observed in males and were more moderate, with a terminal mean body weight of 86.8% ($p \leq 0.01$) of the control value, and reduced food intake from week 6 onwards. Female food intake was reduced only in early lactation.

Administration of 50 mg/kg bw/day sodium bromide had no adverse effect on body weight gain or food intake in males or females of the P generation.

Changes in absolute/relative organ weights (other than reproductive organs) were observed at all dose levels in males and and in high dose females but in absence of histopathological findings these were not considerd adverse (see Annex I).

F1 generation - fertility, parturition and sexual function

In the F1 generation, there was no effect on mating or fertility of males and females at 50 or 175 mg/kg/day. 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). A total of 22/23 (95.6%), 22/22 (100.0%) and 14/15 (93.3%) of the F1 females were pregnant and delivered a litter in the 0, 50 and 175 mg/kg/day dose groups, respectively.

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 or pregnancy rate.

The duration of gestation, gestation index and mean number of implantation sites per dam was not affected by administration of sodium bromide at 50 or 175 mg/kg/day.

F1 generation – reproductive organ weights and histopathology

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

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. The study author considers this to be 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 bw/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 according to the study author.

In males at 175 mg/kg/day, the absolute weight of the left cauda epididymis (88% of control), left testis (91% of control), seminal vesicles with fluid (85% of control), and prostate (82% of control) 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. These organ weight changes reflected the reduced terminal body weight (87% of control) in the group and were therefore probably less adverse.

At histopathological examination, the number of motile sperm ($p \leq 0.01$) and the total count of sperm ($p \leq 0.05$) in the vas deferens were recorded as significantly reduced at 175 mg/kg bw/day compared to the control value. However, there was no significant effect on percent motile or static sperm count from the vas deferens, and cauda epididymal sperm count/density and testicular spermatid count were all comparable to or higher than the concurrent control group and all values were within the historical control range. Three males were observed having a minimal/mild spermatid head retention at histopathology. The toxicological relevans of this effect is unclear since also one control male showed the same effect of mild severity.

Table 44: Selected sperm evaluation parameters - F1 generation males

| | **Dose Level (mg/kg/day)** | | | **HCD[a]** |
|---|---|---|---|---|
| | **0** | **50** | **175** | **Mean**<br>**Min-Max** |
| **No. Rats per Group** | 23 | 22 | 15 | |
| **Vas Deferens** | | | | |
| Number Motile | 521.8 | 504.7 | 389.2** | 481.0<br>308.4 - 821.1 |
| % | 90.4 | 89.2 | 88.8 | 95.6<br>80.3 - 96.0 |
| Static Count | 56.7 | 65.6 | 52.8 | 50.5<br>15.6 - 101.8 |
| Total Count | 578.5 | 570.3 | 442.0* | 523.5<br>348.4 – 852.6 |
| **Caudal Epididymal** | | | | |
| Sperm Count | 323.0 | 347.7 | 370.9 | 281.8<br>64-411 |
| Sperm Density[b] | 1099.05 | 1233.75 | 1454.95 | 1142.5 |

| | | | | |
|---|---|---|---|---|
| | | | | 599.6 – 1713 |
| **Testicular** | | | | |
| Spermatid Density[c] | 112.38 | 99.45 | 99.45 | 122.5<br>27-227 |
| **Morphology (epididymal)** | | | | |
| Normal | 189.2 | 190.5 | 191.7 | 190.4<br>168.8-202.1 |
| % Abnormal | 5.6 | 5.5 | 4.3 | 4.9<br>0.5 – 15.8 |

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).
(a) Historical Control Data generated from 65 studies from 1998 to 2013.
(b) million sperm/mL.
(c) million spermatid/mL.

F1 generation – general toxicity

There were no adverse clinical observations reported for the F1 generation, only minimal, sporadic and transient clinical observations in males and females. Male body weights were significantly reduced ($p \leq 0.01$) after week 9, and mean body weights were 87.9% ($p \leq 0.01$) of the control group value at the end of the dosing period in the 175 mg/kg bw/day dose group. Accordingly, male body weight gain was lower after week 10. Female body weights were not affected and values at the end of the premating period and at the end of gestation were 94.9% and 97.0% of control group values, respectively. Male food intake was reduced from study day 50 onwards, but female food intake was reduced in late gestation and early lactation only.

Administration of 50 mg/kg bw/day sodium bromide had no adverse effect on body weight gain or food intake in males or females of the F1 generation

Conclusion

In conclusion, adverse effects on reproductive capacity were observed in the **P-generation** at **350/500 mg/kg bw/day** 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 was very poor, and a selection for a second generation was therefore not possible. Since the observed adverse effects on fertility and reproductive system of both males and females occurred in presence of significant generalised toxicity (including neurotoxic effects, mortality, marked decreases in body weight gain and food intake), these findings are not considered sufficient as a basis for classification. However, at the next lower dose level where significant generalised toxicity is absent similar effects are seen that can be used as a basis for classification. At **175 mg/kg/day** there were decreases in fertility (approx. 73-74% of control values) in the P-generation in both pairings but less marked effects on clinical condition, body weight and food intake were observed in males and females. During the premating and mating there were no marked or statistically significant increases of clinical observations in males or females, and no statistically significant effects on body weights or body weight gains in females that impacted the reproductive performance. In males, the body weight was >10% lower than control during second mating and there were body weight loss recorded during the first week of mating in both pairings. However, at least in females, the adverse effets on fertility is clearly not considered as being secondary to general toxicity at 175 mg/kg bw/day.

In males there was an increase in the incidence of spermatid retention (mainly described as minimal) in this dose group but there was no direct correlation with other effects. Five females were not pregnant at either pairing, and two of these females had no corpora lutea..

Effects in the **F1 generation** treated at **175 mg/kg/day** were limited to minimal/mild spermatid head retention in three 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.

**Sodium bromide – non-guideline reproductive toxicity studies**

***Three-generation reproductive toxicity study of sodium bromide (Van Leeuwen, F. X. R. et al., 1983)***

In a three-generation reproductive toxicity study (no guideline, not GLP compliant) sodium bromide was administered to rats at dose levels of 0, 75, 300, 1200, 4800 and 19200 ppm (corresponding to 0, 6.75, 27, 108, 432 and 1728 mg/kg bw/day using a default conversion of 1 ppm=0.09 mg/kg bw/day) via the diet. Male rats of proven fertility were mated with females for the first time at the age of 4 months. In three successive generations, at least two litters per female rat were raised. The transplacental transport of bromide was investigated in the third litter of the first generation. In order to investigate the cause of the infertility observed, a cross-mating procedure was performed in which untreated males and females were mated with females and males of the 19200 ppm group. To study the reversibility of the effects, an additional litter was bred with parental animals of the highest dose group which were fed control diets for a period of 3 months after the 7-month treatment period. In addition to the investigation of the reproductive performance of rats, additional studies were carried out to examine the effect of bromide on thyroid and pituitary function.

Fertility, parturition and sexual function

There is no information on mating performance in this study. F0 animals treated at 19200 ppm diet were not fertile (0%), and fertility of the 4800 ppm dose group was reduced (fertility index 25% compared to 70% in control, unclear if statistical significantly different). There was no clear dose-respone relationship in any of the three (F0, F1, F2) generations. In F1 fertility index was 70% at 300 ppm and 85% compared to 62% in control at 1200 ppm (highest dose tested). In F2 the lowest fertility index was 87% at 1200 ppm (highest dose tested). It is noted that the fertility index in the controls of each generation also were low (70, 62 and 52% in F0, F1 and F2 respectively).

In the cross-mating study, only 20% of females treated at 19200 ppm sodium bromide and mated with untreated males and none of the untreated females mated with high dosed males (19200 ppm) became pregnant. Therefore, it appears that the observed effects were due to infertility of male as well as female rats. After being three month on the control diet, these animals were mated again (reversibility study) and a fertility index of 62% was recorded. This indicates some degree of recovery from the infertility effects..

Reproductive organ weights and histopathology

There is no information available on histological changes on any reproductive organ. The relative weights of uterus, ovary, testis, pituitary and adrenals were reported, and there were some statistically significant changes compared to control, but no dose-related or consistent findings. There is no information on sperm parameters, oestrus cycling, pre-coital interval, parturition or pregnancy outcome.

General toxicity

Body weights of the F0 animals at the termination of the study were not statistically significant different from control at 4800 ppm or at any lower dose levels either in males or females. There is no information available on body weights of the highest dose tested, 19200 ppm. There is no information available on clinical observations, mortality or body weight gains for any dose group or generation in this publication.

In F1 animals, the body weights at the termination of the study were not statistically significant different from control in any dose group either in males or females.

In F2 animals, the body weights at the termination of the study were statistically significant different from control at all dose levels tested in males (-15%, $p<0.01$, -10%, $p<0.05$, and -14%, $p< 0.01$ at 75, 300 and 1200 ppm respectively) and at the highest dose tested (1200 ppm, corresponding to 108 mg/kg bw/day) in females (-10%, $p<0.01$).

Body- and organ-weight determination did not reveal a clear pattern of dose-related effects in neither of the three generations. Statistically significantly decreased weights of the adrenals (-15%) of females of the F0 generation were noted at 1200 and 4800 ppm.

Conclusion

In this study there are indications of impaired fertility in F1 and F2 at 1200 ppm (108 mg/kg bw/day) and at 4800 ppm (432 mg/kg bw/day) and 19200 (1728 mg/kg bw/day) in F0, however since the fertility indices were low also in control groups in all three generations (52-70%), and there is no information on clinical observations for any of the animals, and no information on body weight of animals in the dose group of 19200 ppm the quality of the study and the relevance of the findings may be questioned. Moreover, the dose 1728 mg/kg bw/day may be considered to be in excess.

**Sodium bromide – test-guideline repeated dose toxicity studies of relevance for reproductive toxicity**

***90-day oral repeated dose toxicity study of sodium bromide in rats, including recovery assessments (Study report 2016b)***

In an oral repeat dose 90-day toxicity study in rats performed according to OECD TG 408 and with the relevant sections relating to oestrous cycles, sperm evaluation and histopathology according to OECD TG 416 sodium bromide was adminstered daily via oral gavage at doses of 0, 60, 175, 500 mg/kg bw/day. A Sodium Chloride comparator group (284 mg/kg/day) was also included in the assessment.

Organ weights and histopathology

There were statistically significant differences in absolute weights of reproductive organs in males, mainly at 500 mg/kg bw/day (left and right epididymis, left caudal epididymis, left and right testes, seminal vesicles with/without fluid and prostate). These were, however, either not statistically significant different or higher compared to control when adjusted for body weights.

Microscopic examination of testes revealed treatment related findings of retained spermatids at the luminal surface or in basal Sertoli cell cytoplasm in 2/10 in the 175 mg/kg 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 500 mg/kg dose groups had treatment related findings consisting 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. Moreover, at **500 mg/kg/day** there was a reduction (88.6% of control, $p\leq 0.01$) in the number of normal sperm, a reduction in the percent motile sperm from the vas deferens (75.3% of control, $p\leq 0.05$) 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 reduced, although not significantly different (68.5% of control) 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.

At **175 mg/kg/day**, the number of sperm with detached/no head was higher than the concurrent control (5.0 compared to 0.8, p≤ 0.05) and the percent abnormal sperm was increased (3.0% compared to 0.6% in control, p≤ 0.01). The study authors considered it unlikely to have been an effect of treatment since these increases were below the historical control values and the control values were unusually low. However, the DS notes, with regards to the control being unusually low for % abnormal sperm, that the control value is within the range of the submitted historical control data.

There was no effect on sperm count, motility or morphology at 60 mg/kg/day or 175 mg/kg/day.

Table 45: Selected sperm evaluation parameters

| | | Dose Level (mg/kg/day) | | | | | HCD[a] |
|---|---|---|---|---|---|---|---|
| | N | 0 | 284 | 60 | 175 | 500 | Mean<br>Min - Max |
| **Vas Deferens** | | | | | | | |
| Number Motile | 10 | 386.5 | 345.1 | 379.7 | 375.3 | 265.8 (9) | 481.0<br>308.4-821.1 |
| % | 10 | 91.2 | 88.4 | 91.7 | 87.1 | 68.7* (9) | 95.6<br>80.3- 437.8 |
| Static Count | 10 | 41.0 | 40.6 | 37.3 | 52.7 | 110.4* (9) | 50.5<br>15.6- 101.8 |
| Total Count | 10 | 427 | 385 | 417 | 428 | 376 (9) | 531.1<br>348.4- 852.6 |
| **Caudal Epididymal** | | | | | | | |
| Sperm Count | 10 | 270.8 | 241.0 | 259.9 | 219.6 | 185.6 (9) | 299.4<br>230.0-410.5 |
| Sperm Concentration[b] | 10 | 1015 | 922.3 | 987.9 | 872.2 | 912.0 (9) | 1150.6<br>599.6-1713.08 |
| **Testicular** | | | | | | | |
| Spermatid Concentration[c] | 10 | 85.0 | 103.4 | 76.4 | 69.3 | 85.0 (9) | 66.8<br>28.4-100.6 |
| **Morphology (epididymal)** | | | | | | | |
| Normal | 10 | 198.9 | 195.9 | 198.9 | 194.1 | 176.2** (9) | 190.4<br>168.8-202.1 |
| % Abnormal | 10 | 0.6<br>[0-1.5] | 2.0<br>[0-6.5] | 0.6<br>[0-1.5] | 3.0**<br>[0-7.5] | 11.9** (9)<br>[6-21] | 4.9<br>0.5-15.8 |
| Detached Head | 10 | 0.8<br>[0-3] | 3.2<br>[0-9] | 1.0<br>[0-2] | 5.0*<br>[0-15] | 20.6** (9)<br>[7-40] | 6.0<br>1.0-19.4 |
| No Head | 10 | 0.3<br>[0-1] | 0.9<br>[0-4] | 0.1<br>[0-1] | 0.9<br>[0-3] | 3.2** (9)<br>[1-8] | 3.2<br>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.

In females, there was no adverse effect of treatment with sodium bromide on estrous cycles and there were no statistically significant differences in ovary weight (absolute or relative to brain or body weight) in any treatment groups compared with controls reported. 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.

The DS notes that the study author states that 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 and considers that that this may impact on the conclusion of the observation. In contrast, the study pathologist considered that this deviation had no impact on the study results.

The absence of corpora lutea did not correlate with depletion of colloid in the thyroid or with thyroid hormone levels in individual animals.

General toxicity

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 study days 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**, 4 males (of which 2/10 of recovery group animals) had severe clinical signs and required euthanasia: all of these males had shown marked reductions in body weight, and 2/4 had decreased food intake. In surviving males significant increases ($p \leq 0.01$, compared to controls) in clinical signs, consistent with the known sedative effects of sodium bromide, were apparent with increase 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. Females at 500 mg/kg/day 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 (further details on clinical observations are described in STOT RE section 10.12).

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 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, $p \leq 0.01$). Female body weight and weight gain were unaffected during the treatment period. 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 control values ($p \leq 0.05$). At **500 mg/kg/day**, average and relative food consumption in males were significantly reduced ($p \leq 0.01$) over the dosing period (study days 1 to 90) and for each weekly interval after week 1. In females treated at 500 mg/kg/day, within the dosing period there were transient decreases in absolute and in relative food consumption; values for the entire period (study days 1-90) were comparable to controls.

Conclusions

The reproductive toxicity in high dose males treated at 500 mg/kg/day consisted of effects on sperm motility, morphology and sperm count, and statistically significant decreases in absolute reproductive organ weight (but not when adjusted to body weights). The observed adverse effects occurred in presence of severe toxicity characterised by adverse clinical observations, mortality (4/20) and reductions in body weight gain, food intake and water consumption and may therefore not be used as a basis for classification. However, similar effects were also observed at lower dose level. At 175 mg/kg/day, the number of sperm with detached/no head and the percent abnormal sperm was slightly (and statistically significantly) increased compared to control but the clinical signs were less severe compared to the high dose group and there were no deaths or effects on body weights or body weight gains. The effect on sperm parameters at this dose level cannot be considered as secondary consequences of significant generalised toxicity and they furthermore point to a dose related trend in increase in incidences. In females, three out of 10 animals in the 500 mg/kg/day dose group had no corpora lutea in the ovary, but overall follicle counts were not affected. No other adverse effects on reproductive organ weight, histopathology or estrous cycle were recorded in females in this study. The depletion of corpora lutea in female is considered a substance related effect not secondary to general toxicity.

**Sodium bromide –non-guideline repeated dose toxicity studies of relevance for reproductive toxicity**

***Non-guideline study: 90-day oral repeated dose toxicity study (Van Logten et al., 1974)***

In a 90-day feeding study sodium bromide was administered to rats (10/ sex/ group) at dose levels of 75, 300, 1200, 4800, 19200 ppm (0, 6.75, 27, 108, 432, 1728 mg/kg bw/day).

Several effects on the endocrine system (increased admidopyrindemethylase activity; histopathological findings in thyroidea, gonads, adrenals and pituitary; increased activity of thyroids; increased thyroid weight, reduced secretory activity of prostate, decreased spermatogenesis) were observed in this study.

Reduced secretory activity of prostate was recorded starting from 4800 ppm and decreased spermatogenesis at 19200 ppm. Additional histopathological findings in reproductive organs were tendency to decreased number of corpora lutea in females at 19200 ppm and reduced size of tubuli at 19200 ppm.

Other histological findings consisted of changes seen in the adrenals (decreased vacuolisation of zona fasciculate in both sexes at ≥ 75 ppm), pituitary (cysts in males at 19200 ppm), and thyroidea (follicles reduced in size in females at ≥ 4800 ppm; in males at 19200 ppm). In the absence of other effects the histopathological findings in the adrenals noted in males and females of the 75 ppm dosage group were not considered to be adverse effects.

Statistically significant adjusted (to body weight) organ weight changes included reduced prostate weight (33-50% at ≥ 4800 ppm), increased adrenal weight (31% in males at 19200 ppm) and increased thyroid weight (18-92% in females at ≥ 1200 ppm, 57% in males at 19200 ppm).

Clinical signs of neurotoxicity (motor incoordination of the hind legs, depressed grooming) were observed in both sexes at 19200 ppm, and reduced bodyweight gain (23%, $p<0.01$) in males were recorded at 19200 ppm. One female in the high dose group was euthanized after four weeks (tail eaten by cage mate), otherwise there was no mortality in any dose group. Food conversion was decreased in both sexes during the first few weeks.

Conclusion

Besides from the reduced secretory activity of prostate, the histopathological findings relevant for reproductive toxicity in this study (decreased spermatogenesis, decreased number of corpora lutea and reduced size of tubuli) are observed at a very high dose level (1728 mg/kg bw/day). However, they may be considered as supportive evidence for classification. Moreover, the tendency to decreased number of corpora lutea is in concordance with findings in other studies at lower doses. At the dose level of 1728 mg/kg bw/day the animals did not groom themselves sufficiently and exhibited signs of motor incoordination, but no mortality. The male animals showed significant reduced bodyweight gain (23%, $p<0.01$) but the absolute body weight of the animals were not reported. Based on the available information the general toxicity does not appear to be severe, and thus it is assumed that the observed effects on fertility at 1728 mg/kg bw/day are not a secondary consequence of this toxicity.

***Non-guideline study: 90-day oral repeated dose toxicity study on a normal diet and a low chloride diet (Van Logten et al., 1976)***

A 90-day feeding study (low chloride diet) was performed with sodium bromide in rats following administration of 8, 31, 125, 500 and 2000 ppm (0.72, 2.8, 11, 45, 180 mg/kg bw/day).

Several effects on the endocrine system were observed in this study, including increased thyroid activity, decreased spermatogenesis, histopathological findings in gonads, adrenals; reduced pituitary weight; increased adrenal weight.

In males reduced spermatogenesis and increased adrenal weight (22%, $p<0.05$) were recorded at 2000 ppm. Histopathological findings in the adrenals (decreased vacuolisation of the zona fasciculate) were seen in both sexes at 500 ppm. In females, histopathological effects were also recorded at 2000 ppm in ovaries (a decreased number of corpora lutea) and uterus (retardation in maturation). Organ weight changes consisted of reduced pituitary weight (22%, $p<0.05$) at 2000 ppm.

Reduced bodyweight gain were seen on both sexes at 2000 ppm (35%, $p<0.001$ in females and 31%, $p<0.001$ in males). At the same dose level mortalities (3 out of 10 animals both sexes) and clinical signs of neurotoxicity (motor incoordination of the hind legs, depressed grooming) were reported for both sexes.

Conclusion

The relevance of this study is unclear since it is performed using a low chloride diet. Moreover, at the highest dose tested, where effects on reproductive organs were seen, there were excessive toxicity with 30% mortality for both males and females. Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity are not relevant for classification purposes. Nevertheless, a comparison of the results of this study with the results of the 90-day study with sodium bromide in rats with normal diet (Van Logten et al., 1974) shows that low level of chloride in the diet enhances the toxicity of sodium bromide, while the target organs are still the same.

**Summary**

According to CLP Annex I, 3.7.1.3. adverse effects on sexual function and fertility includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems. In the dose-range-finding reproduction toxicity study and subchronic toxicity studies of ammonium bromide and in studies of sodium bromide (OECD TG 416 compliant two-generation reproduction toxicity study, OECD TG 408 compliant 90-day repeated dose toxicity study as well as several non-guideline studies) the following main effects on sexual function and fertility were noted:

Alterations to the female and male reproductive system, gamete production and transport

*Females*

In P females of the two-generation reproductive toxicity study of sodium bromide depletion of corpora lutea was observed in the ovary of 10 from the 500 mg/kg/day group in presence of excessive toxicity, and in 3/24 females in the 175 mg/kg/day dose group where no adverse general toxicity was recorded. In F1 females (tested up to 175 mg/kg bw/day) no depletion of corpora lutea was observed at histopathology, however, the number of estrous stages in the evaluation period was lower than controls and there were some differences in certain follicle types at ovarian examination for which an association with treatment could not be discounted.

Similar effects on the female reproductive organs were observed in the 90-day repeat dose toxicity study on sodium bromide. Depletion of corpora lutea was observed in 3/10 females in the 500 mg/kg/day group, which were all in estrus at termination, and in absence of severe general toxicity.

Also in a non-guideline 90-day repeated dose toxicity study of sodium bromide in rats the number of corpora lutea in the ovaries was found to be decreased, however at a very high dose level (1728 mg/kg bw/day) (Van Logten et al., 1974). These findings were seen in absence of severe general toxicity and are considered as supportive evidence for classification.

*Males*

In the two-generation reproductive toxicity study of sodium bromide at the high dose level (350 mg/kg bw/day) adverse effects on sperm count and morphology were reported. All P males at 350 mg/kg bw/day showed minimal-moderate cellular debris in the epididymis and/or spermatid head retention in the testis and 11/23 males in the 175 mg/kg/day group showed similar but, for the majority, minimal changes. Statistically significantly lower count of motile sperm in vas deferens was recorded in both 350 mg/kg bw/day and 175 mg/kg bw/day dose groups compared to control ($p\leq0.01$ and $p \leq0.05$) and the percentage of sperm with abnormal morphology in epididymis was increased (7.6% and 21.3%, $p\leq0.01$ and $p\leq0.05$ respectively, compared to control). Spermatid head retention in testis of F1 males

(175 mg/kg bw/day; highest dose tested) were not as clear (3 animals) as in P generation, and the total count and number of motile sperm in the vas deferens was lower than controls.

Also in the 90-day repeated dose toxicity study of sodium bromide treatment related findings of retained spermatids in testes in 2/10 in the 175 mg/kg bw/day group and in 9/9 of the 500 mg/kg terminal euthanasia animals were recorded. Four early deaths (of which two from the recovery group) in the 500 mg/kg dose groups had treatment related findings consisting of minimal to moderate spermatid retention in the seminiferous tubule epithelium and in Sertoli cells. Moreover, at 500 mg/kg/day there was a reduction (88.6% of control, $p \leq 0.01$) in the number of normal sperm and percent motile sperm from the vas deferens (75.3% of control, $p \leq 0.05$). At both 500 mg/kg bw/day and 175 mg/kg bw/day the mean number of sperm with detached head or no head were increased compared to the control group values.

The histopathological changes in the gonads observed in the high dose groups of the two-generation reproductive toxicity study and the 90-day repeated dose toxicity study occur in the presence of significant and severe generalised toxcicity and may not be used as a basis of classification, but as supportive information in the total weight of evidence. It could be noted however, that at the intermediate doses (175 mg/kg bw/kg), less adverse changes in the gonads were observed in absence of significant generalised toxicity indicating a treatment-related dose-dependent increase.

Additional supportive information are found in the non-guideline short term toxicity studies of sodium bromide in the rat where effects on testes (decreased spermatogenesis, reduction of tubules, decreased serum testosterone), and epdidymides (reduced weight) were observed at very high doses (1728 mg/kg bw/day) (Van Logten et al., 1974; Loeber et al., 1983). Reduced relative prostate weight (33%, $p \leq 0.01$) and reduced secretory activity of prostate at 432 mg/kg bw/day were also seen in the study by Van Logten et al (1974).

Reduced weight of testes and epididymis were seen both in the 90-day repeated dose toxicity study of ammonium bromide (Study report, 2000a) and the dose range finding reproductive toxicity study of ammonium bromide (Study report, 2001) at doses starting from 100 mg/kg bw/day. There were however no histopathological changes correlated with these weight changes.

Adverse effects on onset of puberty

Available information provide no evidence of adverse effects on onset of puberty.

Reproductive cycle normality

Some indications of effects on estrous cycle were seen in the P generation of the two-generation reproductive toxicity study of sodium bromide: slightly fewer (statistically significant different) estrous stages in the 14 day assessment period in the 500 mg/kg/day dose group compared to the control group, and there were more females with ≥ 6 consecutive days of diestrus (not statistically significant different from control). This effects were seen in at a dose level causing severe generalised toxicity. In F1 generation, 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) in absence of significant generalised toxicity but there was no effect on mating or pregnancy rate.

Sexual behaviour

Markedly impaired mating (although not statistically significant from control group) were observed in the two-generation reproductive toxicity study of sodium bromide at 350/500 mg/kg/bw/day. The decreased mating index is probably due to the neurotoxic effects observed in high dose animals, since this is not seen in the 175 mg/kg/day dose group where clinical signs were less marked and at a lower incidence. Thus, the effect on mating index seen in high dose is likely not treatment related. Similarly, in the dose-range findings reproductive study of ammonium bromide, mating performance was reduced at 503/651 mg/kg bw/day, a dose level where rolling gait were observed.

Fertility

The dose-range finding study of ammonium bromide reported slightly reduced fertility indices at 242/454 mg/kg bw/day (male fertility index: 80%, female fertility index: 90%) and markedly reduced male and female fertility indices at 503/651 mg/kg bw/day (10% compared to 100% in control). Only

one female became pregnant at 651 mg/kg bw/day and the litter produced was dead before day 4 of lactation. At this dose level clinical observations, including rolling gait, was observed but the reduced fertility is not considered to be a secondary consequence of this toxicity.

In the two-generation reproductive toxicity study of sodium bromide fertility was statistically significantly reduced in both cohabitation periods of P generation at 175 mg/kg bw/day (approx. 73% compared to 100% in control) in absence of severe general toxicity. Fertility was also severly reduced at 500 mg/kg/bw/day (approx. 60% compared to 100% in control) in presence of excessive general toxicity (mortality, adverse clinical signs and effects on body weights). In the F1 generation, there was no effect on mating or fertility.

In the three-generation reproductive toxicity study of sodium bromide impaired reproductive capacity was also reported for males and females. F0 animals treated at 1728 mg/kg bw/day were not fertile (0%), and fertility of the 432 mg/kg bw/day dose group was 25% compared to 70% in control (unclear if statistical significantly different). In F1 fertility index was 70% at 27 mg/kg bw/day and 85% compared to 62% in control at 108 mg/kg bw/day. In F2 the lowest fertility index was 87% at 108 mg/kg bw/day. It is noted that the fertility index in the controls of each generation were unusually low (70, 62 and 52% in F0, F1 and F2 respectively). Moreover, since there is no information on clinical observations for any of the animals, and no information on body weight of animals in the dose group of 19200 ppm the quality of the study and the relevance of the findings may be questioned.

Results from the cross-mating study of the 1728 mg/kg bw/day dose group, indicate that the observed effects were due to infertility of male as well as female rats and results from the reversibility study indicate that there is some recovery from the effects on fertility.

Parturition

No effects on parturition were reported in available studies.

Pregnancy outcomes

A slight increase in duration of gestation with increasing amounts of test substance was noted in the dose range finding study in the rat performed with ammonium bromide at 454 mg/kg bw/day and 651 mg/kg bw/day.

Premature reproductive senescence

Available information provide no evidence of premature reproductive senescence of ammonium bromide.

Modifications in other functions that are dependent on the integrity of the reproductive systems

Available information provide no evidence of further modifications in other functions that are dependent on the integrity of the reproductive system.

### 10.10.3 Comparison with the CLP criteria

The criteria for classification in Repr. 1B for adverse effects on sexual function and fertility are considered fullfilled since:

- There are clear evidence of effects on impaired fertility noted in the rat in studies of ammonium bromide and sodium bromide. The effects were severe, dose related and not solely a secondary consequence of general systemic toxicity. Moreover, the observed effects appears to be due to infertility of both male and female rats.
- There are some evidence of effects on the male reproductive organs seen in studies of ammonium bromide and sodium bromide in the rat: decreased organ weights, histopathological changes, adverse effects on sperm count, morphology, and motility. Moreover, there were some evidence indicating reduced spermatogenesis and reduced secretory activity of prostate. These effects were considered not being solely secondary non-sepecific consequences of systemic toxicity.

- There are some evidence for effects on female gonads: a decreased number of corpora lutea were noted in the subchronic toxicity studies of sodium bromide (both guideline and non-guideline) and in the two-generation reproductive toxicity study of sodium bromide. These effects were observed in the absence of severe systemic toxicity in females in the OECD TG 90-day repeated dose toxicity study, and in P females of the intermediate dose group in the two-generation reproductive toxicity study. In females of the high dose group in the two-generation reproductive toxicity study theses effects were seen in presence of excessive maternal toxicity.

  Thus, in a total weight of evidence the available data provide clear evidence of an adverse effect on both male and female sexual function and fertility and there is no mechanistic evidence to indicate that the observed effects are not relevant for humans. Classification in **Repr. 1B, H360F** is therefore warranted.

  Classification in Repr 1A is not appropriate as it should be based on human data and no reliable/robust human data specific of ammonium bromide is available. Moreover, human data on sexual function and fertility is not sufficient for the source substances (bromide salts), and thus read-across for data gap filling is not applicable.

  Classification in Repr 2 is not appropriate as the evidence for adverse effects on sexual function and fertility from existing experimental data on ammonium bromide and the source substances (bromide salts) is considered as clear evidence and not some evidence.

### 10.10.4 Adverse effects on development

Table 46: Summary table of animal studies on adverse effects on development

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| ***Ammonium bromide*** | | | |
| Pre-natal developmental toxicity study<br>OECD TG 414<br>GLP<br>Rat, Sprague Dawley (Charles River CD)<br>Females<br>24/group | Ammonium bromide<br>Vehicle: water<br>Oral (gavage)<br>Dose: 0, 100, 300, 1000 mg/kg bw/day<br>Days 6-19 of gestation | ***Maternal toxicity***<br>↑ mortality (one dam was sacrificed on day 10 of gestation due to the severity of clinical signs) at 1000 mg/kg bw/day<br>↑ maternal clinical signs and neurotoxic effects at 1000 mg/kg bw/day in all animals generally throughout the study<br>↓ maternal bodyweight gain days 6-12 (43% of control, p<0.001) and 6-20 (82% of control, p<0.001) at 1000 mg/kg bw/day<br>***Offspring***<br>↓ mean foetal weight (15% less than control, p<0.001) at 1000 mg/kg bw/day<br>↑ incidence of small foetus at 1000 mg/kg bw/day, foetal incidence: 24% compared to 2% in controls<br>↑ incidence of skeletal abnormalities/variants:<br>- kinked ribs, foetal incidence: 4.5, 9 and 25% at 100, 300 and 1000 mg/kg bw/day, respectively compared to 1.6% in controls | A6.8.1/03, Doc. No. 551-001<br>Study report, 2000b<br>Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | - reduction in size of the 13th ribs at 1000 mg/kg bw/day, foetal incidence: 6.8% compared to 0% in controls<br>- curved scapula at 1000 mg/kg bw/day, foetal incidence: 8.7% compared to 0.5% in controls<br>- slightly kinked ribs at 1000 mg/kg bw/day, foetal incidence: 4% compared to 1% in controls<br>- incomplete ossification of ribs, foetal incidence: 2, 9 and 16% at 100, 300 and 1000 mg/kg, respectively compared to 0% in controls.<br>↑ incidence of visceral abnormalities at 1000 mg/kg bw/day:<br>- kidney abnormalities (reduced/absent/displaced/cystic) of the left kidney often associated with absences of the left adrenal and/or left ureter, foetal incidence: 12.5% compared to 0% in controls<br>- narrowing of the left uterine horn with flattened ovarian end and displaced from ovary, foetal incidence: 7% compared to 0% in controls<br>- spleen abnormalities, flattened and/or reduced in size, foetal incidence: 9% compared to 0% in controls<br>- reduced/absent thyroid, foetal incidence: 3.8% compared to 0.5% in controls<br>↑ incidence of displaced testis, foetal incidence: 4, 8 and 10 % at 100, 300 and 1000 mg/kg bw/day compared to 1.6% in controls (stat. analysis not performed). | |
| Pre-natal developmental toxicity study<br>OECD TG 414<br>GLP<br>Deviations: In addition to the requirements of OECD TG 414 two recovery groups were included in the study. Animals of these additional groups were allowed to | Ammonium bromide<br>Vehicle: water<br>Oral (gavage)<br>Doses: 0, 50, 300, 600, 800 mg/kg bw/day<br>Days 6-19 of gestation | *No statistical analysis of the data performed in this study*<br>*Prenatal study:*<br>***Maternal toxicity***<br>↑ mortality (one dam was sacrificed GD 11 due to the severity of clinical signs) at 600 mg/kg bw/day<br>↑ incidence of maternal clinical signs (neurotoxic effects) at 600 and 800 mg/kg bw/day<br>↓ maternal bodyweight gain (9%) at 800 mg/kg bw/day<br>***Offspring***<br>↑ foetal incidence of kinked ribs: 5.4, 8.5, 6.7% at 300, 600 and 800 mg/kg bw/day compared to 0.4% in controls,<br>↑ foetal incidence of curved scapulae: 1.8, 2.2 and 5.5 % at 300, 600 and 800 mg/kg bw/day compared to 0% in controls,<br>↑ incidence of foetuses with 13 complete ribs: 96%, 87% and | A.6.8.1/04, Doc. No. 551-002<br>Study report, 2007a<br>Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| litter and rear new-borns until weaning.<br><br>Rat, Sprague Dawley (Crt:CD(SD))<br>Females<br>22/group | | 76% at 300, 600 and 800 mg/kg bw/day respectively, compared to 92% in controls<br>↑ foetal incidence of incomplete ossification of ribs: 19%, 29% and 24% at 300, 600 and 800 mg/kg bw/day respectively, compared to 3% in controls<br><br>*Postnatal study:*<br>↓ period of gestation (21.3 days compared to 21.8 days for controls) at 300 mg/kg bw/day (highest dose tested) | |
| Dose range finding study of a reproduction toxicity study<br>GLP<br><br>Rat<br>Sprague Dawley<br>10/sex/group | Ammonium bromide<br><br>Oral (feed)<br>0, 1600, 3200, 6400 ppm<br>Group mean achieved dosages of test material during treatment gestation and lactation: 0, 228, 454 and 651 (during treatment and gestation only) mg/kg bw/day for females<br>Duration: 2 weeks prior mating until the first generation had been weaned | *Note: no statistical analysis conducted due to the small group size. No histopathological examination.*<br><br>***Maternal toxicity***<br>There were no premature deaths during the study.<br>↑ incidences of transient piloerection starting at 228 mg/kg bw/day<br>↑ incidences of rolling gait (6/10) at 454 mg/kg bw/day<br>↑ incidences of neurotoxic effects including rolling gait (all animals), piloerection (5/10), hunched posture (8/10), generally ill condition, staining on the body and an unkempt appearance of the coat (8/10) at 651 mg/kg bw/day displayed during the the whole study period<br>↑ incidences of hyperactivity in females (approx. 50%) at 651 mg/kg bw/day<br><br>***Offspring - F1 generation***<br>↑ pup mortality (all pups in 4 out of 9 litters died before day 21 of lactation) at 454 mg/kg bw/day and at 651 mg/kg bw/day (the only litter produced did not survive to day 4 of lactation)<br>↓ mean weights of litter and pups (>10%) at 454 mg/kg bw/day from lactation day 7 | A6.8.2/01, Doc. No.553-001<br><br>Study report, 2001<br><br>Reliability 1 |
| ***Sodium bromide*** | | | |
| Pre-natal developmental toxicity study<br>OECD TG 414 | Sodium bromide<br>Purity 99.84%<br><br>Vehicle: distilled water | ***Maternal toxicity***<br>↑ mortality (one dam was sacrificed on Day 11 of gestation due to the severity of clinical signs) at 1000 mg/kg bw/day<br>↑ incidence of maternal clinical signs (neurotoxic effects in all animals) at 1000 mg/kg bw/day | A6.8.1/05, Doc. No. 551-003<br><br>Study report, 1995 |

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| Rat, Crl:CD BR VAF/Plus<br>Females<br>25/group | Oral (gavage)<br>0, 100, 300, 1000 mg/kg bw/day<br>Days 6-15 of gestation | ↓ maternal bodyweight gain at 300 mg/kg bw/day (days 16 to 20: 15%, p≤0.01) and at 1000 mg/kg bw/day (days 6-12: 31%, p≤0.05; days 16-20: 16%, p≤0.01)<br>↓ food consumption (9%, days 18-19, p≤0.01) at 1000 mg/kg bw/day<br>***Offspring***<br>↑ fetal incidence of skeletal malformations at 1000 mg/kg bw/day: distorted/ ossification/irregularities of ribs (1.7% compared to 0% in controls). Incidences were 0% at all other dose levels<br>↑ fetal incidence of visceral malformations at 1000 mg/kg bw/day: absent kidney: 3%, absent ureter: 3%, absent uterine horn: 0.7%, narrow uterine horn: 2%, compared to 0% in controls for each malformation. Incidences were 0% at all other dosel levels.<br>↑ fetal incidence of skeletal anomalies at 300 mg/kg bw/day: reduced ossification of one or more cranial centres (14% compared to 4% in controls) and at 1000 mg/kg bw/day: minimally distorted ribs (6% compared to 0% in controls), irregular ossification of the thoracic vertebral centre (9% compared to 3% in controls), shortened/absent 13th ribs (6% compared to 0% in controls), reduced ossification of one or more cranial centres (20% compared to 4% in controls)<br>↑ fetal incidence of skeletal variants at 300 mg/kg bw/day: increased variant sternebrae (57% compared to 41% in controls), unossified sternebrae (40% compared to 28% in controls) and at 1000 mg/kg bw/day: increased variant sternebrae (80% compared to 41% in controls), reduced sternebrae (39% compared to 18% in controls), unossified sternebrae (63% compared to 28% in controls) | Reliability 1 |
| Dose range finding study-developmental toxicity<br>Rabbit, New Zealand White<br>Females<br>6/dosage group and 5 control animals; | Sodium bromide<br>Vehicle: water<br>Oral (gavage)<br>Day 3-28 of gestation<br>0, 100, 200, 400 mg/kg bw/day<br>Pre-study (non- | *Pre- study:*<br>↑ incidence of clinical signs (ataxia and decreased respiration) at 500 and 1000 mg/kg bw/day<br>*Pregnant phase of study:*<br>↑ incidence of clinical signs (ataxia) at 400 mg/kg bw/day<br>No embryotoxic or teratogenic effects recorded | A6.8.1/01, Doc. No. 551-004<br>Study report, 2008a<br>Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| pre-study (non-pregnant phase): 3/group | pregnant animals): 13 days<br>125, 250, 500, 1000 mg/kg bw/day (escalating dosing regimen)<br>500 mg/kg bw/day (fixed dose level) | | |
| Pre-natal developmental toxicity study<br>OECD TG 414<br>Rabbit, New Zealand White<br>Females<br>30/group | Sodium bromide<br>Vehicle: water<br>Oral (gavage)<br>0, 25, 75, 250 mg/kg bw/day<br>Days 6-28 of gestation | No treatment related effects at any dose-level: no maternal toxicity and no embryotoxic or teratogenic effects. | A6.8.1/02, Doc. No. 551-006<br>Study report, 2008b<br>Reliability 1 |
| Two-generation reproductive toxicity study<br>OECD TG 416 (with deviations: Male and female P generation rats were paired twice (excluding high dose group), owing to reduced pregnancy rate in intermediate)<br>Rat, Crl:CD(SD)<br>24/sex/group in P | Sodium bromide<br>Purity: 99.5%<br>Oral (gavage)<br>0, 50, 175, 350/500 (M/F) mg/kg bw/day<br>P generation: once daily beginning 10 weeks before the first cohabitation period, during cohabitation(s) and, gestation, littering and postpartum periods until all F1a and F1b generation pups were weaned and continuing through to the day before euthanasia (>180 dosing days).<br>F1a generation (Subset A, rearing | ***Maternal toxicity - P generation females***<br><u>Mortalities and clinical observations</u><br>↑ mortality: 9/24 females (during pre-mating, mating and lactation) in high dose group and 2/24 females (during gestation and lactation) in intermediate dose group, 1/24 in low dose group (during gestation), 1/24 in control group (during lactation) were euthanized prior to study termination or found dead.<br>↑ incidences of adverse clinical signs, including signs of neurotoxicity (decreased motoractivity, ptosis and/or ataxia) in high dose group during the premating period (3 to 6 females, p≤ 0.01), during gestation (2 to 9, p≤ 0.01) and lactation (1 to 5 females, p≤ 0.01) compared to control group females.<br><u>Body weights</u><br>No stat. sign effects on body weight or body weigh gain during premating phase and mating phase of first paring and no stat. sign. effects on body weight or body weigh gain during second pairing.<br>Gestational phase, first pairing:<br>↓ body weight at high dose level: 89.2% of control weights (p ≤ 0.05) at GD 20.<br>↓ body weight gain at high dose level: 72.3% of control (p ≤ | Study report, 2016a<br>Reliability 1 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| generation mating to produce F1a.<br><br>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.<br><br>23/sex in control group, 22/sex in low dose group, 15/sex in intermediate dose group in F1a generation mating to produce F2. | and mating): 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, and continuing through to the day before euthanasia.<br><br>Any dam in the process of parturition was not given the test or control substance formulations until the following work day. | 0.01) during GD 14-20.<br><br>Lactational phase, first pairing:<br><br>↓ body weight at high dose level: 89.1% and 86.7% of control weights ($p \leq 0.05$, $p \leq 0.01$) at lactation day 7 and 21, respectively.<br><br>↓ overall body weight gain lactation day 0-21 at high dose level: only 1% of control gains ($p \leq 0.05$).<br><br><u>Food consumption</u><br><br>↓ food consumption in first pairing during late gestation (14%, p≤0.01) and during lactation (from 43%, p≤0.01 to 73%, p≤0.01) at high dose, and in during lactation at intermediate dose (from 18%, p≤0.05 to 14%, p≤0.01).<br><br>***Maternal toxicity – F1 generation females***<br><br><u>Mortalities and clinical observations</u><br><br>One female in control was euthanized due to a fractured limb. One female in the 50 mg/kg/day dose group was euthanized due to apparent complications with delivery.<br><br><u>Body weights</u><br><br>*Females:*<br><br>No stat. sign effects on body weight or body weigh gain during premating phase and mating phase.<br><br>Gestational phase:<br><br>↓ body weight (p≤ 0.05) on GD 7 (-7%) and 10 (-6%) in the 175 mg/kg/day dose group<br><br>↓ body weight gain (-20% compared to control group, p≤ 0.05) GDs 0 to 7<br><br>Lactational phase:<br><br>↓ body weight (p≤ 0.01) on LDs 0 (-8%), 4 (-8%), 7 (-8%) and 14 (-6%) in the 175 mg/kg/day dose group<br><br><u>Food consumption</u><br><br>*Females:*<br><br>↓ food consumption in the 175 mg/kg/day dose group during late gestation and lactation reported for GDs 14 to 20 (-10%, p≤ 0.01) and 0 to 20 (-9%, p≤ 0.05), and LDs 0 to 14 (-11%, ≤ 0.01).<br><br>***Offspring***<br><br>**F1a** | |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | ↓ live birth index in in 500 mg/kg bw/day dose group, 88.9% compared to 98.8% in control (p≤ 0.01).<br>↓ viability index PND 0-4 in 500 mg/kg bw/day dose group, 0% compared to 96.6% in control (p≤ 0.01).<br>↑ number of litters and pups with no milk in the stomach, dehydration, cold to touch, not nursing and thin body condition (p≤ 0.01) in 500 mg/kg bw/day dose group from birth to PND 4.<br><br>**F1b**<br>↓ live birth index in 50 mg/kg bw/day dose group (97.5% compared to 100% in control, p≤ 0.01).<br>↑ incidence of bent tail in 175 mg/kg bw/day dose group (one was only apparent on day 21 post-partum) (two pups in two litters, p≤ 0.01) compared to the control group.<br>Tooth eruption in 175 mg/kg bw/day dose group was delayed (p≤ 0.05 to p≤ 0.01) on PNDs 11, 13 and 14 and for the average day 50% of the litters reached criterion.<br><br>**F1b** (post weaning to PND 40)<br>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.<br><br>**F2**<br>↓ viability PND 8-14 in 175 mg/kg bw/day dose group (2 dead pups versus 0 in control, p≤0.01). | |
| Three-generation reproduction study<br><br>Rat (strain not specified)<br>Male/female | Sodium bromide<br><br>Oral (feed)<br><br>0, 75, 300, 1200, 4800, 19200 ppm (corresponding to 0, 6.75, 27, 108, 432 and 1728 mg/kg bw/day based on a default conversion of 1 ppm=0.09 mg/kg | ***Maternal toxicity***<br>No adverse effects on body weights recorded. No information on clinical condition available.<br><br>***Offspring***<br>↓ viability index (viability at postnatal day 5) of F1 pups at 4800 ppm: 32% for the first litter, 61% for the second litter (compared to 90% in control)<br>↓ lactation index (viability at postnatal day 21) of F1 pups at 4800 ppm: 0% for the first litter, 100% for the second litter (compared to 95% in control, unclear if statistical analysis has been performed). | A6.8.2/02, Doc. No. 592-002<br><br>Van Leeuwen et al., 1983<br><br>Reliability 2 |

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>bw/day)</td><td>
<table>
<tr><th></th><th>0 ppm</th><th>75 ppm</th><th>300 ppm</th><th>1200 ppm</th><th>4800 ppm</th><th>19200 ppm</th></tr>
<tr><td colspan="7">Viability index**</td></tr>
<tr><td>F0</td><td>90</td><td>98</td><td>96</td><td>92</td><td>32/61¥</td><td>-</td></tr>
<tr><td>F1</td><td>92</td><td>88</td><td>80</td><td>97</td><td>-</td><td>-</td></tr>
<tr><td>F2</td><td>96</td><td>98</td><td>93</td><td>98</td><td>-</td><td>-</td></tr>
<tr><td colspan="7">Lactation index***</td></tr>
<tr><td>F0</td><td>95</td><td>96</td><td>95</td><td>94</td><td>0/100¥</td><td>-</td></tr>
<tr><td>F1</td><td>93</td><td>85</td><td>72</td><td>80</td><td>-</td><td>-</td></tr>
<tr><td>F2</td><td>99</td><td>99</td><td>99</td><td>99</td><td>-</td><td>-</td></tr>
</table>
** Viability index: No. of pups alive at Day 5 x 100/No. of pups born alive<br>
*** Lactation index: No. of pups alive at Day 21 x 100/No. of pups alive at Day 5<br>
¥ data are given separately for first and second litter<br>
- no breeding
</td><td></td></tr>
</table>

Table 47: Summary table of human data on adverse effects on development

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| There are case reports (see 10.10.5 below) indicating developmental growth retardation (high, weight and skull circumference) in infants exposed to bromide during the entire pregnancy. | | | | |

Table 48: Summary table of other studies relevant for developmental toxicity

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| Pre-natal developmental toxicity (no guideline)<br><br>Rat, Wistar<br>Male and female<br>16-20 offspring | Sodium bromide<br>Vehicle: water | Oral (gavage)<br><br>0, 40, 80, 120 mg/kg bw/day<br><br>Offspring to dams exposed day 3 to day 20 of gestation<br>Postexposure period: 85 days after pups were born | ***Maternal toxicity***<br>No information available<br><br>***Offspring***<br>At 40 mg/kg bw/day:<br>↑ pup mortality (pups died before 20 days of age: 27%, compared to 2.3% in controls) | A6.9.2/02, Doc. No. 592-017<br><br>Harned et al., 1944 |

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| males/group;<br>12-18 offspring females/group | | | At 80 mg/kg bw/day:<br>↑ pup mortality (pups died before 20 days of age: 42%, compared to 2.3% in controls)<br>-reduced learning ability<br>At 120 mg/kg bw/day:<br>↑ pup mortality (pups died before 20 days of age: 58% compared to 2.3% in controls)<br>-reduced learning ability | |
| Postnatal Growth and Brain Development (no guideline)<br>Rat, Sprague Dawley<br>Females<br>18 treated and 14 controls | Sodium bromide | Oral (drinking water)<br>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)<br>Days 5-15 of gestation<br>Measurements were done in samples of blood and brain homogenates obtained from the offspring on postnatal days 1 to 90. | ***Maternal toxicity***<br>No information available<br>***Offspring***<br>At 200 mg/kg bw/day:<br>↓ body weight (15% adult value)<br>↓ brain weight (10% adult value)<br>↓ protein content in the brain (11% at postnatal day 90)<br>↑ size of olfactory glomeruli (30% larger than in control rats at postnatal day 39) | A6.8.1/06; Doc. No. 592-014<br>Disse et al., 1996 |

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

Available studies of developmental toxicity are two pre-natal developmental toxicity studies (OECD TG 414) of ammonium bromide in rat, two pre-natal developmental toxicity studies (OECD TG 414) of sodium bromide in rabbit and rat respectively, and one dose range finding study on sodium bromide in rabbit. Moreover, there is one dose-range finding study for reproductive toxicity of ammonium bromide in rat, one two-generation reproductive toxicity study (OECD TG 416) and one non-guideline multi-generation reproductive toxicity study of sodium bromide in rat. Finally therer are two non-guideline studies of developmental neurotoxicity of sodium bromide in rat and a number of human case reports of infants exposed to bromide during the pregnancy.

**Ammonium bromide– test guideline developmental toxicity studies**

***Pre-natal developmental toxicity study of ammonium bromide in rat (Study report, 2000b)***

In a pre-natal developmental toxicity study (OECD TG 414), performed with ammonium bromide pregnant rats were treated once daily by gavage at dose levels of 0, 100, 300 and 1000 mg/kg bw/day during days 6-19 of gestation.

Adverse effects on the offspring

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 (foetal incidence 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 mid and high dose, was outside historical background range of incidences for this strain of rats at the laboratory in question (historical control range: 0-4.1%) according to the study authors but no historical control data is submitted to confirm this statement. At 1000 mg/kg bw/day major 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). Reduced mean foetal weight (15%), increased incidence of small foetus (24% compared to 2% in controls), increased incidence of foetuses with slightly kinked ribs (4% compared to 1% in controls) and curved scapula (8.7% compared to 0.5% in controls) were also observed at 1000 mg/kg bw/day. Curved scapula 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). Kinked ribs and curved scapula were suggested to be reversible pathological findings as they were not seen in pups three weeks after birth (Study report, 2007a).

Maternal toxicity

Clinical signs (neurotoxic effects) and reduced bodyweight gain (18%) during gestation were recorded in dams at 1000 mg/kg bw/day. The body weight gain minus gravid uterus weight was 65% of control thus pointing to an effect on body weight of the dam, not influenced by the statistically significantly decreased (15% compared to control) mean foetal weight (males and females combined). 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.

Conclusion

Major foetal malformations were reported at 1000 mg/kg bw/day in presence of maternal toxicity (reduced body weight gain and clinical signs of neurotoxicity). 26 foetuses from 7 litters had absent/reduced left kidney, often with associated findings in the left adrenal and/or left ureter. Some of the foetuses also had narrowing of the left uterine horn and/or flattened/small spleen. Also at 1000 mg/kg bw/day curved scapula was observed in 18 foetuses from 8 litters. Foetal effects were also seen at the lower dose levels of 100 and 300 mg/kg bw/day without any indication of maternal toxicity. There was a dose-dependent increase in the incidence of foetuses with kinked ribs, often associated with incomplete ossification of ribs, and a dose-related increased incidence of displaced testis starting from 100 mg/kg bw/day. The major malformations at high dose and the minor abnormalities and variants that were observed from lower dose levels with a dose-dependent increase in incidences are considered as direct effects of the test substance and not secondary effects to maternal toxicity.

***Pre-natal developmental toxicity study of ammonium bromide in rat (Study report, 2007a)***

In a pre-natal developmental toxicity study performed with ammonium bromide pregnant rats were treated once daily by gavage at dose levels of 0, 50, 300, 600 and 800 mg/kg bw/day during Days 6-19 of gestation. In addition two groups were assigned to control and 300 mg/kg bw/day groups to serve as recovery animals (littering phase). Of note is that no statistical analysis was done in this study and no historical control data was available.

Adverse effects on the offspring (pre-natal phase)

There were no obvious effects on embryo-fetal mortality or fetal weights at any dose level tested. Increased incidence of abnormalities and variants was noted in fetuses of the three highest dosage groups. At 300, 600 and 800 mg/kg bw/day, there were increased incidences of fetuses 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 fetuses 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 fetuses 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 increased numbers of fetuses 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).

Adverse effects on the offspring (littering phase)

Among the females treated at 300 mg/kg bw/day that were allowed to litter, 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 and there were no reported incidences of curved scapula, which indicates that the kinked ribs and curved scapulae seen in the fetuses from rats treated at the same dose level may be transient in nature and reversible effects which resolve after birth when administration of the test substance was discontinued (dams were only treated GD 6-19). According to the study authors, a review of 74 scientific papers by Kast (1994) considers kinked/wavy ribs as a reversible pathological finding and reports that wavy ribs were often combined with bent and short scapula.

Mean litter weights at 300 mg/kg/day were slightly lower than control, particularly on Day 21 of lactation (89.5% of control).

Maternal toxicity

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 behavior, slow/irregular respiration, body held low, hunched posture and piloerection. One animal at 600 mg/kg bw/day was sacrificed on Day 11 of gestation due to the severity of these signs. Bodyweight gain at 800 mg/kg bw/day was reduced (9%) when compared to controls (statistical analysis not performed). Bodyweight gain at 300 and 600 mg/kg bw/day was increased (11% and 28%) when compared to controls (statistical analysis not performed).

Conclusion

At 300, 600 and 800 mg/kg bw/day there were increased incidences of foetuses with kinked and/or slightly kinked ribs and of curved scapula. There was also indications of effects on ossification at these dose levels. Similar findings were seen in the previous prenatal toxicity study of ammonium bromide from year 2000 in rat (Study report, 2000b). An increased number of foetuses with fewer than 13 complete ribs were recorded at 600 and 800 mg/kg bw/day and in these dose groups maternal toxicity (clinical signs of neurotoxicity) was evident. Since skeletal variations and retardations were evident at 300 mg/kg bw/day where no severe maternal toxicity was reported (one animal was subdued and a second animal had rolling gait on one occasion; both signs not conclusively attributed to treatment) the relevance of these developmental toxicity findings are not considered to be diminished by maternal toxicity. However, there was no dose-related increase in incidence of kinked ribs in foetuses and the kinked ribs were no longer present in weanlings of the recovery group. Therefore, the level of concern is considered by the dossier submitter to be less serious. For the observed curved scapula in foetuses there was a dose-related increase in incidence at 300, 600 and 800 mg/kg bw/day with no findings recorded at

50 mg/kg bw/day and in control group, but no reported incidence in weanlings of the recovery group. Thus, the level of concern for classification is considered to be moderate for this finding.

**Ammonium bromide– non-guideline developmental toxicity studies**

***Dose-range finding study for reproductive toxicity of ammonium bromide in rat (Study report, 2001)***

In the dose-range finding study for a reproduction toxicity study of ammonium bromide rats were administered (10/sex/group) via food at concentrations of 0, 1600, 3200 and 6400 ppm (corresponding to 0, 127, 242 and 503 mg/kg bw/day in males; 0, 228, 454, 651 mg/kg bw/day in females). Parental animals were treated from two weeks prior to mating until the first generation had been weaned. No statistical analysis was performed due to the small group size.

Adverse effects on the offspring

The fertility in the high dose group was markedly decreased to 10% of control, and consequently there was only one litter produced that did not survive to day 4 of lactation. Pup viability was noticeably decreased in the dose group at 3200 ppm where all pups in 4 out of 9 litters died before day 21 of lactation (found dead days 4 and 14) and included one litter where all pups were born dead, resulting in a reduced gestation index. Moreover, there was a slight increase in pup mortality of surviving litters. Three pups from two litters at 3200 ppm and one pup from one litter at 1600 ppm were killed due to their condition (cold, subdued behaviour, abnormal breathing) on or before day 12 of lactation. There were no marked effects on litter size or survival at 1600 ppm. In control group, all pups in two litters were found dead on lactation day 4. As a result, the viability index (number of pups live on day 4 of lactation/number live on day 0) was 67 %, 92 % and 57% in the control, low dose and intermediate dose groups respectively, and the overall survival index (number of pups live on day 21 of lactation/total number of pups born) was 66%, 89% and 40% in the control, low dose and intermediate dose groups, respectively.

Mean of litter weight was >10% lower than control group at lactation day 1, 7, 14 and 21 in the 454 mg/kg bw/day dose group compared to control group.

Maternal toxicity

Severe clinical observations were recorded in the females of the high dose group (6400 ppm or 651 mg/kg bw/day) including rolling gait which was noted in all animals and persisted throughout the treatment period. In addition, piloerection and hunched posture accompanied this finding. Approximately half of the females showed hyperactivity. The clinical effects observed in the group treated at 3200 ppm (454 mg/kg bw/day) were the same but the effects were less severe. Six out of 10 females (5 out of nine pregnant females) showed rolling gait that appeared later during the treatment period compared to control group. There was no obvious treatment related effect on female bodyweight prior to mating at any dose level, and during gestation in the 1600 and 3200 ppm dose groups there were no differences when compared with control. At 6400 ppm, the body weight gain during gestation were 33% less than control. However, this was based on only one animal due to the poor pregnancy rate at this dose level and for this animal the absolute bodyweight was higher than the control mean body weight throughout the gestation. At the start of lactation body weights at 3200 and 1600 ppm were greater than control, however, by day 14 of lactation, the absolute weights were essentially similar in all dose groups.

There were no severe effects on individual body weights in the 3 dams of the intermediate dose group loosing whole litters during lactation days 4-14 (only one of these dams had approx. 10% lower body weight than the mean group value of control, the other two had higher or no different body weight compared to control). Rolling gait were noted in 3 of 4, piloerection 2 of 4, and hunched posture in 2 of 4 out of the dams with dead litters. The onset of the clinical signs was generally late gestation or during lactation.

Conclusion

It is not clear from this study if the decreased mean litter/pup weights and decreased pup viability during lactation at 454 mg/kg bw/day (3200 ppm) were due to poor maternal care beacause of the observed clinical signs of neurotoxicity in the dams or if there is a direct effect of bromide on the pups. Moreover, the viability indices in the control group were unexpectedly low and therefore it is difficult to conclude on a clear direct effect of ammonium bromide on pup viability.

**Sodium bromide– test-guideline developmental toxicity studies**

***Pre-natal developmental toxicity study of sodium bromide in rat (Study report, 1995)***

In a pre-natal developmental toxicity study (OECD TG 414) performed with sodium bromide, rats were orally exposed by gavage to 0, 100, 300 or 1000 mg sodium bromide/kg bw/day from days 6 through 15 of gestation.

Adverse effects on the offspring

There were no effects on fetal deaths, fetal weight or the sex ratio at any of the tested dose levels. At 1000 mg/kg bw/day there was a higher incidence of fetuses showing malformations (5.3% compared to 1.7% in controls). These malformations were principally visceral, affecting the urogenital system and thoracic skeletal malformations manifest as abnormalities of the ribs. The visceral malformations consisted of increased incidence of fetuses with absent kidney (3% compared to 0% in controls), absent ureter (3% compared to 0% in controls), absent uterine horn (0.7% compared to 0% in controls), narrow uterine horn (2% compared to 0% in controls). The skeletal malformations consisted of increased incidence of fetuses with abnormalities of the ribs (distorted/minimally distorted/ossification irregularities ribs: 1.7% compared to 0% in controls). In addition increased incidence of skeletal anomalies and skeletal variants were noted in the study. At 1000 mg/kg bw/day these skeletal anomalies consisted of increased incidence of fetuses with distorted ribs minimal (6% compared to 0% in controls), shortened/absent 13th ribs (6 % compared to 0% in controls), irregular ossification thoracic vertebral centra (9% compared to 3% in controls) and reduced ossification of one or more cranial centers (20% compared to 4% in controls), and skeletal variants consisted of increased incidence of fetuses with reduced sternebrae (38.8% compared to 17.9% in controls), unossified sternebrae (63.3% compared to 27.9% in controls) and total variant sternebrae (79.7% compared to 41.4% in controls). At 300 mg/kg bw/day these skeletal anomalies consisted of increased incidence of reduced ossification of one or more cranial centers (14% compared to 4% in controls), and skeletal variants consisted of increased incidence of total variant sternebrae (57.1% compared to 41.4% in controls) and increased incidence of unossified sternebrae (40% compared to 28% in controls).

Maternal toxicity

Clinical signs of neurotoxicity were noted in dams at 1000 mg/kg bw/day. The clinical signs consisted of unsteady gait, reduced body tone, poorly coordinated movements, feet falling through the cage grid floor during ambulation, hair loss, increased lacrimation, brown staining on fur, periorbital staining and wet staining around the urogenital region. One animal at 1000 mg/kg bw/day was sacrificed on Day 11 of gestation due to the severity of clinical signs. Bodyweight gain in dams at 300 and 1000 mg/kg bw/day was reduced (days 16-20: 15% and 16%) when compared to controls. At 1000 mg/kg bw/day the food consumption was reduced (9%) in dams during days 18 to 19.

Conclusion

Malformations, affecting the urogenital system and thoracic skeletal malformations manifest as abnormalities of the ribs were observed in the 1000 mg/kg bw/day dose group as well as skeletal anomalies of the ribs. Moreover, reduced ossification of one or more cranial centers, and skeletal variants affecting sternebrae were seen both in 300 and 1000 mg/kg bw/day dose groups with a dose dependent increase in incidences. The fetal weights were not affected at any dose level and therefore the skeletal variants and anomalies cannot be regarded as consequences of retarded growth. In addition, there were increased incidences of malformations of high concern at the highest dose level tested that cannot be considerd as secondary to the observed maternal toxicity (clinical condition).

***Pre-natal developmental toxicity study of sodium bromide in rabbit (Study report, 2008b)***

Subsequent to the dose-range finding study in rabbits (Study report, 2008a), the main study (according to OECD TG 414) on sodium bromide was performed to investigate effects on embryonic and foetal development of the test substance in rabbits at dose levels of 0, 25, 75 and 250 mg/kg bw/day by oral gavage during days 6-28 of gestation.

Adverse effects on the offspring

At 25 mg/kg bw/day one female showed total litter loss in 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 one low dose female only was not considered to be of any toxicological relevance.

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.

At 75 mg/kg bw/day, there was a significant increase (53.2% versus 25.3%, $p \leq 0.01$ compared to control) in irregular ossification of more than one cranial bone. In the absence of an effect at the higher dose level, this finding is considered to be of low toxicological significance.

Maternal toxicity

There were no treatment-related clinical signs, no adverse effects on bodyweight gain or food consumption noted. Increased water intake at 75 and 250 mg/kg bw/day was apparent throughout the treatment period but was only statistically significant for high dosed animals during days 15-18 of gestation and might be due to the high salinity of the dose formulations.

Conclusion

No significant findings of developmental toxicity was seen at any dose level tested.

**Sodium bromide– non-guideline developmental toxicity studies**

***Dose range finding study of a pre-natal developmental toxicity study of sodium bromide in rabbit (Study report, 2008a)***

A dose-range finding study (gavage) was performed in pregnant rabbits in order to select appropriate dose levels for the conduct of a pre-natal developmental toxicity main study on sodium bromide. At first, the maximum tolerated dose was established using non-mated animals (pre-study) and applying an escalating dosing regime (125, 200, 500 and 1000 mg/kg bw/day, with administration of each dose for 3 consecutive days to the same animals) and a fixed dose level of 500 mg/kg bw/day following administration for 13 days. Based on the observations made in this preliminary test with non-pregnant animals, the potential effects on pregnant (6 animals per dosage group and 5 control animals) were investigated in time-mated rabbits at 100, 200 and 400 mg/kg bw/day during days 3 through 28 of gestation.

Maternal toxicity

Treatment of non-pregnant animals at 500 or 1000 mg/kg bw/day was associated with adverse clinical signs (decreased respiration rate and/or ataxia) in the absence of clear effects on bodyweight or food intake. 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

Adverse effects on the offspring

Only limited assessment of litter data was possible due to the low number of litters available, however, there was no indication that embryofoetal survival, growth or development had been affected by maternal treatment at any of the dosages investigated.

**Sodium bromide– test guideline generation reproductive toxicity studies**

***Two-generation reproductive toxicity study of sodium bromide in rat (Study report, 2016a)***

In a two-generation reproduction study (performed according to a protocol similar to OECD TG 416) sodium bromide was administered via oral gavage to Crl:CD(SD) rats at dose levels of 0, 50, 150, 350/500 (male/female) mg/kg bw/day. Male and female P generation rats in control, low and intermediate dose groups were paired twice, owing to reduced pregnancy rate in intermediate dose group. The first cohabitation period (to produce F1a litters) was scheduled after 10 weeks of treatment and a second cohabitation period (to produce F1b litters) was conducted for all but the high dose group. Offspring from F1a formed the F1 generation (a total of 23, 22 and 15 pups per sex per litter from control, 50 mg/kg bw/day and 175 mg/kg bw/day dose groups), dosed from day 21 postpartum and selected for post-weaning assessments (including reproductive assessments and production of the F2 litters). F1-generation male and female rats, selected from the F1a litters, were exposed in utero, via lactation, and via oral gavage after weaning to dose levels of 0, 50 or 175 mg/kg/day for approximately 131 days. The litters (F1b) from the second cohabitation of the P generation rats was terminated at day 40 postpartum.

Adverse effects on the offspring

At 500 mg/kg/day there were reductions in litter size at birth, the number of liveborn pups and pup survival. The number of dams with stillborn pups (3/6) and number of dams with all pups dying before day 4 postpartum (5/6) were significantly increased ($p \leq 0.01$) compared to control. No litters survived after day 5 post-partum in this dose group and there 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.

Delivery and litter observations were not affected in the 50 mg/kg/day and 175 mg/kg/day, dose groups of sodium bromide and there was no effect on foetal weight at PND 1 or later time points up to weaning in any dose group. Macroscopic observations in stillborn pups and pups that died revealed no treatment related abnormalities in the pups. No effect on physical development of the pups (no pups in the 500 mg/kg/day dose group survived to allow evaluation of these end points) were recorded.

For the second cohabitation period to produce F1b, there was no adverse effect of treatment on litter size at birth of the F1b pups or pup viability, growth and physical development. An increased number of dams with stillborn pups at 50 mg/kg bw/day (4/24 compared to 0/23, $p \leq 0.01$) was recorded and consequently also an increased mean percentage of stillborns (2.4% compared to 0% in control, $p \leq 0.01$). However, no effect on mean litter size or mean viable litter size was evident. No effect on viability index or lacataion index was observed at any dose level.

Two pups (2 litters) in 175 mg/kg/day dose group had a bent tail (one was only apparent on day 21 post-partum) ($p \leq 0.01$) compared to the control group. One pup with domed head in low dose group (stillborn) and one pup on day 21 post-partum with a firm mass in the abdomen and slight dilation of the renal pelvis were recorded. Tooth eruption was 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 in the 175 mg/kg/day dose group.

In F1-generation there were no adverse effects of treatment on gestation index or number of dams with stillborn pups and the 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 of the F2 pups were not statistically significant different from control. There was no total litter loss and no intergroup differences in viability index. The number of pups dying between PND 8 and 14 in the 175 mg/kg bw/day dose group was statistically significant increased (2 (1.1%), $p \leq 0.01$) compared to control (0), however, it was not considered biologically significant by the study author as only two pups died and this was within the historical control range of the testing facility

There were no adverse effects on growth or physical development of the F2 litters.

Maternal toxicity

In the P generation at 500 mg/kg/day severe toxicity was reported, characterized by increased mortality (9 females died or were terminated early) and adverse clinical observations ($p \leq 0.01$), including dehydration, ungroomed coat, chromodacryorrhea, hunched posture, ptosis, urine-stained abdominal fur, decreased motor activity, chromorhinorrhea, ataxia, piloerection, low carriage, thin body condition, and bradypnea.. Reduced body weight gain and food intake was observed during late gestation (-10.8%) and lactation (-10.9% to -13.3%). Since the mortality rate of females at 500 mg/kg bw/day in the P generation was > 10%, the general toxicity is considered as excessive and results from this dose group would normally not be acceptable for further evaluation according to the OECD TG 416 and CLP Annex I: 3.7.2.4.4. In the 175 mg/kg bw/day dose group of the P generation, similar clinical signs (dehydration, ungroomed coat, chromodacryorrhea, hunched posture, ptosis, urine-stained abdominal fur, chromorhinorrhea) occurred but they were less marked and at a lower incidence (not statistically significant different from control). Effects on female body weight were not seen and food intake was reduced only in early lactation.

In the F1 generation at 175 mg/kg bw/day, there were no adverse clinical observations reported, only minimal, sporadic and transient clinical observations. Female body weights were not affected, but the food intake was reduced in late gestation and early lactation.

Conclusion

At dose levels where no overt maternal toxicity was present there was no indication of effects of sodium bromide on embryofoetal survival, growth or development treatment in any generation.

**Sodium bromide– non-guideline generation reproductive toxicity studies**

***Three-generation reproductive toxicity study of sodium bromide in rat (Van Leeuwen, F. X. R. et al., 1983)***

In a three-generation reproductive toxicity study (no guideline, not GLP compliant) sodium bromide was administered to rats at dose levels of 75, 300, 1200, 4800 and 19200 ppm sodium bromide via the diet (corresponding to 0, 6.75, 27, 108, 432 and 1728 mg/kg bw/day based on a default conversion of 1 ppm=0.09 mg/kg bw/day). In three successive generations, at least two litters per female rat were raised. It should be noted that 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.

The transplacental transport of bromide was investigated in the third litter of the first generation.

In order to investigate the cause of the infertility observed, a cross-mating procedure was performed in which untreated males and females were mated with females and males of the 19200 ppm group.

To study the reversibility of the effects, an additional litter was bred with parental animals of the highest dose group (19200 ppm) which were fed control diets for a period of 3 months after the 7-month treatment period.

Adverse effects on the offspring

There is no information on gestation index, litter size at birth, altered growth or functional deficiency available to the dossier submitter. The viability of the F1 pups at the 4800 ppm (432 mg/kg bw/day) dose level on postnatal day 5 was markedly reduced in the first litter (viability index 32%) compared to control (viability index 90%) but survival was shown to be greater in the second litter (viability index 61%) when compared to the first litter. During the lactation, 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.

After being three month on the control diet, animals treated at 19200 ppm sodium bromide were mated again (reversibility study). The viability index was still lower (61%) than in the control and a lactation index of 90% was recorded.

No macroscopic anomaly in any pup was observed throughout the investigation. Dams (F0) and 20-day foetuses (F1) examined for bromide concentrations in kidneys showed comparable amounts of bromide in the organs demonstrating that the foetuses had been exposed to bromide in utero.

Maternal toxicity

Body weights of the F0 animals at the termination of the study were not statistically significant different from control at 4800 ppm or at any lower dose levels either in males or females. There is no information available on body weights of the highest dose tested, 19200 ppm. There is no information available on clinical observations, mortality or body weight gains for any dose group or generation in this publication.

In F1 animals, the body weights at the termination of the study were not statistically significant different from control in any dose group either in males or females.

In F2 animals, the body weights at the termination of the study were statistically significant different at the highest dose tested (1200 ppm) in females (-10%, $p<0.01$).

Body- and organ-weight determination did not reveal a clear pattern of dose-related effects in neither of the three generations.

Conclusion

The viability of the F1 pups in the 432 mg/kg bw/day dose group was markedly reduced during both postnatal days 1-4 and during post-natal days 5-21. Maternal body weights were not affected at this dose level but the clinical conditions were not reported, and thus it is not possible to conclude if the pup mortality was a consequence of poor maternal care or a direct effect not secondary to maternal toxicity.

**Developmental neurotoxicity studies**

No developmental neurotoxicity study of ammonium bromide is available but there are experimental studies from the open literature indicating developmental neurotoxic effects of sodium bromide.

***Postnatal Growth and Brain Development study (no guideline) of ammonium bromide (Disse et al., 1996)***

In a published investigation (no guideline study), rats were orally treated with 250 mg % sodium bromide (0.25%) via the drinking water (corresponding to 0.25 g/100 mg or 200 mg/kg bw/day assuming a bodyweight of 250 g per rat throughout the study period with water sonsumption of 20 ml per day) during Days 5-15 of gestation. Controls either received tap water or 250 mg % NaCl. Compared to controls, the pups of the bromide treated rats showed reduced bodyweight (statistically significant from PD 19 onwards, adult values differed by 15%) and brain weight (statistically significant from PD8 onwards, adult values about 10% lower than normal brain weight). Estimation of deficits in development revealed that bromide did not monotonously interfere. Three phases were identified: During phase 1 (postnatal (PD) 1-10) developmental delay was small, but increased during phase 2 (PD10-40); from PD40 on, deficits remained at the highest level reached. Protein contain in brain of bromide-treated animals was reduced, but glomerular profiles were on average 30% larger. 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. Brain homogenates of treated rats contained 3-fold more bromide than the ones in controls on PD1. As in blood, the bromide level decreased constantly and reached control levels until PD10. 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 PD90. In contrast to developmental deficits, the size of olfactory glomeruli was consistently larger in bromide-treated rats during postnatal developmental and glomeruli attained a mean diameter that was 30% larger than in controls at PD39. A small shift to larger diameters was already present at PD 8 and 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. Since after complete excretion of bromide developmental deficits persist and show periods of partial compensation and decompensation, induction of these bromide effects is probably indirect. The exact mechanism of bromide action on developmental processes is unknown and remains to be elucidated according to the study author.

Conclusion

In the study by Disse et al (1996) it was shown that bromide cross the placenta and causes changes in the brain (reduced protein content) and olfactory tract (increased size of olfactory glomeruli) in rats following administration of sodium bromide in dams during gestation days 5-15 at a dose level of 200 mg/kg bw/day (156 mg bromide/kg bw/day). These effects persisted in the offsprings after completed excretion of bromide and showed periods of partial compensation and decompensation.

***Pre-natal developmental toxicity study (no guideline) of sodium bromide in rats (Harned et al., 1944)***

In a published investigation the effects of prenatal administration of sodium bromide to rats was studied 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 sodium bromide/kg bw/day from Day 3 to 20 of gestation or were left untreated as a control. Pups born on Day 22 received bromide only the milk of their mothers and were weaned until 20 days of age. The “120” group consisted of 34 offspring (18 females, 16 males), the “80” group consisted of 30 offspring (12 females, 18 males) and the “40” group consisted of 33 offspring (13 females, 20 males). At the age of 57-60 days the offspring was prepared for learning in the maze and on Days 61-85 each animal was given two trials per day in a five cul-de-sac u-maze.

Mortality among the newborns was high and paralleled the doses given the mothers: 2.3%, 27%, 42% and 58% of pups died before 20 days of age after treatment with 0, 40, 80 and 120 mg/kg bw/day, respectively. Pups from the two highest dosage groups (80 and 120 mg/kg bw/day) showed subnormal growth for a few days but this was considered temporary and of a minor order. Maternal toxicity (body weight and clinical conditions) were not reported in the publication.

The examinations in the u-maze revealed that the groups treated at 80 and 120 mg/kg bw/day made a significantly greater number of errors than each of the other groups. In addition, the 120 mg group was virtually 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 until the 25th day of the test.

Conclusion

Reduced learning ability was noted in rats following administration of sodium bromide in dams during gestation days 3-20. This effect was noted at a dose level of 80 mg/kg bw/day (62 mg bromide/kg bw/day) and at 120 mg/kg bw/day (93 mg bromide/kg bw/day). Dose-dependent increased pup mortality was noted starting from a dose level of 40 mg/kg bw/day (31 mg bromide/kg bw/day).

**Human studies of bromide**

In a publication the transplacental passage of bromide to the foetus with resultant bromism in a 7-day old female infant given birth by a mother severely intoxicated with bromide following ingestion of Miles Nervine (a sedative consisting of sodium bromide, potassium bromide and ammonium bromide) was documented. Serum bromide concentration of the mother was 320% (measured on the sixth postpartum day, corresponding to about 240 mg/kg bw), while the infant´s value was 365% (corresponding to about 1460 mg/kg bw). The infant´s mother experienced some ten days of toxic delirium as a consequence of her ingestion. The infant, in contrast, remained "simply sedated" in spite of a serum bromide concentration which persisted in the "potentially lethal" range until the eighth day of life. The infant responded minimally to painful stimuli; her startle, suck, rooting and grasp reflexes were poor. No tendon reflexes could be elicited; abdominal reflexes were also absent. Pupils reacted very slowly to light. Otherwise the physical examination was normal, and there was no rash. Treatment of infant consisted of extra salt (NaCl) added to the diet in an attempt to hasten renal excretion of bromide. Follow-up examination one month later revealed a healthy infant without obvious sequelae from her "neonatal bromism", and an apparently well-adjusted mother. At 11 months of age, the infant was reported by her physician to continue to do well (Finken and Robertsson, 1963).

Another case report also document transplacental passage of bromide to the foetus with resultant central nervous system depression in a female infant born to a mother severely intoxicated with bromide following ingestion of Nervine (a sedative consisting of sodium bromide, potassium bromide and ammonium bromide). The mother in the 34th week of pregnancy was semiconscious and hyperreflexic on admission. An amniotomy was performed after which the infant was delivered spontaneously. Ten minutes after birth, the infant became hypnotic and could be aroused only with vigorous stimulation. The clinical picture of neonatal bromide intoxication in this case was characterized by marked hypoactivity, reduced cry and suck. Blood bromide level of the mother was 29.5 mg/dL (corresponding to about 22 mg/kg bw assuming a total blood volume of 4.5 L and a body weight of 60 kg), while the infant´s blood level (on the fifth day of life) was 24.2 mg/dL (corresponding to about 120 mg/kg bw assuming a total blood volume of 1 L and a body weight of 2 kg). The infant received antibiotics for seven days, and a solution of 5% glucose and 0.45% sodium chloride was intravenously applied. The intravenous therapy was discontinued on the 14th day of life by which time her neurologic status had become normal. The mother´s treatment consisted of increased intravenous saline administration, ethacrynic acid, and subsequently oral salt tablets. With decreasing blood bromide levels both mother and child gradually improved in health and the infant development was normal by 3.5 months of age. No long-term damages were reported. It was suggested by the author that the neonatal CNS might be less sensitive to bromide, or that the lower level of cortical activity in the neonate might reduce the neuropsychiatric manifestations of bromide intoxication other than overt depression of spontaneous activity and suck reflex (Blackburn and Pleasure, 1975).

A report describes the case of a pregnant mother who had taken a variety of medications including dextroamphetamine and chlorpromazine. She was prescribed bromide-containing drugs (0.3 g total bromide per mL) for psychiatric treatment and took them daily (6 g/day) for 4 days until the day prior to delivery. The infant was born by caesarean section after 37 weeks gestation and described as large, puffy and quiet but in no distress. Neurological examination revealed an infant with a weak, high-pitched cry, poor suck, partial Moro reflex and diminished tone; deep tendon reflexes were absent. The serum bromide levels, first determined on the fifth day, were 200 mg/100 mL in the infant and 310 mg/100 mL in the mother. At 69 days of age the serium bromide level was found to be 23 mg/100 mL in the infant confirming the known slow clearance and increased renal tubular reabsorption of this ion. The discovery of high bromide levels was preceded by signs of improvement of the infant´s condition. After 5 months the patient showed no residual manifestations of bromism (Mangurten H.H. and Ban R., 1974).

Another report describes the case of a pregnant mother who from the beginning of the pregnancy prepared and used photographic chemicals, containing sodium and potassium bromide amongst others. The mother had two episodes of respiratory infection during pregnancy, both accompanied by fever to 39°C, and smoked approximately one package of cigarettes per day, with no alcohol intake. The infant was born by caesarean section after 43-44 weeks gestation and was transferred to intensive care at 90 hours of age because of cyanosis. The infant (a girl) had a head which appeared large in relationship to

the trunk. In addition she had frontal bossing, broadening of the nasal brigde and a dull fixed facial expression. Neurological examination revealed an infant with weak cry, suck and grasp, hyporeflexia, and profound generalised hypotonia. Physical examination at 5 months of age was normal except for residual hypotonia. At 9½ months she demonstrated some residual mild hypotonia of the neck musculature only, speech and social development appeared normal. It cannot be excluded that other chemicals in combination with the bromide salts accounted for the symptoms observed in the newborn. In the report it was stated that the presence of dysmorphic facial features suggests a possible teratogenic effect from fetal bromide exposure during early pregnancy (Mangurten H.H. and Kaye C.I., 1982).

A case of a mother is reported who was treated for hypothyroidism and for anxiety and depression without improvement and one year later started to regularly ingest ("abuse") Bromo-Seltzer for 4 years during which time she gave birth to 2 sons. She had had two other children prior to substance exposure and had a fifth after the exposure was halted, all of which were of normal health. The two sons born during the time of bromide abuse showed growth retardation, still evident at ages 7 – 8 years, but no abnormal neurological signs were noted. One of the boys was microcephalic and had congential heart disease. In the report it was stated that the growth retardation and the bromism may be entirely coincidental but the observation deserves publication since it might point to a link between the two phenomena (Opitz J.M. et al., 1972).

Another case report on transplacental bromide exposure describes a newborn of a mother who regularly ingested bromide-containing medication during the entire pregnancy, the amounts of which were not quantified. At birth, the infant was extremely irritable, showed with a high pitched cry and was very difficult to feed. These symptoms continued for at least 9 weeks after which the child started to improve. After 2½ years, the child showed growth retardation, had only just started to walk, had very brisk knee and adductor jerks, and had not spoken so far. In the report it was stated, that the relation between bromide ingestion and infant developmental retardation is circumstantial but worth recording (Rossiter E.J.R. and Rendle-Short T.J., 1972).

Conclusion

There are human case reports indicating developmental growth retardation (height, weight and skull circumference) in infants exposed to bromide during the entire pregnancy. Moreover, some of the studies only report effects on the infant but no effects in the mother pointing to a higher susceptibility to bromide in the child compared to the mother.

**Summary**

According to CLP Annex I, 3.7.1.4. the major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

In developmental toxicity studies in rats (studies performed with ammonium bromide and sodium bromide) and rabbits (studies performed with sodium bromide) the following main effects on the development of offspring were noted:

Death of the developing organism

Effects on rat pup viability and survival were seen in the dose range finding reproductive toxicity study of ammonium bromide at doses where maternal toxicity (clinical sign of neurotoxicity) was also seen. The only litter produced in the high dose group (651 mg/kg bw/day) did not survive to day 4 of lactation and all pups in 4 out of 9 litters in the dose group at 454 mg/kg bw/day died before day 21 of lactation. Both the viability index and the survival index (number of pups live on day 21 of lactation/total number of pups born) was greatly reduced in the 454 mg/kg bw/day dose group (57% and 40%, respectively), however, since both these indices were unusually low in the control group (67% and 66% respectively) the significance of the effects in intermediate dose group is difficult to evaluate. Moreover, at 454 mg/kg bw/day rolling gait was observed in the four dams

loosing whole litters and a marked effect on the clinical condition (rolling gate, hunched posture, piloerection, hyperactivity) of the dams was evident at 651 mg/kg bw/day.

In the two-generation reproduction toxicity study of sodium bromide in rat, the F1a litter size at birth was lower and pup viability was markedly decreased in the high dose group (500 mg/kg bw/day) compared to control in the first pairing. However, at this dose level the maternal toxicity was excessive (mortality >10%). There were no effects on litter size or pup viability recorded at the lower dose levels or in the F1b generation (second pairing, high dose 175 mg/kg bw/day). There were no adverse effects of treatment on gestation index, litter size at birth, pup viability or survival of the F2 pups.

Effects on the rat pup viability were also seen in the three-generation reproduction toxicity study where the viability of the F1 pups in the 432 mg/kg bw/day dose group was significantly reduced in the first litter (viability index 32% versus 90% in control) compared to control but survival was shown to be greater in the second litter (viability index 61% versus 90% in control) when compared to the first litter. Also pup survival during lactation was completely diminished in the first litter where all the offspring died between post-natal day 5 and 21, while survival was not effected post-natal day 5 to 21 in the second litter. Maternal body weights were not affected at these dose levels but the clinical condition of the dams were not reported in this study and therefore these findings are difficult to evaluate.

Rat pup mortality before 20 days of age was high and dose-dependently increased (27%, 42% and 58% compared to 2.3% in control) in offspring from mothers administered sodium bromide via oral gavage gestation day 3-20 at 40, 80 and 120 mg/kg bw/day respectively (Harned et al., 1944) . Maternal toxicity (body weight and clinical conditions) were not reported in the publication and it is therefore not possible to determine if the observed pup mortality was due to poor maternal care or a direct effect of the test substance on the pups. Moreover, pup-killing/cannibalism of the pups by the dams were reported in the 80 and 120 mg/kg bw/day dose groups.

Structural abnormality

In rats adminstered ammonium bromide, a dose-related increase in the incidence of displaced testis at 100, 300 and 1000 mg/kg bw/day were observed and maternal toxicity (clinical signs of neurotoxiciy and reduced body weight gain) was only seen at the highest dose level. Skeletal abnormalities/variants (kinked ribs, curved scapulae) and incomplete ossification of ribs were noted after administration with ammonium bromide at dose levels without maternal toxicity (100 and 300 mg/kg bw/day) and in presence of maternal toxicity at 1000 mg/kg bw/day (Study report, 2000b). Similar findings was also seen in another prenatal developmental toxicity study of ammonium bromide in rat (Study report, 2007a). However, at weaning (only 300 mg/kg bw/day dose group examined) no increased incidences of kinked ribs wee seen.

At 1000 mg/kg bw/day also major visceral malformations (left kidney, left uterine horn, spleen, thyroid) and increased incidences of skeletal abnormalities/variants (reduction in size of the 13th ribs) were evident in the pre-natal developmental study of ammonium bromide in rat (Study report, 2000b). Increased incidences of similar visceral malformations (kidney, ureter, uterine horn) were seen in the pre-natal developmental study of sodium bromide in rats, and skeletal malformations (ribs) at a dose level (1000 mg/kg bw/day) with maternal toxicity manifested as clinical signs of neurotoxicity and reduced body weigh gain (Study report, 1995). Skeletal anomalies/variants (ribs, sternebrae, cranial centres) were reported from 300 mg/kg bw/day where no adverse maternal toxicity were noted.

No embryotoxic or teratogenic effects were noted in rabbits (Study report, 2008a and 2008b) administered sodium bromide at a dose level of 250 mg/kg bw/day (195 mg bromide/kg bw/day) in the main study and up to 400 mg/kg bw/day in the pre-study. The absence of maternal toxicity at the high dose level in the main study indicates that this dose level might have been too low. In the pre-study clinical adverse signs (ataxia) in does were reported from 400 mg/kg bw/day

Altered growth

Reduced mean weights (>10%) of litter (from lactation day 1) and female pups (from lactation day 7) without any effect on maternal body weight at 454 mg/kg bw/day were reported in the dose range finding reproduction toxicity study of ammonium bromide (Study report, 2001).

Functional deficiency

There are some indications of developmental neuroroxicity as well from the the non-guideline studies by Disse et al (1996) and Harned et al (1944), however not conclusive.

### 10.10.6 Comparison with the CLP criteria

The criteria for classification in Repr. 1B for adverse effects on the development of the offspring are considered to be fullfilled since there is clear evidence of adverse effects on the development of the offspring recorded in studies of ammonium and sodium bromide. The effects were of high concern, dose related and evident also at dose levels where there was no overt maternal toxicity:

- A dose-related increased incidence of displaced testis was noted at 100 and 300 mg/kg bw/day (dose levels without maternal toxicity) and 1000 mg/kg bw/day (dose level with maternal toxicity) in the pre-natal developmental toxicity study of ammonium bromide in rat.
- A statistically significant increase in incidences of visceral malformations (reduction or absence) were seen at a dose level of 1000 mg/kg bw/day in studies of ammonium bromide and sodium bromide in rats. These defects observed in the urogenital system, uterine, spleen and thyroid at a high dose level in two studies are considered to reflect a selective effect on embryofoetal development and not a secondary effect resulting from toxicity to the parent female.
- A dose-dependent increase in incidences of skeletal abnormalities and variants (kinked ribs, curved scapulae and incomplete ossification of ribs) were observed at lower dose levels (from 100 mg/kg bw/day) without associated reductions in foetal weights and without maternal toxicity in two studies of ammonium bromide in rat. In the pre-natal developmental study of sodium bromide in rat skeletal malformations (ribs) were recorded at higher doses (1000 mg/kg bw/day) with maternal toxicity (clinical sign of neurotoxicity) and skeletal anomalies (ribs, cranial centres and sternebrae) were recorded at lower doses (300 mg/kg bw/day) without maternal toxicity. These skeletal abnormalities are also considered to reflect a selective effect on embryofoetal development and not a secondary effect resulting from toxicity to the parent female.
- Marked effects on rat pup viability and survival were seen in the dose range finding reproductive toxicity study of ammonium bromide at doses (454 and 651 mg/kg bw/day) where moderate (454 mg/kg bw/day) and severe (651 mg/kg bw/day) maternal toxicity (clinical signs of neurotoxicity) was also seen. At 651 mg/kg bw/day there was only one litter produced and all pups were dead before day 4 of lactation, and at 454 mg/kg bw/day all pups in 4 out of 9 litters died before day 21 of lactation (found dead days 4 and 14) and included one litter where all pups were born dead. It is not clear from this study if the decreased decreased pup viability during lactation at 454 mg/kg bw/day were due to poor maternal care beacause of the observed clinical signs of neurotoxicity in the dams or if there is a direct effect of bromide on the pups. Moreover, since both the viability index and the survival index were unusually low in the control group the significance of the findings is difficult to evaluate. However, these effects are considered to be some evidence of developmental toxicity.
- In the two-generation reproductive toxicity study of sodium bromide at 500 mg/kg/day, the number of P generation 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 was reduced ($p \leq 0.01$). However, in this dose group severe generalised toxicity was evident.

- Effects on the pup viability were also seen in the three-generation reproduction toxicity study of sodium bromide where both the viability index and lacatation index of the F1 pups in the 432 mg/kg bw/day dose group was significantly reduced in the first litter compared to control but survival was shown to be greater in the second litter when compared to the first litter. Maternal body weights were not affected at these dose levels but the clinical condition of the dams were not reported in this study and therefore these findings are difficult to evaluate. These effects are also considered to be some evidence of developmental toxicity.
- A dose-related increase in pup mortality was noted starting from a dose level of 40 mg/kg bw/day (31 mg bromide/kg bw/day) in the pre-natal developmental toxicity study of sodium bromide in rat (Harned et al., 1944). However, pup-killing/cannibalism of the pups by the dams (unclear to what extent) were also indicated in the 80 and 120 mg/kg bw/day dose groups. Maternal body weights and the clinical condition of the dams were not reported in this study and therefore the observed pup mortality cannot be concluded as not being a secondary consequence of maternal toxicity. These effects are considered to be some evidence of developmental toxicity.
- A reduction (>10%) of mean litter weights (from lactation day 1) and female pup weights (from lactation day 7) without any effect on maternal body weight at 454 mg/kg bw/day were reported in the dose range finding reproduction toxicity study of ammonium bromide (Study report, 2001). However, it is not clear from this study if the decreased mean litter/pup weights during lactation at 454 mg/kg bw/day were due to poor maternal care beacause of the observed clinical signs of neurotoxicity in the dams or if there is a direct effect of bromide on the pups.

Consequently, condsidering the overall weight of evidence of available information there is clear evidence of structural abnormalities and some evidence of both death of the organism and retarded growth. Classification in **Repr. 1B, H360D** is therefore warranted. Moreover, the recorded effects are relevant for humans, and are not considered to be secondary to maternal toxicity.

Classification in Repr. 1A, H360D is not justified since there is no robust human data demonstrating that ammonium bromide or the source substances (bromide salts) have adverse effect on human fetal development. There are only indications from human case reports of developmental growth retardation (height, weight and skull circumference) in infants exposed to bromide during the entire pregnancy

Classification in Repr. 2 is not justified since the evidence for developmental toxicity from existing experimental data on ammonium bromide and the source substances (bromide salts) is considered to be clear and not some evidence of developmental toxicity.

### 10.10.7 Adverse effects on or via lactation

Table 49: Summary table of animal studies on effects on or via lactation

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| No guideline study.<br><br>Bromide transfer through mother's milk and its impact | Sodium bromide<br><br>Potassium bromide<br><br>Vehicle: distilled water | *Maternal toxicity*<br><br>↑ mortality at 300 mg bromide/kg bw/day (one dam) and at 900 mg bromide/kg bw/day (one dam)<br><br>↓ maternal mean bodyweight (18%) at 900 mg bromide/kg bw/day<br><br>↓ food consumption at 900 mg bromide/kg bw/day | A6.8.1/07, Doc. No. 592-015<br><br>Vobecký M., Pavelka S. |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| on the suckling rat<br>Exp. 1: bw of each dam and the corresponding litter and the consumption of diet and drinking water were recorded<br>Exp. 2: determination of production rate of mother´s milk and its elemental composition<br>Exp. 3: measurement of bromide transfer through mother´s milk<br>Rat, Wistar<br>5 dams/group<br>8 pups/dam | <u>Exp. 1 and 2:</u><br>1 or 5 g bromide/L (corresponding to 300 and 900 mg bromide/kg bw/day)<br>Oral (drinking water)<br>Whole lactation period<br><u>Exp. 3:</u><br>1 or 5 g bromide/L (corresponding to 300 and 900 mg bromide/kg bw/day)<br>Single exposure day 12 of lactation | ↓ drinking water at 900 mg bromide/kg bw/day<br>No clinical observations were reported for any dose group.<br>*Effects on or via lactation*<br>↓ milk production<br>-elemental composition of milk changed (about 54% of the chloride in mother´s milk was replaced by bromide)<br>*Offspring toxicity*<br>↓ pup survival at 300 mg bromide/kg bw/day (94.8% of pups survived) and at 900 mg bromide/kg bw/day (56.3% of pups survived) compared to control (100% of pups survived)<br>↓ mean bodyweight in pups (65%) at 900 mg bromide/kg bw/day | and Babický A. 2005<br>Reliability 2 |
| No guideline study, no GLP.<br>Determination of<br>- the production rate of milk in the dams on lacation day 10 an 15.<br>- iodine transfer to sucklings from the mothers on lactation day | Testmaterial unknown, only 'bromide' stated<br>Vehicle: distilled water<br>Oral (drinking water)<br>Group A: 0, 1, 5 g bromide/L during the whole lactation period<br>or<br>Group B: 1 g | *Maternal toxicity*<br>No significant differences in the body weight among the lactating dams.<br>No examination of clinical signs during the study.<br>*Effetcs on or via lactation*<br>↓ milk production rate on days 10 and 15 (-67% and -70% respectively at at 5 g bromide/L, unclear if stat. sign.)<br>*Offspring toxicity*<br>↓ (trend analysis, $p<0.0001$) in pup body weight with increasing bromide intake (-29% compared to control in group A at 5 g bromide/L; -32% compared to control in group B at 5 g bromide/L)<br>↑ (contrast testing linear trend: $p = 0.0062$) relative weight of the thyroids in pups (34% compared to control in group A at 5 g bromide/L; 20% | A6.8.1/08, Doc. No.: 592-056<br>Pavelka et al., 2002.<br>Reliability 2 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| 16.<br>- thyroid hormones in sucklings and mothers.<br><br>Rat, Wistar<br>5-10 dams/group<br>8 pups/dam | bromide/L 14 days prior to mating and 0, 1, 5 g bromide/L during the whole lactation | compared to control in group B at 5 g bromide/L) | |

Table 50: Summary table of human data on effects on or via lactation

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| Case report<br><br>Human | Sodium bromide | Ten patients in the maternity ward at Temple University Hospital having babies at the breast were studied. | Effects as irritability, drowsiness, sleepiness, absence of cry and rash on the face was noted in the child after maternal intake of sodium bromide of 5.4g/day during 3-5 days (beginning on the sixth day following delivery) were noted. | Tyson et al., 1938 |

Table 51: Summary table of other studies relevant for effects on or via lactation

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other studies relevant for effects on or via lactation | | | | |

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

***Postnatal study of sodium bromide and potassium bromide in rat (Vobecký et al., 2005)***

In a non-guideline study bromide transfer through the mother's milk and its impact on the suckling rat was investigated (Vobecký, 2005). Sodium bromide or potassium bromide were administered at dose

levels of 1 or 5 g bromide/L (corresponding to 300 and 900 mg bromide/kg bw/day in dams based on a compound intake of 73 mg and 230 mg bromide/dam/day for the low and high dose group and assuming a bodyweight of 250 g/dam) during the whole lactation period (exp. 1 and 2) or with a single exposure day 12 of lactation (exp. 3).

In the first experiment, the body weight of each dam and the corresponding litter and the consumption of diet and drinking water were recorded. One dam in each dose group (300 mg bromide/kg bw/day and 900 mg bromide/kg bw/day) died and were excluded from the remaining part of the experiment. In the high dose group the difference in body weight between lactation day 2 and 28 in the high dose group was statistically significantly different from control group (-57.2 g compared to 1.8 g, $p = 0.008$). Moreover, decreases in maternal body weight at lactation day 28 (-18%), in food consumption and intake of drinking water during lactation were recorded in high dose group compared to control. However, the study authors state that a marked decrese in body weight was only noticed in two of the five rats. The other three rats had weights comparable to control group. In the low dose group the dams had a total weight gain of 14.6 g between lactation day 2 and 28. The pup survival in high dose group was 56.3% and in low dose group 94.8% of pups survived compared to 100% in control. The general condition in pups of the high dose group was noted as very poor and the mean bodyweight in pups was also lower (less than 40% of the body weight of control pups on lactation day 27) in the high dose group.

In the second experiment, the production rate of mother´s milk and its elemental composition were determined. The production rate in dams of the high dose group produced significantly less milk compared to control (-65% and -61%) and low dose (-58% and -64%) at lactation day 10 and 15, respectivley. Moreover, the elemental composition of the milk changed to about 54% of the chloride in mother´s milk being replaced by bromide.

In the final experiment, potassium bromide was administered on day 12 of lactation and the time-course of transfer of radiolabelled bromide through milk was measured. As soon as 3 h after administration to the mother radioactive bromide was detected (<10% of applied dose) in the offspring and after 25 h the radioactivity in the offspring was approximately 17% of the applied dose.

Thus, bromide can be transferred not only to the foetus but also via mothers milk to their pups. The reduced body weight and survival of the pups in the high dosage level is likely to be a consequence of reduced milk production in dams resulting in a state of malnutrition and lowered viability in pups.

***Postnatal study in rat (Pavelka et al., 2002)***

In an earlier study by Pavelka et al. (2002), dams were given distilled water with the addition of 1 or 5 g bromide/l during lactation with or without the addition of 1 or 5 g bromide/l as early as 14 days before mating, similar effects as in the study from 2005 were noted. Reduced milk production and decreased bodyweight in pups were recorded. There were no significant differences in the body weight among the lactating dams.

Moreover, there were indications (not clear if statistical significant different from control and results presented from two separate experiments showed great variation in concentration of hormones) of reduced levels of T3 and T4 hormones both in the dams and the pups. The relative weight of the thyroids in pups was increased, but no effect on thyroid weight was recorded in the dams. It was also noted that the iodine transfer through mothers milk to the sucklings was decreased by the enhanced bromide intake in the dam with no significant difference between the groups only adminstered bromide during lactation and the group adminstered bromide 14 days premating and during lactation.

***Human case report (Tyson et al., 1938)***

In a human report of ten patients, in the maternity ward at Temple University Hospital, having babies at the breast effects as irritabliblity, drowsiness, sleepiness, absence of cry and rash on face were noted in the child after maternal intake of sodium bromide of 5.4 g/day during 3-5 days (beginning on the sixth day following delivery).

### 10.10.9 Comparison with the CLP criteria

This classification can be assigned on the:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

There were no clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk in the two-generation study of sodium bromide or the dose range finding studies of ammonium bromide and sodium bromide.

However, in available non-guideline studies of sodium bromide or potassium bromide, it was demonstrated that bromide can be transferred via mothers milk to their pups. The milk production was decreased and the elementary composition of the milk was changed in dams administered 900 mg bromide/kg bw/day during lactation. In the same dose group the growth and survival of pups were reduced, which was considered to be a consequence of the reduced milk production in dams resulting in a state of malnutrition and lowered viability in pups. Thus, there is evidence from studies in rat that bromide may cause harm due to its effects on and via lactation. Moreover, there is a weak indication from a human case report on possible effects on the central nervous system of the infant/child after maternal intake of sodium bromide during lactation. Thus, in an overall weight of evidence assessment, classification for effects on or via lactation is considered warranted.

### 10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification of ammonium bromide for adverse effects on sexual function and fertility; adverse effects on development and on adverse effects on or via lactation is warranted: **Repr. 1B, H360 FD and H362**

Specific concentration limits for adverse effects on sexual function and fertility; adverse effects on development or adverse effects on or via lactation are not considered justified since the estimated ED10 values are within the medium potency group (4 mg/kg bw/day < ED10 value < 400 mg/kg bw/day).

## 10.11 Specific target organ toxicity-single exposure

Table 52: Summary table of animal studies on STOT SE

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, route of exposure, dose levels, duration of exposure | Results | Reference |
|---|---|---|---|
| ***Ammonium bromide*** | | | |
| OECD TG 401 (Acute Oral Toxicity)<br><br>EPA OPP 81-1 (Acute Oral Toxicity)<br><br>Rat, CD strain (remote Sprague Dawley origin)<br><br>male/female<br><br>5/sex/group | Ammonium bromide<br><br>Oral: gavage<br><br>2000, 2714, 3684 and 5000 mg/kg bw | 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 some of the animals.<br><br>LD50 males 2868 mg/kg bw<br><br>LD50 females: 2566 mg/kg bw<br><br>LD50 males and females combined: 2714 mg/kg bw | A6.1.1/01, Doc. No. 521-001<br><br>Study report, 1986a<br><br>Reliability 1 |
| ***Sodium bromide*** | | | |
| equivalent or similar to OECD TG 401 (Acute Oral Toxicity)<br><br>EPA FIFRA 81-1<br><br>Rat, CD [Crl: CD(SD)BR]<br><br>male/female<br><br>5/sex/group | Sodium bromide<br><br>Oral: gavage<br><br>3200, 4000, 5000 mg/kg bw | Clinical signs such as piloerection, hunched posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities and prostration were noted within 3 hours of dosing at a dose level of 3200 mg sodium bromide/kg bw.<br><br>LD50 males: 4500 mg/kg bw<br><br>LD50 females: 3900 mg/kg bw<br><br>LD50 males and females combined: 4200 mg/kg bw | A6.1.1/02, Doc. No. 521-002<br><br>Study report, 1988a<br><br>Reliability 1 |

Table 53: Summary table of human data on STOT SE

| Type of data/report | Test substance | Route of exposure<br>Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| ***Bromide*** | | | | |
| Study type: prognosis following poisoning<br>Endpoint addressed: not specified | Sodium, potassium or ammonium bromide | | Treatment of overdoses of sodium, potassium or ammonium bromide includes hydration, the maintenance of a mild water diuresis, and sodium or better, ammonium chloride (10 to 15 g daily in divided doses) with a mercurial diuretic. Hemodialysis may be of value.<br>Acute oral poisoning is rare because single doses are usually promptly rejected by vomiting, but 1 oz has been swallowed and adsorbed sufficiently to cause death.<br>Clinical signs: A blood level of 125 mg per 100 mL is regarded as the minimal intoxicating level. The systemic effects of the bromide ion are chiefly mental: drowsiness, irritability, ataxia, vertigo, confusion, mania, hallucinations, and coma.<br>Other effects include skin rashes, neurological signs, sensory disturbances, and increased spinal fluid pressures. | Gosselin R.E., 1976<br>Reported as supporting study in REACH Registration dossier |
| Study type: prognosis following poisoning<br>Endpoint addressed: not specified | Not reported | | Overdosage with bromides causes slurring of speech, unsteadiness, depression or stupor, and psychosis. Delirium, transitory schizophrenia and hallucinations are characteristic. The literature reveals no uniform effect of overdosage on the eyes, but very commonly the pupils are large and have subnormal reaction to light or accommodation. The pupils return to normal when the patient recovers. | Grant W.M., 1974<br>Reported as supporting study in REACH Registration dossier |

Table 54: Summary table of other studies relevant for STOT SE

| Type of study/data | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other studies relevant for STOT SE. | | | | |

### 10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

There are two available test-guideline studies of acute oral toxicity in rat relevant for assessing STOT SE: one with ammonium bromide and the other with sodium bromide.

**Ammonium bromide - test guideline acute toxicity studies relevant for STOT SE**

***Acute oral toxicity study of ammonium bromide in rats (Study report, 1986a)***

In an oral acute toxicity study performed according to OECD TG 401 ammonium bromide 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 (Study report, 1986a). Mortality, signs of reaction to treatment and body weight gain were recorded during a subsequent 14-day observation period after which LD50 was determined. Clinical signs such as lethargy (5/5 males), decreased motor activity (5/5 males and females), prone or hunched posture (5/5 males), ataxia (5/5 males and 5/5 females), breathing irregularities, unconsciousness and tonic convulsions were observed in rats after oral administration at a dose level of 2000 mg ammonium bromide/kg bw. The duration of signs was 15 minutes to 2 days. These sign were also apparent in all animals at higher dose levels, but at 2714 mg/kg 1/5 males and 4/5 females died within 1 hour after administration and at 3684 mg/kg all rats died within half an hour and 2 days. At 5000 mg/kg all animals were dead within 15 minutes to 1 hour. The mortality rate for ammonium bromide 2000 mg/kg bw in both male and females were zero. Necropsy findings included fur staining, abnormal gastro-intestinal contents, dark areas on the lungs and occasional thymic petechiae. In the surviving animals there were no effects on bodyweight gains and necropsy findings on day 15 were unremarkable.

**Sodium bromide - test guideline acute toxicity studies relevant for STOT SE**

***Acute oral toxicity study of sodium bromide in rats (Study report, 1988a)***

In an oral acute toxicity study in rat similar to OECD TG 401 of sodium bromide clinical signs such as piloerection, hunched posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities and prostration were noted within 3 hours of dosing at a dose level of 3200 mg sodium bromide/kg bw (Study report, 1988a). The mortality rate for sodium bromide was zero for both sexes of rats at 3200 mg/kg bw, at 4000 mg/kg bw 1/5 males and 3/5 females died, and finally at 5000 mg/kg bw 4/5 males and 5/5 females died.

**Human information**

A number of studies of exposure-related observations in humans from clinical or poisoning cases were very sparsley summarised (unknown or not well defined test substance and endpoint not specified) in the REACH-registration. This data was therefore not used in the weight of evidence assessment for comparison with classification criteria.

### 10.11.2 Comparison with the CLP criteria

Lethargy, lack of coordination, loss of righting reflex, and ataxia at 2000 mg/kg bw/day were observed in all rats after administration of ammonium bromide in an acute toxicity study. The duration of signs was 15 minutes to 2 days, and were transient in nature. At the higher dose tested, 2714 mg/kg, 1/5 males and 4/5 females died within 1 hour after administration.

No classification has been warranted for acute oral toxicity (since LD 50 is ≥ 2000) despite significant toxic effect, therefore ammonium bromide should be considered for classification in STOT SE.

Narcotic effects observed at 2000 mg/kg bw/day seen in the oral acute toxicity study of ammonium bromide warrant classification in category 3, H336. Category 2 (2000 ≥ ATE > 300) is not appropriate since the effects were observed to be transient in nature.

In the 90-day repeated dose toxicity study of sodium bromide clinical signs of neurotoxicity in rats were observed from 175 mg/kg bw/day (Study report, 2016b). Effects (including decreased motor activity and ataxia) at 500 mg/kg bw/day were apparent already at 2 hours after dosing on the first day of treatment in males. However, since there was an increase in incidence, duration and severity over the treatment period, these findings point to target organ effects after repeated exposure and not after single exposure. Due to clinical signs of neurotoxicity observed after repeated exposure starting from 100 mg/kg bw/day criteria are also met for STOT RE 2 for effects on the nervous system (see section 10.12).

### 10.11.3 Conclusion on classification and labelling for STOT SE

Based on findings, that were observed to be transient in nature, in an acute toxicity study in rat including lethargy, lack of coordination, loss of righting reflex, and ataxia at 2000 mg/kg bw/day classification in **STOT SE 3, H336** (narcotic effects) is warranted.

## 10.12 Specific target organ toxicity-repeated exposure

Table 55: Summary table of animal studies on STOT RE

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, route of exposure, dose levels, duration of exposure | Results | Reference |
|---|---|---|---|
| ***Ammonium bromide*** | | | |
| Repeated dose toxicity: 4 week dose range-finding study<br><br>GLP compliant<br><br>Rat, Sprague-Dawley male/female<br><br>5/sex/group | Ammonium bromide<br><br>Oral (feed)<br><br>0, 100, 500, 1000 mg/kg bw/day (nominal in diet)<br><br>4 weeks (28 days) | **Mortality and clinical observations**<br><br>↑ clinical signs of neurotoxicity and subdued behaviour at 500 (all males) and 1000 mg/kg bw/day (all males and 4/5 females)<br><br>**Body weight**<br><br>↓ body weight gain (males 49% and females 31%) at 1000 mg/kg bw/day<br><br>**Food consumption**<br><br>↓ food consumption (males 29%) at 1000 mg/kg bw/day<br><br>**Organ weights**<br><br>↓ mean absolute testes weight starting from 100 mg/kg bw/day (-11%, $p<0.05$; -14%, $p<0.05$; -16%, $p<0.01$)<br><br>↓ mean absolute epididymis weights at 500 and 1000 mg/kg bw/day (-14%, $p<0.05$; -16%, $p<0.01$)<br><br>↓ mean absolute kidneys (males) weights at 500 and 1000 mg/kg bw/day<br><br>↓ mean absolute heart, liver and lungs weights at 1000 mg/kg bw/day<br><br>↑ relative liver weight (23%, $p<0.01$; females) at 1000 mg/kg bw/day<br><br>No histopathological examination performed to confirm changes in organ weights. | A6.3.1/01, Doc. No. 532-001<br><br>Study report, 1999<br><br>Reliability 1 |

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|---|---|---|---|
| OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)<br><br>EPA OPPTS 870.3100 (90-Day Oral Toxicity in Rodents)<br><br>GLP compliant<br><br>Rat, Sprague-Dawley male/female<br><br>25/sex/group (control and high doses), 15/sex/group (low and intermediate dose) | Ammonium bromide<br><br>Oral (feed)<br><br>0, 100, 225, 500 (males), 750 mg/kg bw/day (nominal in diet)<br><br>Exposure: 13 weeks (90-days)<br><br>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 | **Mortality and clinical observations**<br><br>3 premature terminations among males at 500 mg/kg bw/day<br><br>↑ clinical signs of subdued behaviour at 225 mg/kg bw/day (11/15 males) and 500/750 mg/kg bw/day (14/15 males and 9/15 females), rolling gait at 225 mg/kg bw/day (4/15 males) and 500/750 mg/kg bw/day (15/15 males and 13/15 females), staggering at 225 mg/kg bw/day (2/15 males) and 500/750 mg/kg bw/day (12/15 males and 11/15 females) and nasal bleeding at 500/750 mg/kg bw/day (5/15 males and 8/15 females)<br><br>**Detailed functional observations**<br><br>↓ hind limb strength at 500/750 mg/kg bw/day (males 33-42% and females 32-51%)<br><br>**Body weight**<br><br>↓ body weight gain at 225 mg/kg bw/day (10% males) and at 500/750 mg/kg bw/day (males 23% and females 22%)<br><br>**Food consumption**<br><br>↓ food consumption 500 mg/kg bw/day (males 7%)<br><br>**Organ weights**<br><br>↓ absolute weight of epididymides at 100 (10%, $p<0.05$), 225 (12%, $p<0.01$) and 500 mg/kg bw/day (22%, $p< 0.001$)<br><br>↓ absolute weight of testes at 225 (10%, $p<0.05$) and 500 mg/kg bw/day (16%, $p< 0.001$)<br><br>**Histopathology**<br><br>No effects reported.<br><br>Ventricular dilation seen in animals from all groups. | A6.4.1/01, Doc. No. 533-001<br><br>Study report, 2000<br><br>Reliability 1 |

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| Dose range finding study of a reproduction toxicity study<br><br>GLP<br><br>Rat, Sprague Dawley<br><br>10/sex/group | Ammonium bromide<br><br>Oral (feed)<br><br>0, 1600, 3200, 6400 ppm<br><br>Group mean achieved dosages of test material during treatment, gestation and lactation:<br><br>0, 127, 242 and 503 mg/kg bw/day for males and 0, 228, 454 and 651 (during treatment and gestation only) mg/kg bw/day for females<br><br>Exposure: 2 weeks prior mating until the first generation had been weaned | **Parental generation – general toxicity**<br><br>**Mortalities and clinical observations**<br><br>*Males and females*<br><br>↑ incidences of clinical signs starting at 127/228 mg/kg bw/day (transient piloerection), at 242/454 mg/kg bw/day 9/10 males and 6/10 females had rolling gait, and at 503/651 mg/kg bw/day all animals displayed clinical signs during the the whole study period (neurotoxic effects; rolling gait, piloerection, hunched posture, generally ill condition of these animals, staining on the body and an unkempt appearance of the coat). About half of the females showed hyperactivity<br><br>**Body weight and food consumption**<br><br>*Males*<br><br>↓ bodyweight gain days weeks 0-8, 13% less than control at 242 mg/kg bw/day and 16% less than control at 503 mg/kg bw/day<br><br>↓ food consumption, 88% of control at 242 mg/kg bw/day and 503 mg/kg bw/day<br><br>*Females*<br><br>↓ bodyweight gain gestation days 0-20 (67% of control or 33% less than control) at 651 mg/kg bw/day<br><br>**Organ weights**<br><br>↓ mean absolute weights of heart, lung and liver at 503 mg/kg, and of kidney at both 242 mg/kg bw/day and 503 mg/kg bw/day<br><br>↓ mean absolute weights of testes at 242 mg/kg bw/day (11%) and at 503 mg/kg (16%) and of epididymides at 242 mg/kg bw/day (11%) and at 503 mg/kg bw/day (11%)<br><br>No effects on relative organ weights and no histopathological examination performed to confirm changes in organ weights | A6.8.2/01, Doc. No.553-001<br><br>Study report, 2001<br><br>Reliability 1 |
| ***Sodium bromide*** | | | |

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| Oral Repeat Dose 90-Day Toxicity Study in Rats, Including Recovery Assessments<br><br>OECD TG 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents) with the relevant sections relating to oestrous cycles, sperm evaluation and histopathology OECD TG 416 (Two-Generation Reproductive Toxicity Study)<br><br>Rat, Crl:CD(SD)<br><br>Male/female<br><br>10/sex/group and an additional 10/sex/group for control and high dose groups were included for the recovery assessment. | Sodium Bromide<br><br>CAS Number: 7647-15-6<br><br>Purity: 100.0%<br><br>Oral (gavage)<br><br>0, 60, 175, 500 mg/kg/day<br><br>A Sodium Chloride comparator group (284 mg/kg/day) was also included in the assessment.<br><br>90 days | **Mortality and clinical observations**<br><br>*Males*<br><br>↑ mortality at 500 mg/kg bw/day (4 animals euthanasied)<br><br>↓ motor activity postdose in 6/10 males in week 2 at 175 mg/kg/day, but all animals recovered before the end of the working day.<br><br>↑ incidences ($p \leq 0.01$) 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) at 500 mg/kg/day.<br><br>*Females*<br><br>↓ motor activity postdose in 8/10 animals, only observed on study days 11-13 at 175 mg/kg/day, and all animals recovered before the end of the working day.<br><br>↑ incidences ($p \leq 0.01$) of 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) at 500 mg/kg/day.<br><br>**Functional observation battery and motor activity**<br><br>*Males*<br><br>↑ ambulations ($p \leq 0.05$) in week 4 (prior to dosing), only at the first 10 minute interval at 175 mg/kg/day and at 500 mg/kg/day.<br><br>↓ incidence of rears at 500 mg/kg/day<br><br>↑ incidences of ataxia (2/10) and abnormal gait predose during week 4 of treatment at 500 mg/kg/day. In week 9 all males exhibited ataxia and abnormal gait.<br><br>*Females*<br><br>↑ motor activity (ambulation) ($p \leq 0.05$) and lack of righting reflex in 5 females and a reduction in hindlimb grip strength at 175 mg/kg/day.<br><br>↑ incidences of ataxia and abnormal gait (6/10 females) week 9 at 500 mg/kg/day<br><br>**Body weight**<br><br>*Males* | Study report, 2016b<br><br>Reliability 1 |

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| | | ↓ body weight at the end of dosing period, 81.2 % of control, ($p \leq 0.01$) at 500 mg/kg/day<br><br>↓ body weight gain for most weekly intervals after week 2, and at the end of the dosing period, 68.8% of control, ($p \leq 0.01$) at 500 mg/kg/day<br><br>*Females*<br><br>↓ body weight gain at the end of the recovery period (66.5% of control values) at 500 mg/kg/day (but body weight and weight gain were unaffected during the treatment period)<br><br>**Food consumption**<br><br>*Males*<br><br>↓ absolute intake over the treatment period, 91.4% of controls ($p \leq 0.05$) at 175 mg/kg/day and at 500 mg/kg/day average and relative food consumption were significantly reduced ($p \leq 0.01$) over the dosing period (study days 1 to 90) and for each weekly interval after week 1.<br><br>↓ average absolute food consumption values ($p \leq 0.01$) in first 2 weeks during the recovery period and relative food consumption values were significantly lower ($p \leq 0.05$) at the start of the recovery period (study days 90 to 92).<br><br>↑ overall intake during the recovery period (study days 90 to 147, $p \leq 0.01$).<br><br>*Females*<br><br>↓ absolute food consumption (study days 43 to 50, $p \leq 0.01$) and relative food consumption (study days 8 to 15, $p \leq 0.01$ and study days 43 to 50, $p \leq 0.05$) at 500 mg/kg/day<br><br>**Thyroid Hormone Analysis**<br><br>↓ T3 levels in males at 60 mg/kg/day (27%, $p \leq 0.05$), 175 mg/kg/day (28%, $p \leq 0.05$) and 500 mg/kg/day (37%, $p \leq 0.01$).<br><br>↓ T4 levels in males at 175 mg/kg/day (28%, $p \leq 0.01$) and at 500 mg/kg/day (52%, $p \leq 0.01$) and in females at 60 mg/kg/day (26%, $p \leq 0.05$), 175 mg/kg/day (34%, $p \leq 0.01$) and at 500 mg/kg/day (47%, $p \leq 0.01$).<br><br>↑ mean TSH levels in males at 175 and 500 mg/kg/day (36% and 74%, respectively, not stat. sign.) and in females at 60 and 500 mg/kg/day (60% and 54%, respectively, not stat. sign.). | |
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| | | **Organ weights**<br>↑ relative to body thyroidea weight (27% increase compared to control, p≤ 0.05) in females at 500 mg/kg/day<br><br>**Histopathology**<br>Mild/moderate depletion of colloid observed in 2 males and 2 females in the groups treated at 175 and 500 mg/kg/day, no correlation between these findings and decreased T3 and T4 levels in individual animals.<br><br>**Reproductive toxicity**<br>Please refer to table 37 and section 10.10. Only effects relevant for STOT RE are discussed here. | |
| Three-generation reproduction study (no guideline)<br>Not GLP-compliant<br>Deviations: no food consumption, pup body weights and litter size determination<br><br>Rat, strain not specified<br>Males and females<br>7-19/sex/ group | Sodium bromide<br><br>Purity: not reported<br><br>Oral (feed)<br><br>Breeding study and thyroid hormone investigation:<br>0, 75, 300, 1200, 4800, 19200 ppm (corresponding to 0, 6.75, 27, 108, 432 and 1728 mg/kg bw/day based on a default conversion of 1 ppm=0.09 mg/kg bw/day) | **Mortality and clinical observations**<br>No information on clinical condition available<br>**Body weights**<br>No adverse effects on body weights recorded<br>**Food consumption**<br>No information on food consumption available<br>**Thyroid Hormone Analysis**<br>↓ thyroxine (T4) serum concentrations in F0 (males: approx. 10-16% at 75 and 300 ppm, 25%, 40%, 60% at 1200, 4800 and 19200 ppm, respectively; in females approx.. 20% and 35% at 4800 and 19200 ppm, respectively) compared to control<br><br>**Organ weights**<br>↓ relative adrenal weight in F0 females at 1200 and 4800 ppm (approx. 15%, p< 0.05 and p<0.01<br><br>**Histopathology**<br>No histopathological examination performed<br><br>**Reproductive toxicity**<br>Please refer to table 37 and section 10.10. Only effects relevant for STOT RE is discussed here. | A6.8.2/02, Doc. No. 592-002<br><br>Van Leeuwen et al., 1983<br><br>Reliability 2 |

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|---|---|---|---|
| No guideline study: 28-day oral repeated dose toxicity study<br><br>Deviations: few animals, one sex, limited parameters investigated. Gross necropsy, organ weight and histopathology were performed for kidney, brain and liver only.<br><br>Rat, Wistar<br><br>female<br><br>4/group | Sodium bromide<br><br>Purity: 99.5%<br><br>Oral (feed)<br><br>0, 300, 1200, 4800 or 19200 ppm (nominal in diet)<br><br>(corresponding to 0, 36, 144, 576, 2304 mg/kg bw/day)<br><br>4 weeks | ↑ clinical signs of neurotoxic effects (motorincoordination of hind legs) at 19200 ppm.<br><br>↑ relative kidney weight at 19200 ppm (21%, $p<0.001$). | A6.4.1/03, Doc. No. 592-007<br><br>van Logten, M. J. et al., 1973<br><br>Reliability 2 |

| | | | |
|---|---|---|---|
| No guideline study: 90-day oral repeated dose toxicity study<br><br>Rat, Wistar<br>male/female<br>10/sex/group | Sodium bromide<br><br>Purity: 99.5%<br><br>Oral (feed)<br><br>0, 75, 300, 1200, 4800 or 19200 ppm (nominal in diet) (corresponding to 0, 6.75, 27, 108, 432, 1728 mg/kg bw/day)<br><br>90 days | **Mortality and clinical observations**<br>↑ clinical signs of neurotoxic effects (motorincoordination of legs) at 19200 ppm (unclear if both males and females)<br>**Body weight and food consumption**<br>↓ body weight gain (23% in males) at 19200 ppm<br>**Clinical chemistry and haematology**<br>↓ erythrocyte counts (males 7%, p≤0.05) at 19200 ppm<br>↑ neutrophilic granulocytes counts (males 73%, p≤0.05 and females 110%, p≤0.05) at 19200 ppm<br>↓ aminopyrine demthylase activity (males 53%, p≤0.05) at 19200 ppm<br>**Organ weights**<br>↑ relative thyroidea weight in females at 1200 ppm (32%, p≤0.01), 4800 ppm (18%, p≤0.01) and in males and females at 19200 ppm (57%, p≤0.001 and females 48%, p≤0.001)<br>↓ relative thymus weight (females 24%, p≤0.05) at 19200 ppm<br>↑ relative spleen weight (males 25%, p≤0.01) at 19200 ppm<br>↓ relative prostate weight at 4800 (33%, p≤0.01) and 19200 ppm (50%, p≤0.001)<br>↑ relative adrenal weight (males 31%, p≤0.001) at 19200 ppm<br>**Histopathology**<br>(Note: quantification, severity, or incidences not reported in the publication)<br>↑ increased vacuolisation of zona fasciculate in adrenals in males and females at 75, 300, 1200, 4800 and 19200 ppm<br>↓ size of follicles in thyroidea (males and females) at 19200 ppm<br>↑ thyroid activity at 4800 ppm (females) and 19200 ppm (males and females)<br>↓ number of corpora lutea at 19200 ppm<br>↓ size of tubuli at 19200 ppm<br>↑ pituitary cysts (males) at 19200 ppm<br>↓ spermatogenesis (tendency) at 19200 ppm<br>↓ secretory activity of prostate (tendency) at 4800 and 19200 ppm | A6.4.1/04, Doc. No. 592-005<br><br>van Logten, M. J., et al., 1974<br><br>Reliability 2 |

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|---|---|---|---|
| No guideline study: 90-day oral repeated dose toxicity study on a low chloride diet<br><br>Rat, Wistar<br>male/female<br>10/sex/group | Sodium bromide<br><br>Oral (feed)<br><br>0, 8, 31, 125, 500 and 2000 ppm (low chloride diet) (corresponding to 0, 0.72, 2.8, 11, 45, 180 mg/kg bw/day)<br><br>90 days | *Note: the reults from the 90-day repeated dose toxicity on normal chloride diet is given in the study van Logten, M. J., et al., 1974. Here only results from the low chloride study is presented.*<br><br>**Mortality and clinical observations**<br><br>3 males and 3 females at 2000 ppm (low chloride diet) died during the study period<br><br>↑ motor incoordination of hind legs (no incidences given and unclear if both males and females) at 2000 ppm (low chlrodie intake)<br><br>**Body weight and food consumption**<br><br>↓ body weight gain after 12 weeks at and at 2000 ppm (males 31% and females 35%)<br><br>**Clinical chemistry and haematology**<br><br>↑ neutrophilic granulocytes in females at 2000 ppm<br><br>↑ total leukocyte counts in females at 2000 ppm<br><br>↓ monocytes in males at 2000 ppm<br><br>**Organ weights**<br><br>↓ relative (to heart weight) pituitary weight in females at 2000 ppm<br><br>↑ relative (to heart weight) adrenal weight in males at 2000 ppm<br><br>**Histopathology**<br><br>↑ thyroid activity at 500 and 2000 ppm<br><br>↓ vacuolisation of the zona fasciculate in the adrenals at 500 and 2000 ppm<br><br>↓ zymogen granulae in pancreas at 500 and 2000 ppm<br><br>↓ spermatogenesis at 2000 ppm<br><br>↓ corpora lutea at 2000 ppm<br><br>Retardation of maturation of uterus at 2000 ppm<br><br>Hyperaemic brain at 2000 ppm<br><br>Degeneration in myocard of the heart at 2000 ppm<br><br>Granulocytes along blood vessels in the lung at 2000 ppm<br><br>↓ secretory activity of salivary gland at 2000 ppm | A6.4.1/05, Doc. No. 592-006<br><br>Van Logten M.J. et al., 1976<br><br>Reliability 2 |

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| No guideline study: Sub-chronic toxicity, oral (Limited parameters investigated in the study. Few animals used. Gross necropsy, organ weight and histopathology were not performed. Haematology and clinical chemistry parameters were not investigated except for blood bromide levels and total halide levels.)<br><br>Dog, Mongrel<br>Male/female<br>4 dogs/group | Sodium bromide<br><br>Oral (gelatine capsules)<br>Group 1: 100 mg/kg bw/day<br>Group 2 and 3: initial doses of 100 (group 2) and 200 mg/kg bw/day (group 3) increments of 100 or 200 mg/kg bw/day at intervals of 6 weeks until death resulted<br>Group 4: initial dose of 400 mg/kg bw/day, when necessary this dose was increased but always before the 6th week of administration of the previous dose.<br>Treatment with 100, 200, 300, 400 and 500 mg NaBr/kg bw/day (corresponding to 78, 156, 234, 312 and 390 mg bromide/kg bw/day) resulted in a mean blood bromide concentration of 32.2, 36.3, 45.9, 51 and 49.6 mEq/L, respectively.<br><br>44-185 days | 100 mg/kg bw/day:<br>↑ clinical signs indicating gastrointestinal toxicity<br>↓ body weight (body weight loss: 15.4% in week 10 of administration with increasing doses of sodium bromide beginning with 100 mg/kg bw/day (group 2))<br><br>200 mg/kg bw/day:<br>↑ mortalities<br>↑ clinical signs of neurotoxicity<br>↑ clinical signs indicating gastrointestinal toxicity<br>↓ body weight (body weight loss: 15.4% in week 10 of administration with increasing doses of sodium bromide beginning with 200 mg/kg bw/day (group 3))<br>↑ skin lesions<br><br>400 mg/kg bw/day:<br>↑ mortalities<br>↑ clinical signs of neurotoxicity<br>↑ clinical signs indicating gastrointestinal toxicity<br>↓ body weight (body weight loss: 15.4% in week 10 of administration<br>↑ skin lesions | A6.4.1/07. Doc. No. 592-027<br><br>Rosenblum I. 1958.<br><br>Reliability 3 |
| *Potassium bromide* | | | |

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|---|---|---|---|
| No guideline study: sub-chronic toxicity, oral<br>GLP compliance: no<br>Dog, Beagle male/female 3/sex/group | Potassium Bromide<br>Oral (feed)<br>30 mg/kg bw (equivalent to 20 mg Br-/kg bw) No control animals<br>115 days (daily). Plus 5 days for an additional test.<br>(No post-exposure period) | Mean steady-state serum bromide concentration: 245 mg/dL:<br>Electrodiagnostic test revealed only subtle changes over time. No clinical neurotoxic signs.<br>397 mg bromide/dL:<br>Two of the dogs showed caudal paresis and ataxia and two of the dogs were agitated to hyperexcitable without signs of weakness or ataxia. | A6.10/17, Doc. No. 592-032<br>March P.A., Podell M. and Sams R.A. 2002.<br>Reliability: 2 |
| ***Studies with reliability 3, included in the draft CAR for BAC and/or REACH registration but not considered in the assessment by the DS*** | | | |
| Gaskill, C. L.; Cribb, A. E. 2000. Pancreatitis associated with potassium bromide/phenobarbital combination therapy in epileptic dogs. Can Vet J 41, 555-558 | | | |
| Grier, R. L.Year:1967. Quaternary Ammonium Compound Toxicosis in the Dog. JAVMA, Vol. 150, No.9, 984-987 | | | |
| Underhill, F. P. (1913). Studies on the Metabolism of Ammonium Salts. I. The elimination of ingested Ammonium Salts in the Dog upon an adequate mixed diet. J Biol Chem 15, 327-335. | | | |

Table 56: Summary table of human data on STOT RE

| **Type of data/report** | **Test substance** | **Route of exposure**<br>**Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| ***Sodium bromide*** | | | | |

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| Type of effects studied: endocrine system modulation (and effects on central nervous system) (in vivo)<br><br>Human female | Sodium bromide | Oral (capsule)<br>0, 4, 9 mg/kg bw/day (actual ingested)<br>Exposure: The experiment covered six menstrual cycles. Bromide administration during the first three cycles | **General signs/observations:**<br>Medical histories, physical examinations and haematological biochemical and urine analyses after completion of the experiment showed no treatment-related abnormalities except for some incidence of nausea associated with bromide-capsule ingestion (nausea noted in 3 volunteers of the 4 mg/kg bw/day group and 11 of the 9 mg/kg bw/day group).<br>**Endocrine system:**<br>The concentrations of T4, fT4, TBG, T3 and TSH withing normal limits at each point of time (start of experiment, after 3 and 6 cycles).<br>**Neurophysiology (EEG):**<br>No changes in δ1-, δ2-activities; significant changes in α1-band and β activity at 4 and 9 mg/kg bw/day. | A6.10.05, Doc. No. 592-001<br><br>Van Gelderen C.E.M. et al., 1993<br><br>Reliability 2 |
| Type of effects studied: endocrine system modulation (in vivo)<br><br>Human male/female | Sodium bromide | oral: capsule<br>1 mg/kg bw (actual ingested)<br>Exposure: 8 weeks or two full cycles (daily) | **General signs/observations:**<br>In two females a short lasting itching dermatosis with small vesicles was observed at the beginning of the experiment (reversible effect).<br>**Endocrine system:**<br>No significant differences in T4, fT4, TBG and T3 determined in serum at the start and at the end of the experiment; no differences in serum concentrations of cortisol, testosterone, estradiol, progesterone, TSH, prolactin, LH, FSH at the start and at the end of the experiment; no differences in the response to administration of TRH and LHRH at the start and at the end of bromide administration. | A6.10/03, Doc. No. 592-012<br><br>Sangster B., et al., 1982<br><br>Reliability 2 |

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| Type of effects studied: endocrine system modulation (and effects on central nervous system) (in vivo)<br><br>Human male/female | Sodium bromide | oral: capsule<br>0, 4 and 9 mg bromide/kg bw/day (actual ingested)<br>Exposure: three month or three full cycles (daily) | **General signs/observations:**<br><br>Medical histories, physical examinations and haematological, biochemical and urine analyses after completion of the experiment showed no treatment-related abnormalities except for some incidence of nausea associated with bromide-capsule ingestion (nausea noted in 2 persons at 4 mg/kg bw/day and in 5 persons at 9 mg/kg bw/day).<br><br>**Endocrine system:**<br><br>At 9 mg/kg bw/day, a slight but significant increase in T4 and T3 in females only (individual concentrations of T4 and T3 in this group within normal limits at the start and the end of the investigation); no changes on all other parameters (TBG, fT4, cortisol, oestradiol, progesterone or testosterone, thyrotropin, prolactin, luteinizing hormone and follicle-stimulation hormone) at 4 and 9 mg/kg bw/day.<br><br>**Neurophysiology (EEG and visual evoked response):**<br><br>Decrease in δ1- and δ2-activities and increases in β-activities and in mean frequency (mobility parameters) at 9 mg/kg bw/day only; increased α1-activity at 4 mg/kg bw/day. | A6.10/04, Doc. No. 592-013<br><br>Sangster B., et al., 1983<br><br>Reliability 2 |
| Study type: prognosis following poisoning<br>Endpoint addressed: not specified | Not reported | | Bromide intoxicated patients can show one or more of the following signs and symptoms: drowsiness, lethargy, dysarthia, weakness, ataxia, various skin disorders, memory loss, disorientation, psychosis with delirium and hallucinations. This condition can easily masquerade for many psychotic states and should be suspected whenever a clinical diagnosis is not clear. | McDanal C.E., Owens D. and Bolman W.M., 1974<br><br>Reported as supporting study in REACH Registration dossier |

Table 57: Summary table of other studies relevant for STOT RE

| Type of study/data | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| *Sodium bromide* | | | | |

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| Endocrinology<br>4 or 12 weeks feeding study, alterations in the endocrine system were investigated<br><br>Rat, Wistar<br>male<br>10/group | Sodium bromide | Oral (diet)<br>0, 20, 75, 300, 1200 and 19200 mg/kg diet (corresponding to 0, 1.8, 6.75, 27, 108 and 1728 mg/kg bw/day)<br><br>Exposure: 4 or 12 weeks<br><br>The study was initiated to ascertain whether alterations in the endocrine system in the rat detected during a semi chronic feeding study could be detected in male rats after exposure to high dietary concentrations of sodium bromide and moreover whether histopathological and immunocytochemical findings could be correlated with serum-hormone levels | 20, 75, 300 ppm:<br>No treatment related effects.<br><br>1200 ppm:<br>↑ thyroid weight (38%, p<0.01 at 4 weeks)<br>↓ T4 level (23%, p<0.01 at 4 weeks)<br>↑ FSH level (21%, p<0.05 at 12 weeks)<br><br>19200 ppm:<br>↓ bw (9%, p<0.05 at 4 weeks; 21%, p<0.001 at 12 weeks)<br>↑ thyroid weight (71%, p<0.05 at 4 weeks and 82%, p<0.001 at 12 weeks)<br>↑ incidences of histopathological changes in thyroidea at 4 and 12 weeks (increase of follicles and a decrease in their size)<br>↑ incidences of histopathological changes in testes at 12 weeks (7/10 males with reduction of tubule diameter)<br>↓ spermatogenesis (at 12 weeks)<br>- changes in immunoreactivity in pituitary gland (↓ GH at 4 and 12 weeks, ↑ TSH at 12 weeks, ↑ ACTH at 12 weeks)<br>- changes in hormone levels in serum (↑ TSH at 4 and 12 weeks, ↑ FSH at 4 and 12 weeks, ↑ insulin, ↓ T4 level at 4 and 12 weeks, ↓testosteron at 4 and 12 weeks, ↓ LH at 4 weeks, ↓ corticosterone at 4 and 12 weeks) | A6.10/09; Doc. No. 592-036<br><br>Loeber et al., 1983<br><br>Reliability: 2 |

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| Endocrinology<br>4 week (dietary), effects on the thyroid were investigated<br>Low iodine diet<br><br>Rat, Sprague-Dawley<br>male/female<br>12/sex/group. | Sodium bromide | Oral (feed)<br>4, 8 and 16 g/kg (nominal in diet (corresponding to 200, 400 and 800 mg/kg bw/day with 1 ppm = 0.05 mg/kg bw/day for older rats)<br>Exposure: 4 weeks (daily)<br>Animals underwent a 2-week pre-tre-treatment period and a 4-week treatment period. | 16 g/kg, low-iodine:<br>Clinical signs (hypoactivity, ruffled fur and emaciation), reduced bodyweight, reduced food consumption, iodine-deficiency, decreased free T4 and total T4 levels, increased thyroid weight, decreased T4, T3 and reverse T3 in thyroid gland, all male animals found dead or had to be killed (mostly Day 37, all by Day 42), four females sacrificed moribund or found dead.<br>8 g/kg, low-iodine:<br>Clinical signs (hypoactivity), reduced bodyweight, reduced food consumption, iodine-deficiency, decreased free T4 and total T4 levels, increased thyroid weight, decreased T4, T3 and reverse T3 in thyroid gland, one male and one female found dead.<br>4 g/kg, low-iodine:<br>Iodine deficiency, decreased free T4 and total T4 levels, increased thyroid weight, decreased T4, T3 and reverse T3 in thyroid gland.<br>Low-iodine:<br>Iodine-deficiency, decreased free T4 and total T4 levels, increased thyroid weight, decreased T4, T3 and reverse T3 in thyroid gland. | A6.10/14; Doc. No. 592-041<br><br>Buchberger W., Holler W. and Winsauer K., 1990<br><br>Reliability: 2 |

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| Endocrinology<br>Mechanism of action<br><br>Rat, Wistar<br>male<br>8/group | Sodium bromide | Oral (feed)<br>19 g NaBr/kg (nominal in diet)<br>(corresponding ot 950 mg sodium bromide/kg bw/day) or 11 g NaCl/kg diet as a control for two weeks).<br>Exposure: 14 days (daily) | 19 g sodium bromide/kg diet:<br>↓ bodyweight,<br>↑ thyroid weight<br>↓ uptake of 125-I by the thyroid gland,<br>↓ T4 level,<br>↑ TSH level<br>↓ I-TPO activity<br>↓ guaicol-TPO activity,<br>↑ NADH cytochrome c reductase activity. | A6.10/15; Doc 592-042<br><br>Van Leeuwen F.X.R., Hanemaaijer R. and Loeber J.G., 1988<br><br>Reliability: 2 |
| Neurotoxicity<br>Behaviour effects were investigated (no guideline study)<br><br>Mouse, NMRI<br>male | Sodium bromide | Oral: dietary<br>0, 400, 1200, 3600 and 10800 ppm (corresponding to 0, 80, 240, 720 and 2160 mg/kg bw/day)<br><br>36 day(s) (Daily)<br>(postexposure period: 50 days) | 400 ppm:<br>No treatment related effects<br><br>1200 ppm:<br>-behavioural effects (↓ evasion time)<br><br>3600 ppm:<br>-behavioural effects (disturbance of the normal nocturnal rhytm of motility, ↓ evation time)<br><br>10800 ppm:<br>-behavioural effects (disturbance of the normal nocturnal rhytm of motility, disturbance of the normal behaviour on the treadmill, ↓ evation time)<br>↓ bodyweight (>10%) | A6.9.2/01, Doc. 592-016<br><br>Hansen K. and Hübner H., 1983<br><br>Reliability 2 |

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| Neurotoxicity<br>Prenatal exposure (in utero)<br><br>Rat, Wistar<br>male/female<br>16-20 offspring males/group;<br>12-18 offspring females/group | Sodium bromide | Oral: gavage<br>0, 40, 80, 120 mg/kg bw/day<br>Offspring to dams exposed day 3 to day 20 of gestation<br>(postexposure period: 85 days after pups were born) | 40 mg/kg bw/day:<br>↑ pup mortality (pups died before 20 days of age: 27%, compared to 2.3% in controls)<br><br>80 mg/kg bw/day:<br>↑ pup mortality (pups died before 20 days of age: 42%, compared to 2.3% in controls)<br>↓ learning ability<br><br>120 mg/kg bw/day:<br>↑ pup mortality (pups died before 20 days of age: 58% compared to 2.3% in controls)<br>↓ learning ability | A6.9.2/02, Doc. No. 592-017<br><br>Harned et al., 1944 |
| Neurotoxicity | Sodium bromide | Rat, Sprague-Dawley<br>male/female<br>Long-lasting microinfusion into the superior cervical ganglion of adult rats.<br>10 µL of 500 mM solution<br>Exposure period: 30 minute(s) (Single exposure) | 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. | Joo, F., Dames W., Wolff J.R., 1979<br><br>Reported as supporting study in REACH Registration dossier |

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| Neurotoxicity<br><br>Mouse (murine C1300 neuroblastoma cells)<br><br>in vitro study | Sodium bromide | 0, 10-4, 10-5 or 10-6 M sodium bromide<br><br>Sodium bromide was applied in vitro to mouse neuroblastoma cells of different ages for short and long periods (2 h to 10 days). | Morphological changes induced were observed in murine neuroblastoma cells after sodium bromide treatment. Coated vesicles proliferated and originated by pinching off from the Golgi complex and from 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 were formed. There was a distinct increase in the number and area of specialized contacts 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, mitochondria appeared within the varicosities and primitive contacts were formed between the terminal swellings and potential postsynaptic elements, which were absent in controls. | Spoerri P.E. and Wolff J.R., 1982<br><br>Reported as supporting study in REACH Registration dossier |

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| Neurotoxicity<br><br>in vitro study | Sodium bromide | Mouse (C1300 mouse neuroblastoma cells, clone Neuro-2a)<br>0, 10-4, 10-5 and 10-6 M<br>Exposure period: 2 day(s) (Continuous exposure) | 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. | Eins S., Spoerri P.E. and Heyder E., 1983<br><br>Reported as supporting study in REACH Registration dossier |
| Neurotoxicity<br><br>The effects of sodium bromide on the bullfrog sympathetic ganglion were studied by extracellular and intracellular recording techniques.<br>in vitro study | Sodium bromide | Bullfrog, Rana catesbiana<br><br>112 mM sodium bromide | Equimolar replacement of sodium chloride by sodium bromide in Ringer's solution caused hyperpolarization of ganglion cells and antidromically evoked spikes showed increased rates of rise as well as prolonged post spike positivity. | Montoya G.A. and Riker W.K., 1982<br><br>Reported as supporting study in REACH Registration dossier |
| *Potassium bromide* | | | | |

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| Endocrinology: The aim of the study was to provide information on the possible goitrogenic effect of bromide on the functional morphology of the rat thyroid at concentrations resembling an actual possible increase in the environmental Br- level.<br><br>16 or 66 day administration of potassium bromide in drinking water<br><br>Rat, Wistar<br>Male<br>6 animals/group | Potassium bromide | Oral (drinking water)<br>The following dose levels were used in the study:<br>0, 10, 50 and 100 mg Br-/L (corresponding to 0, 0.5, 2.5 and 5 mg Br- /kg bw/day and 0, 0.745, 3.73, 7.45 mg KBr/kg bw/day 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) | 10, 50, 100 mg Br-/L in drinking water:<br><br>Histopathological changes in thyroidea at all dose levels, with a dose-related increase in extent.<br><br>Tissue of the thyroid gland of animals displayed a marked growth activation of the follicular epithelial component, mitoses in the follicular cells were more frequent and microfollicular tissue rearrangement was observed.<br><br>Additional changes: lowering in the portion of colloid in the thyroid tissue, slight to moderate thyroglobulin-positivity of collid tissue, decreased plasma T4 level, slightly decreased plasma T3 level. The plasma TSH level was statistically significantly increased compared to control at 66 days (but not after 16 days).<br><br>The concentration of bromide in the thyroid increased with the amount of bromide intake, while at the same time the molar ratio of iodine/bromide decreased. | A6.10/11; Doc. No. 592-037<br><br>Velický et al., 1997a<br><br>Reliability: 2 |

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| Endocrinology<br>16, 66 or 133 days of administration, effects on the thyroid were investigated<br><br>Rat, Wistar<br>Male<br>10 animals/group | Potassium bromide | Oral (drinking water)<br>The following dose levels were used in the study: 0, 10, 50, 100, 200 and 400 mg Br-/L (corresponding to 0, 0.5, 2.5, 5, 10 and 20 mg Br- /kg bw/day and 0, 0.745, 3.73, 7.45, 14.90, 29.81 mg KBr/kg bw/day 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)<br>The experiment were carried out in three series:<br>(1) Four groups of ten animals each received 0, 10, 50 and 100 mg Br-/L drinking water. Exposure time 16 days.<br>(2) Four groups of ten animals each received 0, 10, 50 and 100 mg Br-/L drinking water. Exposure time 66 days.<br>(3) Four groups of ten animals each received 0, 100, 200 and 400 mg Br-/L drinking water. Exposure time 133 days. | 10, 50, 100, 200, 400 mg $Br^-$ /L in drinking water:<br>Increased mitotic activity of follicular cells (increased values of mitotic index with increasing bromide concentrations) in thyroids.<br>Increase in bromine content and a concomitant decrease of the I/Br molar ratio in the thyroid tissue .<br>Histopathological changes in thyroidea (microfollicular rearrangement of the follicular epithelium, reduction of the amount of colloid).<br>LOAEL: 0.5 mg bromide/kg bw/day | A6.10/10, Doc. No. 592-039<br>Velický J. et al., 1997b<br>Reliability: 2 |

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| Endocrinology<br><br>133 day administration of potassium bromide in drinking water, effects on the thyroid were investigated<br><br>Rat, Wistar<br><br>male<br><br>10 animals/group | Potassium bromide | Oral (drinking water)<br><br>0, 100, 200 or 400 mg/L (nominal in water) (corresponding to 0, 3.3-5, 6.7-10, 13-20 mg Br- /kg bw/day and 0, 4.92-7.45, 9.99-14.90, 19.37-29.81 mg KBr/kg bw/day based on an assumed average bodyweight of 300 g/rat over the study period and taking into account a water consumption of 10-15 mg/animals/day).<br><br>Exposure: 133 days (daily) | 100 mg Br-/L drinking water:<br><br>Follicular rearrangement, changes in rought endoplasmic reticulum, lysosomes and microvilli in thyrocytes, mitoses in thyrocytes more frequent, numerous and dilated capillaries, decreased thyroglobulin (Tg) immunoreactivity, decreased amount of intrafollicular colloid, slightly increased TSH level, decreased T4 level.<br><br>200 mg Br-/L drinking water:<br><br>Follicular rearrangement, changes in rought endoplasmic reticulum, lysosomes and microvilli in thyrocytes, mitoses in thyrocytes more frequent, numerous and dilated capillaries, decreased amount of intrafollicular colloid, slightly decreased TSH level, decreased T4 level.<br><br>400 mg Br-/L drinking water:<br><br>Follicular rearrangement, changes in rought endoplasmic reticulum, lysosomes and microvilli in thyrocytes, mitoses in thyrocytes more frequent, numerous and dilated capillaries, loss of Tg immunoreactivity, decreased amount of intrafollicular colloid, slightly decreased TSH level, decreased T4 level.<br><br>Increasing concentrations of Br- in the drinking water caused an increased bromine concentration in the thyroid, a decreased iodine content and a decreased I/Br molar ratio.<br><br>LOAEL: 100 mg Br-/L drinking water (3.3 mg bromide/kg bw/day) | A6.10/13, Doc. No. 592-034<br><br>Velický, J., 1998<br><br>Reliability: 2 |

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| Endocrinology<br><br>16, 66 or 133 day administration of potassium in drinking water, effects on the thyroid were investigated<br><br>Rat, Wistar<br><br>male<br><br>10 animals/group | Potassium bromide | Oral (drinking water)<br><br>0, 10, 100, 200 and 400 mg Br-/L (corresponding to 0, 0.5, 5, 10 and 20 mg Br-/kg bw/day and 0, 0.745, 7.45, 14.90, 29.81 mg KBr/kg bw/day based on an assumed average bodyweight of 300 g/rat over the study period and taking into account a water consumption of 15 ml/animals/day) via the drinking water.<br><br>Exposure: 16, 66 or 133 days (daily) | 10 mg Br-/L drinking water:<br><br>Many small follicles with very small lumina, hypertrophic granular ER and dilated cisterns with light material, changes in Golgi apparatus, irregular-shaped nuclei, higher density of nuclear chromatin, sporadic thyrocytes with signs of necrosis, colloid droplets were rarely formed.<br><br>50 mg Br-/L drinking water:<br><br>Numerous very small follicles with very small lumen, markedly increased ER with highly dilated cisterns, irregular-shaped nuclei, condensed nuclei, condensed nuclear chromatin, poor colloid droplets.<br><br>100 mg Br-/L drinking water:<br><br>Highest proliferation rate of thyrocytes, intracellular cavities, dilated ER cisterns, enlarged Golgi complex, poor colloid droplets, irregular-shaped nuclei (133 days treatment), increased density of nuclear chromatin, sporadic thyrocytes with signs of necrosis.<br><br>200 mg Br-/L drinking water:<br><br>Microfollicles predominated, markedly reduced colloid in the small lumina, increased number of granules, cisterns of ER and dense bodies (lysosomes), irregular-shaped nuclei, higher density chromatin, well developed golgi apparatus, desmosomes and junctional complexes between neighbouring cells.<br><br>400 mg Br-/L drinking water:<br><br>Small follicles with reduced lumina, dilated cisterns of ER, increased number of spherical structures and granules. | A6.10/12; Doc. No. 592-038)<br><br>Velický, J. et al 2004<br><br>Reliability: 2 |

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| Endocrinology<br>The purpose of the study was to evaluate the effects of potassium bromide on the canine thyroid gland<br><br>Dog, Laboratory Hound dog<br>2 males and 3 females per group (control and treatment) | Potassium bromide | Oral (drinking water)<br>Loading dose of 100 mg/kg bw/day for two days and a maintaining dose of 30 mg/kg bw/day for 180 days.<br>Dose adjustment was performed on Day 120. If serum concentration of bromide was lower than 250 mg/dL potassium bromide dose was increased by 5 mg/kg bw/day for the remaining study.<br>Basal total thyroxin (TT4), free thyroxine (fT4) and thyroidea stimulating hormone (TSH) concentration were evaluated over a 6 month period. Thyrotropin-releasing hormone (TRH) stimulation test was also performed in all dogs at Day 182. Thyroid tissue wet weights were taken. Thyroids were subjected to gross and histopathological examinations. Increased microfollicular development (MFD) and decreased intrafollicular colloid staining (IFS) and vascularity were scored and mitotic index was calculated. | Neither clinical signs of hypothyroidism nor evidence of bromism were identified in any of the dogs. No adverse side effects were noted in the study. | A6.10/18, Doc. No. 592-022<br><br>Paull et al., 2003<br><br>Reliability: 2 |

### 10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

No repeated dose toxicity studies performed with ammonium bromide in animals by the dermal and inhalation routes are available.

There is one 90-day oral repeated dose toxicity study in rat according to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) and one 4-week oral dose-range finding study in rat of ammonium bromide available.

In addition, there are a number of repeated dose toxicity studies on sodium bromide available, including one very recent 90-day oral repeated dose toxicity study in rat according to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) and several non-guideline studies focused on investigation of effects on nervous system or the endocrine system (thyroid). Studies of Potassium bromide with focus on the endocrine system are also included.

Moreover, information from reproductive toxicity studies of both ammonium bromide and sodium bromide are included here but are discussed in full in section 10.10; only effects that are considered relevant for STOT RE are discussed in this section.

**Ammonium bromide – test guideline repeated dose toxicity studies**

***90-day oral repeated dose toxicity study of ammonium bromide in rats (Study report, 2000a***)

A 90-day feeding study which included also a neurotoxicity screening battery was performed with ammonium bromide in rats following administration of 100 and 225 mg/kg bw/day in both sexes and 500 mg/kg bw/day (males) as well as 750 mg/kg bw/day (females), respectively. Main study and neuropathology animals were treated continuosly for at least 13 consecutive weeks. Recovery animals were fed treated diet for 13 weeks, and were then fed untreated diet for a period of at least 4 weeks.

Clinical observations

Clinical signs of subdued behaviour and neurotoxic effects (abnormalities of gait) 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. The signs generally became apparent after approximately 8 weeks of treatment, and persisted until necropsy (main study animals) or at least the third week of the recovery period. 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. In the absence of histopathological findings it was considered that all neurotoxicological effects were probably reversible.

Body weight, food and water consumption

Reduced bodyweight gain were recorded in males at ≥ 225 mg/kg bw/day and in females at 750 mg/kg bw/day. Food consumption was reduced in males at 500 mg/kg bw/day.

Hematology and clinical chemistry

Minor changes in haematology parameters in males at 500 mg/kg bw/day and females at 750 mg/kg bw/day, minor changes in biochemical parameters (males at ≥ 225 mg/kg bw/day; females at ≥ 100 mg/kg bw/day) and decreased urine pH (females at 750 mg/kg bw/day).

Organ weights and histopathology

At 500 mg/kg bw/day following the 4 week recovery period epididymides weights (absolute 15% and adjusted 14%) were significantly lower than control. Absolute prostate (-24% $p<0.05$) and testes (-9%, $p<0.01$) weights were significantly reduced, although the statistical significance had disappeared after covariance adjustment. No treatment related necropsy or histopathological findings were observed.

**Ammonium bromide – non-guideline repeated dose toxicity studies**

***Dose-range finding study for a 90-day oral repeated dose toxicity study of ammonium bromide in rats (Study report, 1999)***

A 4-week dose-range finding study was performed in rats with ammonium bromide concentrations of 100, 500 and 1000 mg/kg bw/day mixed with the diet.

Clinical observations

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 (mainly females at mid-and high dose), subdued behaviour (all males at mid- and high dose, 4/5 females at high dose), rolling gait (all males and females at both mid and high dose), hunched posture, piloerection and

eyes partially closed (all females at high dose). To a lesser extent unkempt coat and irregular breathing were noted.

Body weight, food and water consumption

Statistically significant reduced body weight gains (males: 49%; females: 31%), reduced food consumption (males: 29%) and increased relative liver weight (females) were noted in addition in animals of the high dose group (1000 mg/kg bw/day).

Organ weights and histopathology

High dose males showed marked decreased mean epididymides (-16%), heart, kidney, liver, lung and testes absolute weights compared to control group. Intermediate dose group males also showed decreases in mean epididymides, kidneys, and testes absolute weights when compared to the control group. However, due to the decreased mean body weight of the intermediate and high dose males (statistical significant different from control in high dose) these apparent effects disappeared after covariance analysis. No histophatological examination was done and therefore no association between changes in organ weight and histopathological effects can be done.

**Sodium bromide – test guideline repeated dose toxicity studies in rat**

***90-day oral repeated dose toxicity study of sodium bromide in rats, including recovery assessments (Study report, 2016b)***

In an oral repeat dose 90-day toxicity study in rats performed according to OECD TG 408 and with the relevant sections relating to oestrous cycles, sperm evaluation and histopathology according to OECD TG 416 sodium bromide was adminstered daily via oral gavage at doses of 0, 60, 175, 500 mg/kg bw/day. A Sodium Chloride comparator group (284 mg/kg/day) was also included in the assessment.

Administration of 285 mg/kg/day of sodium chloride at an equivalent osmolarity as 500 mg/kg/day of sodium bromide produced no significant toxicity (other than mean thyroid weights higher than any other group), indicating that any toxicity observed in the sodium bromide-treated groups was probably due to exposure to bromide.

Clinical observations

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 study days 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**, 4 males had severe clinical signs and required euthanasia on study days 52, 55, 86 or 107. All of these males had marked reductions in body weight, and 2/4 had decreased food intake. 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 study day 120.

Females at 500 mg/kg/day 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 study day 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.

Functional observations and motor activity

Functional observations and motor activity generally reflected the clinical observations. 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 for functional observations and motor activity postdose in week 9, ataxia and abnormal gait were observed in all (10/10, $p \leq 0.01$) males and in 6/10 ($p \leq 0.01$) females of the high dose groups, 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 the number of males showing ataxia was slightly lower (7, $p \leq 0.01$), but 5 ($p \leq 0.01$) animals showed unusual posture and 8 showed lack of air righting response (landing on their side). Also 3/10 males in the **175 mg/kg bw/day** dose group had difficulty in air righting, and although the increase was not statistically significantly different from control, this was considered as associated with treament beacause of the similar finding in high dose group. Moreover, males in high dose group had signs indicating lack of grooming (unkempt, urine/fecal staining) and 9/10 males urinated in the open field. In females, ataxia was observed in 8 ($p \leq 0.01$) females and abnormal gait and unusual posture was slightly increased. 8/10 ($p \leq 0.01$) females were sleeping in the home cage prior to examination both at 175 mg/kg bw/day and 500 mg/kg bw/day. Decreased fine movement was observed in high dose 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, food and water consumption

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 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 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 (study days 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 (study 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 (study days 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 (study days 43 to 50, $p \leq 0.01$) and in relative food consumption (study days 8 to 15, $p \leq 0.01$ and study days 43 to 50, $p \leq 0.05$) values for the entire period (study days 1-90) were comparable to controls. Over the entire recovery period (study days 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.

There was no adverse effect on water intake in any group during the first period measured (study days 1-7). In the second period (study days 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).

Hematology and clinical chemistry

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 and demonstrated 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. Mean TSH levels were 36% and 74% higher than controls in the 175 and 500 mg/kg/day male groups, respectively but not statistically significantly different. 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.

Organ weights and histopathology

Single animals in the intermediate and high dose group groups also showed depletion (mild/moderate) 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. 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. No other significant organ weight changes were observed that were considered of toxicological relevance. The absolute brain weight in males was statistically significantly decreased (5-9%, $p \leq 0.05$ and $p \leq 0.01$) at 60, 175 and 500 mg/kg bw/day. But the relative (to body weight) weights were not statistically significant different from control at 60 and 175 mg/kg bw/day, or increased (16%, $p \leq 0.01$) at 500 mg/kg bw/day.

In addition to the depletion of colloid in thyroid, other histopathological findings were found in the liver (minimal/mild periportal vacuolation and tension lipidosis) and lung (minimal/mild macrophage aggregation and minimal/mild/moderate mixed cell infiltration) in both males and females at all dose levels.

Effects on the reproductive organs are not reported here but discussed in 10.10.1 Adverse effects on sexual function and fertility.

**Sodium bromide – other test guideline studies of relevance for repeated dose toxicity in rat**

***Two-generation reproductive toxicity study of sodium bromide in rat (Study report, 2016a)***

In a two-generation reproduction study (performed according to a protocol similar to OECD TG 416) sodium bromide was administered via oral gavage to Crl:CD(SD) rats at dose levels of 0, 50, 150, 350/500 (male/female) mg/kg bw/day.

Clinical observations

In the high dose groups of males and females (350/500 mg/kg bw/day) severe toxicity was reported, characterized by increased mortality (4 males and 9 females died or were terminated early) and adverse clinical observations ($p \leq 0.01$), including 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 bw/day dose group, similar clinical signs occurred but they were less marked and at a lower incidence, especially in females.

There were no adverse clinical observations, reported for the F1 generation.

Body weight, food and water consumption

In the 350/500 mg/kg bw/day dose group reduced body weight gain was observed in males from week 6 onwards and body weight at the end of the dosing period was 74% ($p \leq 0.01$) of control values: food intake was also lower from week 4 onwards. In females, reduced body weight gain and food intake was observed only during late gestation and lactation.

In the 175 mg/kg bw/day dose group, effects on body weight were only observed in males and were more moderate, with a terminal mean body weight of 86.8% ($p \leq 0.01$) of the control value, and reduced food intake from week 6 onwards. Female food intake was reduced only in early lactation.

Administration of 50 mg/kg bw/day sodium bromide had no adverse effect on body weight gain or food intake in males or females of the P.

Organ weights and histopathology

In the P-generation, the absolute weight of the brain, liver, kidneys, adrenals, spleen, thymus and thyroid/parathyroid were all unaffected in males by the 50 mg/kg bw/day dose of sodium bromide. A reduction in the ratio of the kidney weight to the body and brain ($p \leq 0.01$) weights in the 50 mg/kg bw/day dose group was considered not adverse, as there was no effect on the absolute weight and no findings at histopathology. Absolute brain weight was decreased ($p \leq 0.01$) and the ratio to the terminal body weight was increased ($p \leq 0.01$) at 175 and 350 mg/kg/day. These changes were considered not adverse as the relative weight was increased and there was no effect on the histopathology of the brain.

There were dosage-related reductions ($p \leq 0.05$ to $p \leq 0.01$) in the absolute and relative (to brain and or body weight) weight of the liver, kidneys, adrenals, thymus and thyroid/parathyroid in the males of 175 and 350 mg/kg/day dose groups compared to the control group values, but they were considered not adverse as there was correlation with histopathological findings in these organs. In addition, the reduced ($p \leq 0.05$) absolute weight of the spleen in the 350 mg/kg/day dose group was not considered adverse as the relative weight was increased ($p \leq 0.01$) and there was no effect on histopathology of the spleen.

In females, there were reductions in absolute weight of the brain ($p \leq 0.01$) and thymus ($p \leq 0.05$) in the 500 mg/kg/day group without any concomitant histological changes in these organs. Any other organ weight differences observed in females treated with 500 mg/kg bw/day were considered incidental and there was no effect of treatment at 50 or 175 mg/kg bw/day.

In the F1-generation, 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. In females, a significant reduction ($p \leq 0.01$) in the brain weight at 175 mg/kg bw/day was not considered adverse as there was no effect on the histopathology of the brain. There was no adverse effect on organ weights at 50 mg/kg bw/day.

Effects on the reproductive organs are not reported here but discussed in 10.10.1 Adverse effects on sexual function and fertility.

**Sodium bromide – non-guideline repeated dose toxicity studies in rat**

***28-day oral repeated dose toxicity study of sodium bromide in rats (Van Logten et al., 1973)***

A 28-day feeding study was performed with sodium bromide in female rats only following administration of 300, 1200, 4800 and 19200 ppm (0, 36, 144, 576, 2304 mg/kg bw/day). Clinical signs, body weight, food intake and water consumption were recorded and halide content of blood and some organs (brain, liver, kidneys) determined. At termination, liver, kidneys and brain were weighed and studied histopathologically in the control and highest dosage group. During the first week of the experiment the plasma concentration of bromide increased rapidly (higher plasma concentrations with higher bromide-amount in food), thereafter a plateau was reached by the third week. Bromide concentration in the brain and liver was lower than in the kidneys and in plasma. In the highest dosage group (19200 ppm) about 50% of the chloride in plasma, brain, kidney and liver had been replaced by bromide. In the other treatment groups there was also a dose-related replacement of chloride by bromide. In addition, high dosed animals showed clinical signs (motor incoordination of their hind legs, insufficient grooming) and increased kidney weight.

***90-day oral repeated dose toxicity study of sodium bromide in rats (Van Logten et al., 1974)***

A 90-day feeding study was performed with sodium bromide in rats following administration of 75, 300, 1200, 4800, 19200 ppm (0, 6.75, 27, 108, 432, 1728 mg/kg bw/day. In this study the halid content of the blood was investigated and it was reported that the bromide concentration in the plasma rose to a plateau within 3 weeks. In all groups except in the highests dosage group, these plateaus were directly proportional to the bromide concentrations in the diets, as where the bromide concentrations in brain and kidneys after 13 weeks. Total molar halogen concentration in plasma, remained constant throughout the investigation. The average bromide concentration in the brain was lower than the kidneys. At the highest dose group about 25% of the chloride in the brain and 50% in the kidneys had been replaced by bromide.

Clinical signs of neurotoxicity (motor incoordination of the hind legs), depressed grooming, both sexes at 19200 ppm) and several effects on the endocrine system (increased admidopyrindemethylase activity; histopathological findings in thyroidea, gonads, adrenals and pituitary; increased activity of thyroids (females at ≥ 4800 ppm; males at 19200 ppm), increased thyroid weight, reduced secretory activity of prostate (at ≥ 4800 ppm), decreased spermatogenesis (at 19200 ppm)) were observed in this study.

Organ weight changes consisted of increased thyroid weight (females at ≥ 1200 ppm, males at 19200 ppm), reduced prostate weight (≥ 4800 ppm), increased spleen weight (males at 19200 ppm), increased adrenal weight (males at 19200 ppm) and reduced thymus weight (females at 19200 ppm). Histological findings consisted of findings made in the adrenals (decreased vacuolisation of zona fasciculate in both

sexes at ≥ 75 ppm), pituitary (cysts in males at 19200 ppm), ovaries (tendency to decreased number of corpora lutea in females at 19200 ppm), testis (tubuli reduced in size) and thyroidea (follicles reduced in size in females at ≥ 4800 ppm; in males at 19200 ppm). In the absence of other effects the histopathological findings in the adrenals noted in males and females of the 75 ppm dosage group were not considered to be adverse effects.

One female in the high dose group was euthanized after four weeks (tail eaten by cage mate), otherwise there was no mortality in any dose group. Male body weights were decreased compared to control during the entire study, and body weight gain was reduced to 23% ($p \leq 0.01$ compared to control). Food conversion was decreased in both sexes during the first few weeks.

***90-day oral repeated dose toxicity study of sodium bromide in rats (Van Logten et al., 1976)***

A 90-day feeding study (low chloride diet) was performed with sodium bromide in rats following administration of 8, 31, 125, 500 and 2000 ppm (0.72, 2.8, 11, 45, 180 mg/kg bw/day). Animals were observed for clinical signs, bodyweight gain, food intake, and halide content of blood and some organs (brain, kidneys) were determined. Haematological examinations (total leucocytes and differential count) and clinical chemistry (bromide level and total halogenide content) were performed. After sacrifice rats were subject to gross and histopathology and weights of several organs were taken. The bromide concentration in the plasma rose to a plateau within 8 weeks. In all groups except in the highests dosage group, these plateaus were directly proportional to the bromide concentrations in the diets, as where the bromide concentrations in brain and kidneys after 13 weeks. The average bromide concentration in the brain was lower than the kidneys. At the highest dose group about 75% of the chloride in the brain and 80% in the kidneys had been replaced by bromide. Clinical signs of neurotoxicity (motor incoordination of the hind legs, depressed grooming) and several effects on the endocrine system (increased thyroid activity, decreased spermatogenesis, histopathological findings in gonads, adrenals; reduced pituitary weight; increased adrenal weight) were observed in this study.

Mortalities (both sexes) were reported at 2000 ppm, clinical signs of neurotoxicity (both sexes) at 2000 ppm, reduced bodyweight gain (both sexes at 2000 ppm), increased thyroid activity (both sexes) at ≥ 500 ppm, reduced spermatogenesis at 2000 ppm, changes in haematological parameters (both sexes) at 2000 ppm, changes in organ weights (both sexes) at 2000 ppm and histopathological changes at ≥ 500 ppm. Organ weight changes consisted of reduced pituitary weight (females at 2000 ppm) and increased adrenal weight (males at 2000 ppm). Histopathological findings consisted of findings made in the adrenals (decreased vacuolisation of the zona fasciculate, both sexes at 500 ppm), ovaries (decreased number of corpora lutea at 2000 ppm), uterus (retardation in maturation at 2000 ppm), brain (hyperaemic at 2000 ppm), heart (degeneration in myocard at 2000 ppm), lungs (granulocytes along blood vessels at 2000 ppm), pancreas (decreased zymogen granulae at 2000 ppm) and salivary gland (reduced secretory activity at 2000 ppm). A comparison of the results of this study with the results of the 90-day study with sodium bromide in rats with normal diet (Van Logten *et al*., 1974) shows that low level of chloride in the diet enhances the toxicity of sodium bromide, while the target organs are still the same.

***Three-generation reproductive toxicity study of sodium bromide in rats (Van Leeuwen, et al., 1983)***

In a three-generation reproduction study (publication) dose levels of 75, 300, 1200, 4800 and 19200 ppm sodium bromide (corresponding to 6.75, 27, 108, 432 and 1728 mg/kg bw/day using a conversion of 1 ppm=0.09 mg/kg bw/day) were administered to rats via the diet. In three successive generations, at least two litters per female rat were raised. In addition to the investigation of the reproductive performance of rats, additional emphases were placed on the thyroid gland.

Females of the F0 generation showed a dose-dependent decrease in relative weight of adrenals (statistically significant at 1200 and 4800 ppm). Investigation of thyroid hormone levels revealed a dose-dependent statistically significant decrease in T4 levels in serum of parental male animals of the F0 generation after 6 weeks. In parental female animals of the F0 generation T4 levels in serum was statistically significant decreased at 4800 and 19200 ppm only. LOAEL was set based on reduced

relative adrenal weight noted in females at ≥ 1200 ppm (108 mg/kg bw/day) and reduced thyroid hormone (T4) noted in males at ≥1200 ppm and in females at ≥ 4800 ppm (432 mg/kg bw/day). During Technical Meeting III 2011 it was decided that effects on the endocrine system noted at the dose level of 1200 ppm (108 mg/kg bw/day) should be considered adverse.

***4 and 12 weeks oral repeated dose toxicity of sodium bromide in rats (Loeber et al., 1983)***

The study was initiated to ascertain whether alterations in the endocrine system in the rat detected during a semichronic feeding study (Doc. IIIA 6.4.1/04, Doc. IIIA 6.4.1/05) could be detected in male rats after exposure to high dietary concentrations of sodium bromide and moreover whether histopathological and immunocytochemical findings could be correlated with serum-hormone levels. Furthermore a range of lower dietary concentration of sodium bromide was studied to investigate whether a previously observed decrease in the serum thyroxine level (Doc. IIIA 6.8.2/02) could be confirmed. Male Wistar rats were fed a normal or sodium bromide-enriched diet for 4 or 12 weeks. Sodium bromide concentrations were 0, 20, 75, 300, 1200 and 19200 mg/kg diet (corresponding to 0, 0.18, 6.75, 27, 108 and 1728 mg/kg bw/day based on a default conversion of 1 ppm=0.09 mg/kg bw/day. At the end of the experiments the pituitary gland, thyroid and testes were examined by histopathological and immunocytochemical techniques, while serum hormone levels were established by radioimmunoassay. Thyroid-stimulating hormone (TSH), growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroxin (T4) and testosterone were determined in the tissues. TSH, GH, luteinising hormone (LH), insulin and follicle-stimulating hormone (FSH) were measured in sera of the treated animals. In a separate 12-week experiment five animals receiving 19200 mg NaBr/kg diet and control animals were submitted to a release test using thyrotropin-releasing hormone (TRH) in a dose of 1 µg/kg bodyweight.

Reduced bodyweight was noted at 19200 ppm. Animals of this high dose group also showed a statistical significant increase in thyroid weight. Increased thyroid weight was also noted in animals of the 1200 ppm group after 4 weeks but not after 12 weeks. In the pituitary gland of the 19200 ppm group rats 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. Histopathological changes were noted in thyroidea (increase of follicles and a decrease in their size) and in testes (reduction of tubule diameter) in animals of the 19200 ppm group, and decreased spermatogenesis were noted in this dose group animals after 12 weeks. The concentration of thyroxin, testosterone and corticosteron in the serum appeared to be decreased at the highest dose level. Reduced T4 level was also noted in animals of the 1200 ppm group (at 4 weeks only). FSH level was increased at 19200 ppm (at 4 and 12 weeks) and at 1200 ppm (at 12 weeks only). In addition TSH levels and increased insulin levels were noted at 19200 ppm. No histopathological changes could be detected in the haematoxylin/eosin stained sections of the pituitary glands in neither dosage group.

***Four weeks oral repeated dose toxicity of sodium bromide in rats (Buchberger, 1990)***

To investigate the toxicological potential of sodium bromide in dependency of iodide intake, a 4-week study in rats with 4, 8 and 16 g sodium bromide/kg diet was performed (corresponding to 480, 960 and 1920 mg sodium bromide/kg bw/day). 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. Body weight and food consumption were recorded weekly. Urine samples were collected and analyzed for iodide content. Blood samples were 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. Iodine intake was checked by determining the urinary iodide secretion. Iodine deficiency was observed for all animals receiving the low-iodine diet and there were no differences between the 4, 8 and 16 mg bromide-groups. 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 with exception of the highest dosage group showing lower thyroid weights than the lowest dosage group. 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. In addition animals treated with 16 g/kg showed hypoactivity, ruffled fur and emaciation, reduced bodyweight, reduced food consumption and decreased free T4 and total T4 levels in serum. All animals of this dosage group were found dead or had to be killed. For rats of the 8 g/kg group hypoactivity, reduced bodyweight, reduced food consumption and decreased free T4 and total T4 levels were observed. At the lowest dosage group (4 g/kg diet) animals showed decreased free T4 and total T4 levels in serum. As a 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.

***Two weeks oral repeated dose toxicity of sodium bromide in rats (Van Leeuwen, 1988)***

An oral toxicity investigation was performed to study the mechanism of action of bromide on the thyroid gland in rats. Male rats were fed a diet containing 19 g NaBr/kg diet or 11 g NaCl/kg diet as a control for two weeks. Animals were sacrificed and blood was collected for determination of T4 and TSH levels. Thyroid glands were weighed and homogenized and thyroid peroxidase (TPO) activities, peroxidase 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 NaI and 125-I for the determination of 125-I uptake 24 hours after intubation. Animals treated saline containing NaI and 125-I for the determination of 125-I uptake 24 hours after intubation. Animals treated with 19 g/kg of sodium bromide showed decreased bodyweight, increased thyroid weight, decreased T4 level, increased TSH level, decrewased I-TPO activity, decreased guaiacol-TPO activity as well as increased NADH cytochrome c reductase activity. The uptake of 125-I by the thyroid gland was significantly lower compared to control animals. As a conclusion it was shown in the study that bromide inhibits the uptake of iodide in the thyroid gland, the oxidation of iodide to iodine and thus incorporation of iodine in thyrosine residues and the coupling of thyrosine residues to thyronine. Bromide causes an increase in NADH cytochrome c reductase activity, probably as the result of an increased TSH stimulus.

**Sodium bromide – non-guideline repeated dose toxicity studies in mouse**

***36 days oral repeated dose toxicity study of sodium bromide in mice (Hansen and Hübner, 1983)***

In order to investigate the effects of bromide on the behaviour of mice sodium bromide was administered with the diet at doses of 400, 1200, 3600 and 10800 ppm (corresponding to 80, 240, 720 and 2160 mg sodium bromide/kg bw/day) on Days 43-78 (36 days in total) of the experiment which lasted for 128 days (postexposure period was 50 days). In this published investigation (no guideline study) a fully automated system was used to record and process measurements of the nocturnal motility of the mice, together with three other variables (evasion time, spontaneous treadmill performance and body weight). The equipment used to measure spontaneous motility recorded movements simultaneously for a maximum of 120 individual caged mice. Evation 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. Behavioural effects were noted in mice treated with sodium bromide at ≥ 1200 ppm, and reduced bodyweight was noted in mice at 10800 ppm. Behavioural effects consisted of disturbance of the normal nocturnal rhythm of motility (≥ 3600 ppm or 720 mg/kg bw/day), decrease in evasion time (≥ 1200 ppm or 240 mg/kg bw/day) and disturbance of the normal behaviour on the treadmill (10800 ppm). The reduction in evasion time and the disturbance of the normal nocturnal rhythm of motility indicate a disinhibition. Treatment with the

highest dosage used (10800 ppm) showed alterations of behaviour and marked effects on bodyweight (>10%) which were not completely reversible after sodium bromide was withdrawn from the diet.

**Sodium bromide – non-guideline repeated dose toxicity studies in dog**

***Six weeks oral repeated dose toxicity study of sodium bromide in dogs (Rosenblum, 1958).***

An oral toxicity study was performed with the objective to receive information on the course of sodium bromide intoxication in dogs. Mongrel dogs were treated with either constant or increasing doses of sodium bromide mixed with the diet (Group 1: 100 mg/kg bw/day; Group 2 and 3: initial doses of 100 (group 2) and 200 mg/kg bw/day (group 3) increments of 100 or 200 mg/kg bw/day at intervals of 6 weeks until death resulted (between 44 and 185 days); Group 4: initial dose of 400 mg/kg bw/day, when necessary this dose was increased but always before the 6th week of administration of the previous dose). Animals were observed for bodyweight gain, signs of toxicity and mortality. Furthermore, blood bromide concentrations were measured. Treatment with 100, 200, 300, 400 and 500 mg NaBr/kg bw/day (corresponding to 78, 156, 234, 312 and 390 mg bromide/kg bw/day) resulted in a mean blood bromide concentration of 32.2, 36.3, 45.9, 51 and 49.6 mEq/L, respectively. Rapid elevation of the blood bromide level increased the lethality of bromide. Clinical signs of neurotoxicity were noted in dogs at the dose level of 200 mg/kg bw/day and above. The usual progression as the blood bromide level rose was as follows: slight ataxia, stupor, severe ataxia and coma. In addition shivering and salivation was noted in some dogs. The shivering which was seen in some dogs may also have been related to central nervous system toxicity while the salivation may have been a result of the irritant effect of bromide. Reduced bodyweight was noted at > 100 mg/kg bw/day. Signs of gastrointestinal toxicity were noted in dogs at all dose levels (bloody stool at ≥ 100 mg/kg bw/day, vomiting at ≥ 100 mg/kg bw/day; diarrhea at ≥ 400 mg/kg bw/day). Skin lesions were observed on the hind limbs, head and sternum in some of the animal receiving 200 mg/kg bw/day and above, and were characterised mainly by nonsuppurative white macules overlaid by scales and varying in size. Emaciation and body weight loss was noted in dogs at all dose levels.

**Potassium bromide – non-guideline repeated dose toxicity studies in rat**

***16 and 66 days oral repeated dose toxicity study of potasssium bromide in rats (Velický et al., 1997a)***

An investigation with potassium bromide in rats was performed to provide information on the possible goitrogenic effect of bromide on the functional morphology of the rat thyroid at concentrations resembling an actual possible increase in the environmental $Br^-$ level. Following dose levels were used in the study: 0, 10, 50, 100 mg $Br^-$/L corresponding to 0, 0.5, 2.5, 5 mg $Br^-$/kg bw/day 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 as applied in the previous investigation (above). After 16 or 66 days of treatment, rats were sacrificed and thyroid lobes excised. Tissue of one part of the lobe was prepared for serial sections which were stained with haematoxylin-eosin. Tissue of the other part of the lobe was fixed and further processed for electron microscopy. Radioimmunassay and instrumental neutron activation analysis were performed. Administration of bromide resulted in morphological changes in the thyroid gland. The extent of the changes 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. Tissue of the thyroid gland of animals exposed to bromide displayed a marked growth activation of the follicular epithelial component, mitoses in the follicular cells were more frequent and microfollicular reorganization was observed. Additional changes in bromide-treated animals compared to controls were lowering in the proportion of colloid in the thyroid tissue, slight to moderate thyoglobulin-positivity of colloid tissue, decreased plasma T4 level, slightly decreased plasma T3 level, concentration and/or duration of treatment dependent rise in bromine level in thyroid tissue

and decreased I/Br molar concentration ration in thyroid tissue. The results of this investigation indicate that even the lowest amount of bromide administered (10 mg/L) can induce changes comparable with parenchymatous goiter 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.

***16, 66 and 133 days oral repeated dose toxicity study of potasssium bromide in rats (Velický et al., 1997b)***

The toxicological potential of potassium bromide was assessed in rats. The aim of the study was to find out to which extent the hyperplasia resulting from increased mitotic activity of thyroid follicular cells participates in the changes observed in a previous study. Animals were treated with 0, 10, 50, 100, 200 and 400 mg $Br^-$/L (corresponding to 0, 0.5, 2.5, 5, 10 and 20 mg $Br^-$/kg bw/day 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) via the drinking water. The experiment were carried out in three series: (1) Four groups of ten animals each received 0, 10, 50 and 100 mg $Br^-$/L drinking water. 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. Proliferation activity was evaluated. All animals treated with potassium bromide showed microfollicular rearrangement of the follicular epithelium, reduction of the amount of colloid and increased values of mitotic index with increasing bromide concentrations. Based on the findings made in this investigation 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 increase of the I/Br molar ratio in the thyroid tissue after administration of $Br^-$ in drinking water was demonstrated.

***133 days oral repeated dose toxicity study of potasssium bromide in rats (Velický et al., 1998)***

Subsequent to the previous study, the investigations were continued and extended to 133 days using potassium bromide concentrations of 0, 100, 200 and 400 mg/L drinking water (corresponding to 0, 3.3-5, 6.7-10, 13-20 mg/kg bw/day based on an assumed average bodyweight of 300 g/rat over the study period and taking into account a water consumption of 10-15 mg/animals/day). The animals were divided into 3 experimental and 1 control group, each consisting of 10 animals. After 133 days of treatment rats were sacrificed and thyroid lobes excised. A portion of one lobe was used for preparation of serial sections, the other lobe was processed for electron microscopy. Thyroglobulinassay was performed, concentration of thyroid hormones and TSH in plasma, and bromine and iodine levels in the thyroid gland dry weight determined. With an increasing concentration of bromide, the amount of bromide in the thyroid dry weight showed a concomitant decrease in the molar I/Br ratio. Animals treated with 100 mg/L showed follicular rearrangement, decreased thyroglobulin (Tg) immunoreactivity, decreased amount of intrafollicular colloid, slightly increased TSH levels and decreased T4 levels. Mitoses in thyrocytes were more frequent than in the controls. The capillaries were numerous and dilated. Rats of the 200 and 400 mg/L groups showed a similar histopathological picture. The electron microsopy examination showed changes in the localization and extent of the Golgi apparatus, rough endoplasmic reticulum, lysosomes, and microvilli in thyrocytes. The results demonstrated that 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.

***16, 66 and 133 days oral repeated dose toxicity study of potasssium bromide in rats (Velický et al., 2004)***

In a further study with potassium bromide, the investigations were continued and extended to the electron microscopic level using the same experimental design as mentioned above. The following dose levels were used in the study: 0, 10, 100, 200 and 400 mg $Br^-$/L (corresponding to 0, 0.5, 5, 10 and 20 mg $Br^-$/kg bw/day based on an assumed average bodyweight of 300 g/rat over the study period and taking into account a water consumption of 15 ml/animals/day) via the drinking water. The animals were divided into 9 experimental and 3 control groups, consisting of 10 animals each. After 16, 66 or 133 days of treatment rats were sacrificed and thyroid lobes excised and process for electron microscopy. Histopathological findings in the thyroid were noted at all dose levels. The most important finding in the cytoplasm of thyrocytes was the hypertrophy and hyperplasia of the endoplasmatic reticulum, 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. Colloid droplets were only rarely found. It was 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.

**Potassium bromide – non-guideline repeated dose toxicity studies in dog**

***115 days feeding study of potassium bromide in dogs (Paull et al., 2003)***

In a placebo-controlled experiment the effect of potassium bromide on the canine thyroid gland was investigated. Laboratory Hound dogs were treated with a loading dose of 100 mg/kg bw/day for two days and a maintaining dose of 30 mg/kg bw/day for 180 days (2 males and 3 females for treatment and control group each). Dose adjustment was performed on Day 120. If serum concentration of bromide was lower than 250 mg/dL, potassium bromide dose was increased by 5 mg/kg bw/day for the remaining study. Basal total thyroxine (TT4), free thyroxine (fT4), and thyroid stimulating hormone (TSH) serum concentrations were evaluated over a 6-month period. Thyrotropin-releasing hormone (TRH) stimulation test was also performed in all dogs at beginning and conclusion of the study. Unilateral thyroidectomy was performed in all dogs on Day 182. Thyroid tissue wet weights were taken and stained sections thereof prepared. Increased microfollicular development (MFD) and decreased intrafollicular colloid staining (IFC) and vascularity were scored and mitotic index was calculated. 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. Both control and experimental groups 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 was found between experimental and control dogs. In the scored categories of microfollicular development and intrafollicular colloid staining, no significant difference was found between the KBr-treated and control dogs. The degree of vascularity identified in thyroid sections was not different between the treatment and control groups. No differences in normal follicular nor microfollicular regions of thyroid sections were observed. 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. Based on the results of this study it is concluded that potassium bromide 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.

***Subchronic toxicity feeding study of potassium bromide in dogs (March, Podell and Sams, 2002)***

Administration of potassium bromide to Beagle dogs was performed in order to develop a multidose method of bromide administration that targets serum bromide concentrations in the range of 200-300 mg/dL. Recommended therapeutic doses of 30-40 mg potassium bromide/kg bw/day (20-27 mg Br/kg

bw/day) in dogs will hypothetically produce a steady-state bromide concentration of 100-200 mg/dL. Dogs were administered 30 mg/kg of potassium bromide mixed with canned food over 12 hours and for a period of 115 days. A dose adjustment was made on Day 115 to rapidly increase and maintain bromide serum concentrations at 400 mg/dL. There were no adverse neurological side-effects from Day 0-115 in any of the dogs treated with 30 mg/kg/day of sodium bromide. Following dose adjustment, two of the dogs showed caudal paresis and ataxia and two of the dogs were agitated to hyperexcitable without signs of weakness or ataxia. The steady-state serum bromide concentration was variable (range: 178-269 mg/dL) with a mean of 245 mg/dL. The mean median t ½ was 15.2 days. Median apparent total body clearance was 16.4 mL/kg/day, median apparent volume of distribution was 0.4 L/kg, median renal clearance was 8.2 mL/kg/day and median serum bromide concentration post dose adjustment was 397 mg/dL. The cerebrospinal fluid to serum bromide concentration ratio was 0.63 at Day 9, 0.77 at Days 45 and 115 and 0.86 at Day 121. Electrodiagnostic test revealed only subtle reductions in power with increasing bromide concentrations. These changes were not considered to be adverse effects. Mean brainstem auditory evoked response latencies of waves I and V increased significantly ($p<0.05$) from day 0 to 9, but not after this time point (up to 121 days). I-V interpeak latencies were statistically significantly increased between days 0 and 121. According to the study author, the increases in latencies concided with appearance of clinical neurological deficits. Muscle and nerve biopsies from all dogs were normal.

**Human studies relevant for repeated dose toxicity**

***Study of sodium bromide in human volunteers (Sangster et al., 1982)***

A study was performed to determine whether ingestion of a dose of bromide equal to the acceptable daily intake (1 mg bromide/kg bw/day as determined by the WHO) might induce effects in humans of both sexes. Bromide was orally administered (capsules) at concentrations of 1 mg bromide/kg bw/day as sodium bromide to 21 healthy volunteers (10 males and 11 females not using oral contraceptives and not being pregnant) during 8 weeks or 2 full cycles. Special attention was paid to the endocrine system. At the start and end of the study a full medical history, the results of a physical examination, haematological studies and standard clinical chemistry and urine analyses were recorded for each subject. In the study concentrations of thyroxin (T4), free thyroxin (fT4), thyroxin binding globulin (TBG), triiodothyronine (T3), cortisol, testosterone, estradiol, progesterone, thyroid stimulating hormone (TSH), prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone releasing hormone (TRH) and luteinizing hormone releasing hormone (LHRH) were determined in serum before and after the daily ingestion of $Br^-$ during 8 weeks. At the end of the investigation, plasma bromide levels in the range of about 10% of the therapeutic plasma concentration of 6-12 mmol/L and also about 10% of the mean plasma concentration in rats of 7.7±1.1 mmol/L in which effects were observed were determined. Plasma half-life of bromide in man is about 12 days. Thus, it was expected that at the end of the experiment the plasma bromide concentration would have reached a steady state during only the last week. The results of the medical histories, the physical examinations and the haematological, biochemical and urine analyses after completion of the experiment showed no abnormalities when compared to pre-experimental values. In two females a short lasting itching dermatosis with small vesicles was observed at the beginning of the experiment (reversible effect). Daily ingestion of 1 mg bromide/kg bw did not change the serum concentration of any of the hormones measured, produced by thyroid, adrenals, gonads and pituitary gland.

***Study of sodium bromide in human volunteers (Sangster et al., 1983)***

In another study bromide was orally administered (capsules) at concentrations of 0, 4 or 9 mg bromide/kg bw/day as sodium bromide to 14 healthy volunteers (7 males and 7 females not using oral contraceptives and not being pregnant) during three months or three full cycles. Special attention was paid to the endocrine system and the central nervous system. At the start and end of the study a full medical history, the results of a physical examination, haematological studies and standard clinical

chemistry and urine analyses were recorded for each subject. In the study concentrations of thyroxin (T4), free thyroxin (fT4), thyroxin binding globulin (TBG), triiodothyronine (T3), cortisol, testosterone, oestradiol, progesterone, thyrotropin, TSH, prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH) were determined in serum before and after the daily ingestion of bromide during 12 weeks or 3 full cycles. A quantitative analysis of the EEG and the evoked response at the start and end of the investigation was performed to obtain information on the functioning of the CNS. Mean plasma bromide concentrations at the end of treatment were 0.08, 2.14 and 4.30 mmol/l for males and 0.07, 3.05 and 4.93 mmol/l for females of the 0-, 4- and 9-mg bromide/kg bw/day groups, respectively. The two groups receiving sodium bromide showed bromide concentrations that gradually increased during the first six weeks of the experiment and then remained stable except in the males receving 9 mg bromide/kg bw/day. In the male volunteers taking 9 mg bromide/kg bw/day the plasma bromide concentration decreased slightly from week 8 to week 12. Whether this decrease was induced by changes in chloride intake, by physiological variation or by the inaccurate taking of the capsules could not be established. Plasma concentrations were shown to have increased until week 8 after which a non-significant decrease was observed. No significant differences were found between females and males at either dose level. The mean plasma bromide concentrations for the two dose levels of bromide differed significantly from each other both for the females and for the males. Considering the fact that a substance, when regularly administered, usually reaches a steady state in plasma after four times the plasma half-life and that in this study a steady state for bromide was reached after 6 weeks, it may be concluded that in these volunteers the plasma half-life of bromide was about 10 days. Medical histories, physical examinations and haematological, biochemical and urine analyses after completion of the experiment showed no treatment-related abnormalities except for some incidence of nausea associated with bromide-capsule ingestion (nausea noted in 2 persons at 4 mg/kg bw/day and in 5 persons at 9 mg/kg bw/day). The nausea was considered to be an effect of the relatively large amount of bromide administered in one capsule to the stomach and not a systemic effect. A decrease in mental concentration and an increased need of sleep were reported in 5 males at 4 mg/kg bw/day and by one female and one male at 9 mg/kg bw/day. However, on consideration of the relation between the mentioning of this symptom and the dose administered, association with the bromide ingested seemed unlikely. Further on, these symptoms could not be replicated in another study using a larger amount of subjects (Van Gelderen *et al*., 1993). The results of the serum biochemical and urine analyses at the start and end of the study were within normal limits and no significant changes were observed except that three subjects showed an increase in the concentration of γ-glutamyl transpeptidase. In two this appeared to be related to the consumption of ethanol-containing beverages. Visual inspection of the EEG records did not reveal overt differences caused by bromide. An alteration in neurophysiological parameters was evident at 9 mg bromide/kg bw/day and was characterised by a decrease in $\delta_1$ and $\delta_2$-activities, an increase in β-activities and an increase in mean frequency, expressed in the mobility. Increased $\alpha_1$-activity was noted at 4 mg/kg bw/day.The changes never exceeded normal limits according to the study author but reflected a shift in background EEG activity. The changes were most pronounced in the fronto-temporal and central areas. As concluded by The European Agency for the Evaluation of Medicinal Products EMA; Committee for Veterinary Medicinal Products, Bromide, sodium salt, summary report EMEA/MRL/182/97-FINAL, March 1997) the effect on EEG measurements noted at 4 mg/kg bw/day was not considered adverse. In the female subjects taking 9 mg bromide/kg bw/day, a significant increase in T4 and T3 was observed, although the individual concentrations of T4 and T3 in this group were within normal limits at the start and the end of the investigation. No changes on all other parameters (TBG, fT4, cortisol, oestradiol, progesterone or testosterone, thyrotropin, prolactin, luteinizing hormone and follicle-stimulation hormone) were noted at 4 and 9 mg/kg bw/day.

***Study of sodium bromide in human female volunteers (Van Gelderen et al., 1993)***

A limited replication study was carried out to confirm the findings in the former study. Bromide was orally administered (capsules) at concentrations of 0, 4 or 9 mg bromide/kg bw/day as sodium bromide to 45 healthy female volunteers during three menstrual cycles. After the administration period the 45 females were observed for another three cycles. At the start and end of the administration period and at the end of the experiment a medical history, physical examination and haematological and routine

clinical chemistry tests were performed. Before and at the end of the experiment the thyroid hormones (thyroxin (T4), free thyroxin (fT4), thyroxin binding globulin (TBG), triiodothyronine (T3) and thyrotropine (TSH)) were analysed. Before, after three menstrual cycles and at the end of the experiment an EEG with a Visual Evoked Response was recorded. Mean plasma bromide concentrations at the end of the treatment were 0.07, 3.22 and 7.99 mmol/l, respectively. Medical histories, physical examinations and haematological biochemical and urine analyses after completion of the experiment showed no treatment-related abnormalities except for some incidence of nausea associated with bromide-capsule ingestion (nausea noted in 3 volunteers of the 4 mg/kg bw/day group and 11 of the 9 mg/kg bw/day group). The nausea was considered to be an effect of the relatively large amount of bromide administered in one capsule to the stomach and not a systemic effect. In none of the three groups were significant changes observed in the serum thyroxin concentration (T4), free thyroxin (fT4), triiodothyronine (T3), thyroxine-binding globulin (TBG) and thyrotropine (TSH). Clinical observation did not show effects on the thyroid or on the central nervous system. After three cycles one subject of each of the groups complained about drowsiness and vertigo. Another three of each group complained about sleepiness. Quantitative analysis of the electroencephalogram (EEG) showed no changes in $\delta_1$-, $\delta_2$- activities but significant changes in $\alpha_1$-band and β- activity in both groups (the quantitiative analysis of the spectral values was not given in the report). The changes in the quantitative EEG were borderline effects at both dosages according to the study author. As concluded by EMA the effect on EEG measurements noted at 4 mg/kg bw/day was not considered adverse.

In the REACH Registration there are also additional human case reports on observations following poisoning. They point to symptoms of neurotoxicity but are very meagrely reported and are included in the table 56, but not considered further in the evaluation.

**Mechanistic data**

A number of mechanistic studies of sodium bromide (Table 49) were reported as supporting studies in the REACH Registration dossier. These indicate morphological changes in rat nerve cells and neuroblastoma cell lines after sodium bromide treatment. Hyperpolarization in bullfrog sympathetic ganglion was also reported. The relevance of these effects in supporting classification in STOT RE for neurotoxic effects is however not clear.

**Summary**

Based on the available data from oral repeted dose toxicity of ammonium bromide, sodium bromide and postassium bromide it is concluded that the nervous system and the endocrine system (thyroid, adrenal, pituitary) appears to be the main target organs in rats. In dogs, the nervous system was the main target organ. One study in mouse also indicated that the nervous system is the main target organ.

Nervous system

The observed depression of the central nervous system after administration of bromide salts in experimental animals is in agreement with the known depressive effect on the central nervous system of bromide and formed the principal basis for the former use of bromide compounds as antidepressants and anticonvulsants in humans and its sustained application for the treatment of seizures in dogs. The underlying mechanism of the anticonvulsant activity of bromide is suggested to be due to the disturbance of the active as well as the passive transport of chloride across nerve cell membranes, leading to hyperpolarisation of these cells (e.g. Van Leeuwen and Sangster, 1987).

Clinical signs of depression of the central nervous system, behavioural effects and effects on function and motor activity were observed in OECD testguideline compliant repeated dose toxicity studies in rats and supporting data demonstrating similar findings are available in non-guideline repeated dose studies in rat, dog and mouse, and in reproductive and developmental toxicity studies of rat and rabbit. The

relevance of the neurofunctional changes in the animals studies is obscured in the presence of general toxicity in some cases, and in the absence of neuropathological changes. Nevertheless, the effects (clinical signs of depression of the central nervous system, behavioural effects and effects on function and motor activity) are clear and consistent and demonstrate an increase in severity related to exposure and duration. It is noted in the study by Joo, Dames and Wolff (1979) that electron microscopic findings after sodium bromide microinfusion were reported and described to be similar in principle to that found in GABA-treated ganglia. Since GABA is a neutrotransmitter depressing the synaptic transmission, and effects the chloride, potassium and calcium channels in the ganglion cells, sodium bromide may be assumed to have the same mechanism for inhibitory effect on the central nervous system. But it is unclear if the findings in this study have any functional significance. In addition, the relevance of the the morphological changes after sodium bromide treatment in the in vitro studies by Spoerri and Wolff (1982) and Eins, Spoerri and Heyder (1983) described as being similar to those described after GABA treatment are also not clear to the dossier submitter. However, in the open literature a non-guideline study by Safdari et al (2017) reported reduced discrimination index and recognition index as well as decreased frequency of exploration of new objects after 28-day treatment of potassium bromide (at 50 mg/kg bw/day) indicating an impaired memory function in male rats. These effects are in line with the bromide-induced inhibitory effects of the central nervous system mediated via GABA receptor activity. There is no information on the general systemic toxicity of the rats available in the publication and the study is considered as supportive information.

There is some information on neurotoxic effects of ammonium bromide in humans reported in the open literature (Sangster et al., 1983, Van Gelderen et al., 1993). At a dose level of 4 and 9 mg bromide/kg bw/day (oral administration) statistically there was an effect in the quantitative EEG. However, as concluded by EMA (The European Agency for the Evaluation of Medicinal Products) the electrophysical changes noted at 4 mg/kg bw/day (Sangster et al., 1983) were not considered adverse. The dossier submitter concurs with EMA that the findings cannot be considered as adverse, but considers the findings as supportive information for the nervous system as target organ.

Thyroid

In several reports from the open literature the bromide ion was shown to interfere with the morphology and function of the thyroid and thyroid hormones in rodents administered sodium bromide or potassium bromide. The activation of the thyroid gland was characterised by an increase in weight of the organ and a histopathologically observed reduction in follicle size and increase in height of the follicular epithelium. These were accompanied by a decrease in serum thyroxine (T4), resulting in a sustained increase in the synthesis and secretion of TSH, which occurs via the negative feedback system of the pituitary gland to stimulate thyroid function (Loeber *et al*., 1983; Velický et al., 1997a; Velický *et al*., 1998; Velický *et al*., 2004).

The effects on the thyroid are very likely due to the ability of bromide in replacing iodide within the thyroid gland, which had been demonstrated by lower molar concentration ratios of I/Br in the thyroid after bromide treatment (Velický et al., 1998). In a mechanistic study it was suggested that the changes caused by bromide treatment of rats in the thyrocytes (hypertrophy and hyperplasia of the endoplasmic reticulum, combined with dilated cisterns and tubules, and reduction in the amount of colloid) points 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 (Velický et al., 2004). In another mechanistic study it was concluded that bromide not only inhibits the uptake of iodide by the thyroid gland but also inactivates thyroid peroxidase, thus inhibiting iodide incorporation in tyrosine residues and coupling of iodotyrosine residues to iodothyronine (Van Leeuwen et al., 1983). In another study it was hypothesized that the bromide ion acts directly on the thyroid, thereby inducing alterations in the pituitary gland by feedback mechanisms (Loeber et al., 1983).

In contrast to rats, investigations in dogs did not reveal effects on the thyroid gland (with exception of a decrease in serum total thyroxine and free thyroxine over time which were within the reference ranges and was observed both in control and treated animals and therefore not clearly attributable to treatment), at a dosages of 240 mg/kg bw/day (Paull et al., 2003). The higher sensitivity of rats to bromide

treatment with regards to thyroid effects could be due to differences in the regulation, plasma protein binding and half lives of thyroid hormones in rats on the one hand and in dogs on the other.

With regards to differences between rats and humans it is noted that in the 'Guidance for the identification of endocrine disruption in biocides and pesticides, Appendix A – Additional considerations on how to assess the potential for thyroid disruption for human health' when interpreting data from experimental animals *"Substances inducing histopathological changes (i.e. follicular hypertrophy and/or hyperplasia and/or neoplasia) in the thyroid, with or without changes in the circulating levels of thyroid hormones, would pose a hazard for human thyroid insufficiency in adults as we all as pre- and post-natal neurological development of offspring*." In this case of ammonium bromide and bromide salts, histopathological changes (i.e. follicular hypertrophy and/or hyperplasia) in the thyroid, and changes in the circulating levels of thyroid hormones have been reported. Thus, human relevance of thyroid disruption of ammonium bromide cannot be ruled out.

In the study of human female volunteers, at the dose level tested in man (9 mg bromide/kg bw/day) statistically significant increased serum thyroxine and triiodothyronine concentrations were noted although the levels were within normal limits at the start and the end of the investigations (Sangster et al., 1983; Van Gelderen et al., 1993).

Adrenals

In short term toxicity studies in the rat effects (but not adverse) of bromide on the adrenal gland were noted at low dosages levels (histopathological changes were noted at a dose level of 4.4 mg bromide/kg bw/day) (Van Logten et al., 1974). At 108 mg/kg bw/day the effects on the adrenals were considered significant/severe.

Table 58: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

| **Study reference** | **Effective dose (mg/kg/d)** | **Length of exposure** | **Extrapolated effective dose when extrapolated to 90-day exposure** | **Classification supported by the study** |
|---|---|---|---|---|
| ***Nervous system*** | | | | |
| Study report, 2000a | 100 mg/kg bw/day ammonium bromide in males<br>Slight limpness in 3 males; of these, only one showed the finding on more than one occasion | 90 days | The effect level is at the guidance value (100 mg/kg bw/day) for the exposure length 90 days. | STOT RE 2<br>Nervous system |
| Study report, 2007a | 600 mg/kg bw/day ammonium bromide<br>Neurotoxic effects including staggering, rolling gait, and subdued behaviour | 13 days | The effect level is below the extrapolated guidance value range for 13 days of exposure (690 mg/kg bw/day) for STOT RE 2 | STOT RE 2<br>Nervous system |
| Study report, 2001 | 242/454 mg/kg bw/day ammonium bromide<br>Rolling gait was observed (males and females) | 8 weeks = 56 days | The effective dose is above the extrapolated guidance value (150 mg/kg bw/day) for STOT RE 2 for the exposure | No classification |

| Study reference | Effective dose (mg/kg/d) | Length of exposure | Extrapolated effective dose when extrapolated to 90-day exposure | Classification supported by the study |
|---|---|---|---|---|
| | | | length of 54 days. | |
| Hansen and Hübner, 1983 | 240 mg/kg bw/day sodium bromide<br>Disturbance of normal behaviour in mice (decrease in evasion time) | 26 days | The effect level is below the extrapolated guidance value of 300 mg/kg bw/day (28-day) for STOT RE 2 | STOT RE 2<br>Nervous system |
| Study report, 2016b | 500 mg/kg bw/day sodium bromide<br>Clinical signs of neurotoxic effects, 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) | 90 days | The effect level is above the guidance value of 100 mg/kg bw/day (90-day) for STOT RE 2 | No classification |
| Logten et al., 1973 | > 1500 mg/kg bw/day sodium bromide<br>Clinical signs of neurotoxicity | 28 days | The effect level is above the extrapolated guidance value of 300 mg/kg bw/day (28-day) for STOT RE 2 | No classification |
| Rosenblum, 1958 | 200 mg/kg bw/day potassium bromide<br>Clinical signs of neurotoxicity (ataxia, shivering, coma) were noted in dogs | Initial exposure for 6 weeks (= 42 days) at 200 mg/kg bw/day (thereafter increasing dosages) | The effective dose is below the extrapolated guidance value (approx. 200 mg/kg bw/day) for STOT RE 2 for the exposure length of 42 days. | STOT RE 2<br>Nervous system |
| ***Thyroidea*** | | | | |
| Loeber et al., 1983 | 108 mg/ kg bw/day sodium bromide<br>Histopathological changes in thyroidea and decreases levels of thyroxin | 28 days | The effect level is below the guidance value of 300 mg/kg bw/day (28-day) for STOT RE 2 | STOT RE 2<br>Thyroid |
| Velický et al., 1997a | 0.5 mg bromide/kg bw/day; corresponding to 0.673 mg ammonium bromide /kg bw/day | 16 or 66 day | The effective dose is below the guidance values for STOT RE 1 for exposure | STOT RE 1<br>Thyroid |

| Study reference | Effective dose (mg/kg/d) | Length of exposure | Extrapolated effective dose when extrapolated to 90-day exposure | Classification supported by the study |
|---|---|---|---|---|
| | Changes in the thyroid gland (including marked growth activation of the follicular epithelial component, frequent mitoses in the follicular cells, microfollicular tissue rearrangement, lowering in the portion of colloid in the thyroid tissue, slight to moderate thyroglobulin-positivity of collid tissue) and decreased plasma T4 level as well as slightly decreased plasma T3 level. | | lengths of both 90 days and 28 days. | |
| Velický et al., 1998 | 3.3-5 mg bromide/kg bw/day or 4.90-6.13 mg ammonium bromide/kg bw/day<br>Follicular rearrangement, decreased thyroglobulin (Tg) immunoreactivity, decreased amount of intrafollicular colloid, increased frequencies of mitoses in thyrocytes, and slightly increased TSH levels and decreased T4 levels. | up to 133 days | The effective dose is below the extrapolated guidance value (approx.. 6.5 mg/kg bw/day) for STOT RE 1 for exposure length of 133 days | STOT RE 1<br>Thyroid |

### 10.12.2 Comparison with the CLP criteria

The findings of the oral studies in rats, mice, dogs and humans that are specific to the target organs nervous system and thyroid are compared with the CLP criteria for STOT RE below.

Nervous system

Signs of central nervous system depression are evident in several test guideline repeated dose toxicity studies. The effects appear to be reversible and both immediate at high dose levels and delayed at lower dose levels. After repeated exposure of higher dose levels around and above 500 mg/kg bw/day mortality due to severity of adverse clinical signs is reported.

- Clinical signs of neurotoxicity of (increased limpness, decreased alertness, increases in landing foot splay and decreases in fore and hind limb grip strength) were noted during the detailed neurotoxicity examination of the 90-day feeding study of ammonium bromide in rats at ≥ **100 mg/kg bw/day** in males and in females at ≥ 225 mg/kg bw/day (Study report, 2000a). The effects observed at 100 mg/kg

bw/day in males, slight limpness in 3 males (of these, only one showed the finding on more than one occasion), warrant classification in STOT RE 2 for effects on the nervous system.

- Clinical signs of neurotoxic effects (abnormalities of gait) were noted in males at **≥ 225 mg/kg bw/day** and in females at 750 mg/kg bw/day in the 90-day feeding study of ammonium bromide in rats. The onset of the clinical signs were delayed, and persisted generally until necropsy (Study report, 2000a). These effects are at doses above the guidance value for classification and do not warrant classification.
- Clinical signs of neurotoxic effects were noted in male and female rats at a dose level of **500 mg/kg bw/day** and above in the 4-week dose-range finding study of ammonium bromide in rats with. The observed clinical signs consisted of agitated, nervous and hyperactive behaviour, rolling gait, hunched posture, piloerection and eyes partially closed (Study report, 1999). These effects do not warrant classification at an effect level of 500 mg/kg bw/day.
- Clinical signs of neurotoxic effects, 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) were reported at **500 mg/kg bw/day** (corresponding to **476 mg ammonium bromide/kg bw/day**) in the 90-day oral repeated dose toxicity study of sodium bromide in rats (Study report 2016b). These effects do not warrant classification at an effect level of 500 mg/kg bw/day.
- Neurotoxic effects at 600 mg/kg bw/day (1/22 dead) in the pre-natal developmental toxicity study (exposure GD 6-19) of ammonium bromide in rat (Study report, 2007a). The extrapolated guidance value range for 13 days of exposure is 690 mg/kg bw/day, and therefore the effects at 600 mg/kg bw/day warrant classification in STOT RE 2 for effects on the nervous system.

Non-guideline studies of repeated dose toxicity studies supporting effects on nervous system:

- Disturbance of normal behaviour in mice (decrease in evasion time) at a dose level of **240 mg/kg bw/day** sodium bromide (corresponding to **228 mg ammonium bromide/kg bw/day**) (26 days exposure) (Hansen and Hübner, 1983). This effect supports classification in STOT RE 2 since it is below the guidance value of 300 mg/kg bw/day (28-day).
- Clinical signs of neurotoxicity were noted in dogs at the dose level of **200 mg/kg bw/day** potassium bromide (corresponding to **243 mg ammonium bromide/kg bw/day**) (initial dose for 6 weeks, thereafter increasing dosages at intervals of 6 weeks) and above (Rosenblum, 1958). This effect supports classification in STOT RE 2 since it is intermediate in exposure length of 90-days and 28-days and guidance values 100 mg/kg bw/day (90-days) and 300 mg/kg bw/day 28-days).
- Clinical signs of neurotoxicity were evident from high doses (> 1500 mg/kg bw/day) of sodium bromide (corresponding to 1576 mg ammonium bromide/kg bw/day) in the 28-day study by Logten et al (1973). These effects do not warrant classification at the effect level of 1500 mg/kg bw/day (28-day).

Supporting data found in reproductive toxicity studies pointing to effects on the nervous system and the specific profile of toxicity, but not sufficient for classification since the effective doses are above the extrapolated guidance value for classification:

- Rolling gait was observed at **242/454 mg/kg bw/day** in 9/10 males and 6/10 females, and in all animals at 503/651 mg/kg bw/day in the dose range finding study of a reproduction toxicity of ammonium bromide (8 weeks; Study report, 2001). About half of the females showed hyperactivity at 503/651 mg/kg bw/day.
- Clinical signs of neurotoxicity was observed in the two-generation reproductive toxicity study of sodium bromide, less severe at **175 mg/kg bw/day** (corresponding to **184 mg ammonium bromide/kg bw/day**) (181-186 dosing days), and severe (in both sexes and in all males) at 350/500 mg/kg bw/day) (Study report, 2016a).

- Neurotoxic effects at **1000 mg/kg bw/day** (1/24 dead) in the pre-natal developmental toxicity study (exposure GD 6-19) of ammonium bromide in rat (Study report, 2000b).
- Ataxia at **500 and 1000 mg/kg bw/day** (corresponding to **525 and 1052 mg ammonium bromide/kg bw/day**) in the dose range-finding study of a pre-natal developmental toxicity study (exposure GD 3-28) of sodium bromide in rabbit (Study report, 2008a).
- Neurotoxic effects at **1000 mg/kg bw/day** (1/25 dams sacrificed) in the pre-natal developmental toxicity study (exposure GD 6-15) of sodium bromide (corresponding to **1052 mg ammonium bromide/kg bw/day**) in rat (Study report, 1995).

In human studies the following indications of effects on the nervous system were reported that may be considered as supporting evidence, although not severe effects:

- Neurophysiological changes (decreases in δ1- and δ2-activities and increases in β-activities and in mean frequency (mobility parameters)) at 9 mg/kg bw/day and at 4 mg/kg bw/day (increased α1-activity) in a 3-month study by Sangster et al., 1982.
- Significant changes in α1-band and β activity at 4 and 9 mg/kg bw/day but no changes in δ1-, δ2-activities in the study by Van Gelderen et al., 1993 that included administration during the 3 first menstrual cycles out of 6 cycles.

In a total weight of evidence assessment, and based on findings in animal studies at dose levels below the guidance values for classification supported with findings in humans, ammonium bromide should be classified in STOT RE 2 for effects on the nervous system.

It should be noted that the criteria are also met for classification in STOT SE 3 of the substance for causing transient narcotic effects after single doses at higher dose levels (see section 10.11).

Thyroid

The majority of available studies performed with bromide salts and effects in the thyroid gland are in rats. In available studies of dogs there were no effects reported on the thyroid. Adverse findings in the thyroid in rat at dose levels at or around guidance values for classification in STOT RE 2 according to the CLP criteria ar listed below.

- Statistical significant reduction in T3 (males only) and in T4 (males and females) from **175 mg** sodium bromide **/kg/day (**corresponding to **167 mg ammonium bromide/kg bw/day) (**week 4) in the 90-day repeated dose toxicity study in rat (Study report, 2016b). Single animals in these groups also showed depletion (mild/moderate) 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.

Non-guideline studies of repeated dose toxicity studies supporting effects on the thyroid:

- Changes in the thyroid gland (including marked growth activation of the follicular epithelial component, frequent mitoses in the follicular cells, microfollicular tissue rearrangement, lowering in the portion of colloid in the thyroid tissue, slight to moderate thyroglobulin-positivity of collid tissue) and decreased plasma T4 level as well as slightly decreased plasma T3 level were observed in rats even at low bromide doses **(0.5 mg bromide/kg bw/day**; corresponding to **0.673 mg ammonium bromide /kg bw/day**) where no other clinical signs or systemic effects related to treatment were noted in a (non-guideline) (16 or 66 day) repeated dose toxicity study of potassium bromide in drinking water (Velický et al., 1997a). These functional and morphological changes in the thyroid, with exposure-related increase in extent are below the guidance values for STOT RE 1 for both 90-day and 28-day studies and thus supports classification.

- Similar changes in the thyroid gland as observed in the study by Velický et al., 1997(a) and in addition, slightly increased TSH level and decreased T4 level in rats that were adminstered potassium bromide at 100 mg Br-/L in drinking water up to 133 days, corresponding to **3.3-5 mg bromide/kg bw/day** or **4.90-6.13 mg ammonium bromide/kg bw/day**( (Velický et al., 1998).
- Statistical significant increase in thyroid weight noted at ≥ **108 mg sodium bromide /kg bw/day (corresponding to 103 mg ammonium bromide/kg bw/day**), histopathological changes in thyroidea noted at 1728 mg/kg bw/day, and changes in hormone levels in serum noted at ≥**108 mg/kg bw/day** (decreased thyroxin) after four weeks administration of sodium bromide in rat (Loeber et al., 1983). This effect supports classification in STOT RE 2 since it is below the guidance value of 300 mg/kg bw/day (28-day).
- Statistical significant increase in thyroid weight from **108 mg sodium bromide /kg bw/day** (corresponding to **103 mg ammonium bromide/kg bw/day**) in females, decreased size of follicles in thyroidea in females at ≥ 432 mg/kg bw/day; increased activity of thyroids in females at ≥ 432 mg/kg bw/day in the 90-day repeated dose toxicity study of sodium bromide in rats (Van Logten *et al*., 1974).

Supporting data pointing to effects on the thyroid and the specific profile of toxicity, but not sufficient for classification, was also found in the three-generation reproductive toxicity study of sodium bromide in rats (van Leeuwen, 1983) where reduced thyroid hormone (T4) in males at **≥108 mg/kg bw/day** (corresponding to **103 mg ammonium bromide/kg bw/day**) and in females at ≥ 432 mg/kg bw/day were reported.

In human studies the following indications of effects on the thyroid were reported in the study by Sangster et al (1982): slight but significant increase in T3 and T4 in females only (20% and 14%, p,<0.01) at **9 mg bromide/kg bw/day** after 3 months intake of oral capsules (Sangster et al., 1982). However, since the levels of both T3 and T4 were within normal limits at the start and the end of the investigation they are considered by the dossier submitter to be of less significant toxicological relevance and as supportive information for classification in STOT RE with the thyroid as target organ.

Based on significant changes, which clearly indicate functional disturbance and morphological changes which are toxicologically relevant, at dose levels below the guidance values for classification both for category 1 (Velický et al., 1997a, Velický et al., 1998) and 2 (Loeber et al., 1983) seen in three non-guideline studies in rat with with a focus on alterations in the endocrine system, and considering the limitations of the studies, using expert judgement and a total weight of evidence assessment of all available information, a classification of ammonium bromide in STOT RE 2 for effects on the thyroid is considered warranted.

### 10.12.3 Conclusion on classification and labelling for STOT RE

Classification of Ammonium bromide in **STOT-RE 2; H373 (nervous system, thyroid)** is warranted.

There is no need of setting a specific concentration limit.

### 10.13 Aspiration hazard

Not evaluated in the current CLH report.

## 11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in the current CLH report.

## 12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in the current CLH report.

## 13 ADDITIONAL LABELLING

Not relevant.

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## 15 ANNEXES

Annex I to the CLH report

CONFIDENTIAL Annex: CAR of BAC and Ammonium bromide, Document III, Sections A6