Committee for Risk Assessment
RAC

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

Ammonium bromide

EC Number: 235-183-8
CAS Number: 12124-97-9

CLH-O-0000006899-51-01/F

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

Adopted
8 October 2020

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: -

Contact details for dossier submitter:

Swedish Chemicals Agency

Esplanaden 3a, P.O Box 2

SE-172 13 Sundbyberg, Sweden

kemi@kemi.se

+46 8 519 41 100

Version number: 2   **Date: 190222**

CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON AMMONIUM BROMIDE

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 12124-97-9	Min. 98.3% (98.3-99.8)	No entry in Annex VI Table 3.1	Eye Irrit. 2 H319 STOT SE 3 H335 Skin Irrit. 2 H315 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 Repr. 1B Lact. STOT SE 3 STOT RE 2	H319 H360FD H362 H336 H373 (nervous system, thyroid)	GHS07 GHS08 Dgr	H319 H360FD H362 H336 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 Repr. 1B Lact. STOT SE 3 STOT RE 2	H319 H360FD H362 H336 H373 (nervous system, thyroid)	GHS07 GHS08 Dgr	H319 H360FD H362 H336 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.

> ## RAC general comment
>
> Ammonium bromide is a substance registered under REACH at 1000 - 10000 tonnes per annum, with carcinogenic, mutagenic and/or reprotoxic properties and widespread use, it was therefore prioritized for harmonised classification and labelling. Industrial uses identified for ammonium bromide include flame retardants and uses 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, which is used as a preservative for liquid-cooling and processing systems.
>
> Ammonium bromide does not have a current entry in the Annex VI of the CLP Regulation.

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	1.3×10^{-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) (*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	CRC Handbook data,	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
	not given 145.6 g/l at 100°C, pH not given	1991-1992 and 2007-2008 (AmBr A.35/01, and 05) and Cambell and Marsh, 1959	
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.
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). The substance has a low propensity to become airborne with a maximum attainable concentration of 0.1 mg/L. 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 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) Human 6 males and 4 women Oral (drinking water) Single administration 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) Tissues and body fluids sampled: blood Time and frequency of sampling: twice daily for a period of 4 days	Absorption: highest concentration was reached within 1-4 hours after dosing. Distribution: The ratio red blood cells to serum of bromide ions was about 0.45. Excretion: effective half-life was 32 hours; biological half-life was 12 hours	Ammonium bromide	A6.2/10, Doc. No. 592-010 Söremark, R., 1960 Reliability 2
Mouse			
Distribution (no guideline study, prior to GLP-implementation) Mouse, albino (strain not specified) I.v. in tail vein Single administration A 20 mg mouse received 75 µCi, corresponding to 1 mg Br, in the form of a water solution of NH_4Br^{82} 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. 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 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 Human 7 males Oral and i.v. Single administration 1 ml/kg of 3% sodium bromide, equivalent to 30 mg/kg bromide. 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. 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). 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 Vaiseman N., Koren G. and Pencharz P. 1986 Reliability 2
Rat			
Kinetics and distribution (no guideline study) Rat, Wistar 5 males per group Oral (drinking water), subcutaneous injection or intragastric administration 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. 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. Half-lifes: Thyroid: 94.3 ± 14.6 h Liver: 235.0 ± 88.9 h Whole body: 197.8 ± 22.2 h	Sodium bromide	A6.2/17, Doc. No. 592-075 Pavelka S. et al., 2000a Reliability 2

Method	Results	Remarks	Reference
Exposure regimen: 12-396 hours Biological half-lives of bromine in 15 different organs and tissues were determined at different time intervals			
Distribution (no guideline study), no GLP compliance Rat, Wistar 5 males per group s.c. and oral (in drinking water) 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. 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) Rat, Wistar 4 females per group Oral (feed) 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. 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 van Logten, M. J. et al., 1973 Reliability 2
90-day oral repeated dose toxicity study (no guideline study) Rat, Wistar male/female 10/sex/group Oral (feed) 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. 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 van Logten, M. J., et al., 1974 Reliability 2

Method	Results	Remarks	Reference
	mmole/kg, respectively in control.		
Dog			
Intravenous and oral administration to healthy Beagle dogs (no guideline study) Dog, Beagle 4 females/group Oral and i.v. 20 mg/kg bw Single treatment.	Absorption of bromide was rapid, reaching peak serum concentrations of 65 to 96 mg/L after 30-45 minutes. The mean bioavailability of bromide was estimated to be approximately 46%. 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. Mean apparent elimination half life ($t_{\frac{1}{2}}$) 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 Trepanier L.A. and Babish J.G. 1995. Reliability 2
Potassium bromide			
Dog			
Bromide pharmacokinetics in vivo (no guideline) Dog, Beagle male/female 3/sex/group Oral (feed) 30 mg/kg bw/day (actual ingested) (equivalent to 20 mg Br-/kg bw) 115 days (daily)	The mean steady-state serum bromide concentration was 245 mg/dL (range from 178 to 269 mg/dL). The mean elimination t1/2 using a one-compartment model was 15.2 days. A majority of dogs reached a serum bromide concentration that was 75% of the apparent steady-state concentration by 30 days and at least 90% of the apparent steady-state concentration by 60 days. Apparent total body clearance was 16.4 mL/day/kg and volume of distribution was 0.40 L/kg. 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 March, P.A. Podell, M., Sams, R.A. 2002. 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 Pig Rabbit	as permeable as skin in situ. Low amounts of bromide penetrated human skin (<1%).		Tregear R.T., 1966 Reliability 2
Distribution study in vivo (no guideline study) Guinea pig, 'Sabra' albino 9-11 males per group Dermal and inhalation Dermal: 30 mins Inhalation: 5 mins Dermal: 15% Dead Sea bath salt solution with 10 Ci/L $Na^{82}Br$ 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. Reliability 2
Dermal penetration in vivo Human (Healthy volunteers and psoriatic patients were examined) Guinea pig (males/females) 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. humans: 30 minutes/bath 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). ^{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 Shani, J. et al., 1985 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 Dog 10 females Oral (feed) 10 mmol/kg 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 Rats and rabbits Males: 18-45 mg/kg bw (average 28 mg/kg bw) Females: 18-45 mg/kg bw (average 28 mg/kg bw 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 Dogs and humans Oral (feed) Exposure time for dogs: at least 10 days 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 Rabbit Infusion into the marginal ear vein Males: 2-15 mmol/kg 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 Dog 2 females Oral (gelatin capsules mixed with the diet) 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 Underhill, 1913

Method	Results	Remarks	Reference
	elimination		
Distribution Dog Oral (drinking water) Single treatment and treatment over several days (the treatment period is not further specified in the report), daily Doses/conc.: - 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) - 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. 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

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

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

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) EPA OPP 81-1 (Acute Oral Toxicity)	Rat, CD strain (remote Sprague Dawley origin male/female 5/sex/group	Ammonium bromide Vehicle: distilled water	Oral (gavage) 2000, 2714, 3684 and 5000 mg/kg bw	LD50 males: 2868 mg/kg bw LD50 females: 2566 mg/kg bw LD50 males and females combined: 2714 mg/kg bw	A6.1.1/01, Doc. No. 521-001 Study report, 1986a 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 ≤ 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) EPA OPP 81-2 (Acute Dermal Toxicity) (Sub-Division F) EEC Directive 92/69/EEC, Annex V, Test B3, July 1992	Rat (Sprague-Dawley) male/female 5/sex/group	Ammonium bromide	2000 mg/kg bw (comparable to 40 mg/cm2) Coverage: semiocclusive 24 hours	LD50: > 2000 mg/kg bw (male/female)	A.6.1.2/01, Doc. No. 522-001 Study report, 1998a 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 ≤ 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) EU Method B.2 (Acute Toxicity (Inhalation)) EPA OPP 81-3 (Acute inhalation toxicity)	Rat (Sprague-Dawley) male/female	Ammonium bromide Dust aerosol MMAD: 2.61 µm	Nominal concentration: 37.96 mg/L Measured concentration: 0.1 mg/L (maximum attainable concentration) inhalation (nose only) 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 Study report, 1998b 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.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed no classification for acute toxicity via the oral, dermal or inhalation route as the respective CLP criteria were not met.

Comments received during general consultation

These endpoints were not commented during the general consultation.

Assessment and comparison with the classification criteria

Acute oral toxicity

For the assessment of acute oral toxicity, the DS provided one animal study with CD rats considered as reliable without restriction or Klimisch 1. In the 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 bw 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 LD_{50} 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/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 body weight gains and necropsy findings on day 15 were unremarkable. The mortality rate at 2000 mg/kg bw in both males and females was zero, at 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.

The acute oral LD_{50} value of ammonium bromide was determined to be 2868 mg/kg bw for males, 2566 mg/kg bw for females and 2714 mg/kg bw for combined sexes. As the determined LD_{50} values are above the oral acute toxicity estimate (ATE) values for category 4 (300 mg/kg bw < ATE ⩽ 2000 mg/kg bw), RAC concludes that **no classification is warranted for ammonium bromide for acute oral toxicity**.

Acute dermal toxicity

For the assessment of acute dermal toxicity, the DS provided one animal study with SD rats considered as Klimisch 1. In the study performed according to OECD TG 402, five male and five female rats were exposed to a 24 h dermal application of 2000 mg/kg bw of ammonium bromide which was applied under occlusive water moistened patches onto the skin which was clipped the day before (Study report, 1998a). The rats were observed for 14 days after dosing. Loose faeces, test side slightly red and wet perigenitals were observed after dermal application.

None of the animals died during the study, and the acute dermal LD_{50} of ammonium bromide was determined to be > 2000 mg/kg bw. As the LD_{50} is above the dermal ATE values for the category 4 (1000 mg/kg bw < ATE ≤ 2000 mg/kg bw), RAC concludes that **no classification is warranted for ammonium bromide for acute dermal toxicity**.

Acute inhalation toxicity

For the assessment of acute inhalation toxicity, the DS provided one animal study with SD rats considered as "reliable without restriction or Klimisch 1". In the study performed according to OECD TG 403 five male and five female rats were exposed to aerosolized ammonium bromide (dust) with the maximum attainable concentration of 0.1 mg/L as a single 4 h treatment (nose only). All animals were observed for clinical signs for a period of 14 days post-exposure. All animals were subjected to necropsy at the termination of the study. Lungs were removed and weighted to allow calculation of lung to body weight ratio. No deaths or clinical signs of toxicological importance were demonstrated. There were no treatment-related effects on body weights, lung to body weight ratios or necropsy findings.

The acute inhalation LC_{50} for dust aerosol of ammonium bromide was considered to be > 0.1 mg/L. Since no higher dust aerosol concentrations of ammonium bromide than 0.1 mg/L could be generated due to rapid sedimentation of the relatively heavy ammonium

bromide particles, and no deaths or clinical signs of toxicological importance were demonstrated at the highest tested dose, the CLP criteria for classification with respect to acute inhalation toxicity are not met. RAC concludes that **no classification for ammonium bromide is warranted for acute inhalation toxicity**.

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 -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Ammonium bromide					
OECD TG 404 GLP	Rabbit New Zealand White Female Six animals	Ammonium bromide	0.5 g/animal Duration of exposure: 4 hours Post exposure period: 3 days	Average score 24, 48 and 72 h: - Erythema 0.0 - Oedema 0.0 Reversibility: not applicable, there were no erythema or oedema observed. Results: Non-irritant to skin	A6.1.4/01, Doc. No. 565-001 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.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation because no dermal reactions were recorded in any rabbit tested for ammonium bromide at any reading time in the available study performed in accordance with OECD TG 404 and GLP.

Comments received during consultation

This endpoint was not commented during the consultation.

Assessment and comparison with the classification criteria

DS provided one study on skin corrosion/irritation with New Zealand White rabbits (six females) performed according to OECD TG 404 (Klimisch score was not stated) (Study report, 1996a). The dorsal area of each rabbit was clipped between the limb girdles (two test sites 6 x 6 cm) 24 hours before dosing. A quantity of 0.5 g of ammonium bromide was applied by semi-occlusive application for four hours. Dermal reactions (erythema and oedema) were assessed 1, 24, 48 and 72 hours after removal of the dressings. No dermal irritation responses were observed in any animal at any reading time during the observed period.

As no dermal reactions were recorded in any animal tested for ammonium bromide at any reading time, RAC agrees with the DS that CLP classification criteria for skin corrosion/irritation are not fulfilled and **no classification is warranted for ammonium bromide for skin corrosion/irritation**.

10.5 Serious eye damage/eye irritation

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

Method, guideline, deviations	Species, strain, sex,	Test substance,	Dose levels duration of	Results - Observations and time point of onset - Mean scores/animal	Reference

if any	no/group		exposure	- Reversibility	
Ammonium bromide					
EPA OPP 81-4 (Acute Eye Irritation) equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion) GLP	Rabbit, New Zealand White 6 females	Ammonium bromide	0.1 g of test substance Reactions of conjunctivae, iris and cornea were examined 1, 24, 48, and 72 hours after treatment and on day 8.	Mean scores 24-72 h Corneal opacity score: 0 in all six animals Iritis score: 0, 0, 0.3, 0.3, 0, 0.3 (fully reversible within 48 hours) Conjunctivae redness score: 2, 2, 0.6, 2, 2, 1.3 (fully reversible within 8 days) Chemosis score: 0, 0.3, 0, 0.6, 0.6, 0 (fully reversible within 72 hours)	A6.1.4/03, Doc. No. 566-002 Study report, 1986b 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.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify ammonium bromide as Eye Irrit. 2, H319 based on a mean score of 2 for conjunctival redness in 4 out of 6 rabbits in an acute eye irritation study with New Zealand White rabbits. The effects were fully reversed within 21 days.

Comments received during consultation

One MSCA supported classification of ammonium bromide as Eye Irrit. 2, H319.

Assessment and comparison with the classification criteria

DS provided one study on acute eye irritation with New Zealand White rabbits (six females) performed similar to OECD TG 405 and considered as Klimisch 1 (Study report, 1986b). Six rabbits were subject to a single ocular instillation of 0.1 g of ammonium bromide into the right eye. The left eye remained untreated and served as a control. Reactions of conjunctivae, iris, and cornea were examined 1, 24, 48 and 72 hours after the treatment and on day 8. Mean 24 – 72 hours examination score for corneal opacity, iritis, conjunctivae redness and chemosis was calculated for each animal and is summarised in the table below:

Type of reaction	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6
Corneal opacity	0	0	0	0	0	0
Iritis	0	0	0.3	0.3	0	0.3

Conjunctivae redness	2	2	0.6	2	2	1.3
Chemosis	0	0.3	0	0.6	0.6	0

Iritis and chemosis were fully reversible within 72 h, and conjunctivae redness was reversible within 8 days. No corneal lesions were observed.

As the score for conjunctival redness at least in 4 out of 6 rabbits was 2 and the effect was fully reversible within 21 days, RAC concludes that **classification as Eye Irrit. 2, H319 (Causes serious eye irritation) 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 OECD Guideline 406 (Skin Sensitisation) EPA OPP 81-6 (Skin Sensitisation) EU Method B.6 (Skin Sensitisation)	Guinea pig, Dunkin-Hartley Female 20 in test group 10 in control group	Ammonium bromide	Induction: 5% test substance for intradermal injection, 55% test substance for topical application (maximum practicable concentration) Challenge: 55% test substance for topical application (maximum practicable concentration) Evaluation: 24h, 48h after challenge; dermal reactions were also assessed 24h after intradermal and topical induction application	not sensitising No. with positive reactions: 1st reading: 0 out of 19 (test group); 24 h after chall.; dose: 5 % / 55 % 2nd reading: 0 out of 19 (test group); 48 h after chall.; dose: 5 % / 55 % 1st reading: 0 out of 9 (negative control); 24 h after chall. 2nd reading: 0 out of 9 (negative control); 48 h after chall. Positive controls: 100% of the test group animals reacted positively following challenge	A6.1.5/01, Doc. No. 567-001 Study report, 1998c 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.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin sensitisation because under the conditions of the available Guinea pig maximisation test none of the animals showed a positive sensitisation response. Thus, the available information did not meet the CLP criteria for classification for skin sensitisation.

Comments received during consultation

Classification of this endpoint was not commented during the consultation.

Assessment and comparison with the classification criteria

The DS provided one Guinea pig maximisation test with ammonium bromide performed according to the OECD TG 406 and considered as "reliable without restriction or Klimisch 1" (Study report, 1998c).

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, 5% intradermal and 55% topical induction concentrations of ammonium bromide were selected for the main study because 5% intradermal concentration was the highest non-irritating dose and 55% topical concentration was the maximum practicable concentration. No reactions were noted at concentrations up to 55%.

For the induction exposure in the main study, 20 Guinea pigs were exposed to the test material via 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. The 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 the induction and all 19 surviving Guinea pigs showed a negative response also after the challenge exposure. RAC agrees with the DS that the available information **does not meet the CLP criteria for classification for skin sensitisation**.

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) OECD TG 471	*Salmonella Typhimurium* (TA98, 100, 1535, 1537) *Escherichia Coli* (WP2 uvrA)	Ammonium bromide	17-5000 µg/plate	negative	negative	No cytotoxicity Positive controls demonstrated the sensitivity of the assay	A6.6.1/01, Doc. No. 557-002 Study report, 1998d

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

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

Test system Method Guideline	Species Strain Sex No/group Vehicle	Test substance	Frequency of application	Sampling times	Dose levels	Results	Remarks	Reference
Mammalian erythrocytes micronucleus test	Mouse CD1 M&F 5/sex	Ammonium bromide	2	48 h after first application	400-1600 mg/kg bw/day	negative	-	A6.6.4/01, Doc. No. 557-003

OECD TG 474	(negative control) 5 M (low and mid dose group) 5 M&F (high dose group) 5 M (positive control) Vehicel.: water								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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity, because in the available *in vitro* studies on 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. It was noted however, that exposure of the bone marrow was not demonstrated.

Comments received during general consultation

This endpoint was not commented during general consultation.

Assessment and comparison with the classification criteria

The DS provided two *in vitro* tests and one *in vivo* test with ammonium bromide and three *in vitro* tests with sodium bromide.

Bacterial Reverse Mutation Test (Ames test) on 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 according to OECD TG 471. Bacteria were exposed at concentrations ranging from 17 to 5000 µg ammonium bromide per plate in the presence and absence of S9 mix in triplicate plates each. A positive control was applied. No cytotoxicity (clearing of background lawn) or precipitation was observed up to and including the maximum concentration of 5 mg/plate. No mutagenic activity was observed in any of the 5 bacterial strains tested both in the absence and presence of S9 mix.

Mammalian Cell Gene Mutation Test on ammonium bromide in mouse lymphoma cells L5278Y
(Study report, 1998e):

Ammonium bromide was examined in the test according to OECD TG 476 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.

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 assays with and without metabolic activation.

In vivo micronucleus study on ammonium bromide in mice
(Study report, 1998f):

Mammalian erythrocyte micronucleus test on ammonium bromide according to OECD TG 474 was performed on CD-1 mice. Animals were orally exposed at 400, 800 and 1600 mg/kg bw/day 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. 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei and the frequency of micronucleated PCEs was determined. The polychromatic erythrocytes/normochromatic erythrocytes ratio (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 the maximum tolerated dose of the substance was judged to be around 600 mg/kg bw/day.

No micronucleus induction was detected in bone marrow erythrocytes of mice treated with ammonium bromide concentrations of 400, 800 and 1600 mg/kg bw/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 with cyclphosphamide induced a significant increase in the number of micronucleated PCE.

Other studies on sodium bromide

In addition, Ames test on sodium bromide according to OECD TG 471 (Study report, 1988f), mammalian chromosome aberration test on sodium bromide in cultured human lymphocytes according to OECD TG 473 (Study report, 1988g) as well as unscheduled DNA repair synthesis study on sodium bromide in HeLa S3 epithelioid cells of human cervical lymphoma (Study report, 1988h) gave no indication of mutagenic activity.

Comparison with the classification criteria

In the available *in vitro* studies on 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. There was no micronucleus induction *in vivo* in mice caused by ammonium bromide, however, it was noted that exposure of the bone marrow was not clearly demonstrated.

RAC concludes that **no classification 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) Rat, Fischer (F344) 60/sex/group	Potassium bromide or bromine by methyl bromide fumigation Oral (feed) 104 weeks (interim sacrifice at 52 weeks) Potassium bromide: 500 ppm (16.5 mg bromine/kg bw/day in males; 20 mg bromine/kg bw/day in females) Methyl bromide: 80, 200 or 500 ppm (2.67, 6.77, 16.9 mg bromine/kg bw/day for	Potassium bromide: 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) 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) LOAEL:>500 ppm (>16.5 and >20 mg bromine/kg bw/day for males and females, respectively) Methyl bromide: Slight depression of body weight gain from wk 60 onwards in males of the 500 ppm group (3-6%) Tumour incidence rate was unaffected. LOAEL:>500 ppm (16.9 and 20.2 mg bromine/kg bw/day, respectively)	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			
	males; 3.23, 8.29, 20.2 mg bromine/kg bw/day for females)		

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 HCD mean: 91/389 (10.89%)	No	No information	No information	Single (females)	No	Oral, via diet	MoA not known

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
	HCD range: 3.8% to 16.3%. (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)							

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.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There were no chronic toxicity/carcinogenicity studies on ammonium bromide. A chronic toxicity and carcinogenicity study with potassium bromide and methyl bromide fumigated diet was assessed by the DS to evaluate the carcinogenic properties of ammonium bromide. In this study, a statistically significant increase in the incidence of mononuclear cell leukaemia 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 respectively). However, an increased incidence of tumours 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 respectively). According to the DS, F344 rats are known to have a high spontaneous tumour incidence of mononuclear cell leukaemia and the incidence of mononuclear cell leukaemia was within the historical control data range. However, the DS was not able to evaluate the appropriateness of the historical control data. The DS proposed no classification for carcinogenicity due to insufficient data.

Comments received during consultation

This endpoint was not commented during the consultation.

Assessment and comparison with the classification criteria

There is no human data on carcinogenicity of ammonium bromide or any other bromide salts and there are no carcinogenicity studies on ammonium bromide in animals available. The DS provided one chronic toxicity and carcinogenicity study with potassium bromide and methyl bromide fumigated diet performed with Fischer (F344) rats similar to OECD TG 453 and considered as Klimisch 2 (Mitsumori *et al*., 1990).

The study was conducted by feeding diets containing 80, 200 or 500 ppm total bromine following fumigation with methyl bromide (corresponding to 2.67, 6.77, 16.9 mg bromine/kg bw/day for males and 3.23, 8.29, 20.2 mg bromine/kg bw/day for females) as well as a diet containing 500 ppm potassium bromide (corresponding to 16.5 mg bromine/kg bw/day in males and 20 mg bromine/kg bw/day in females) to groups of 60 males and 60 females for up to 2 years.

Ten males and ten females from each group were killed after week 52 and 104 for urinalysis, haematology, blood biochemistry and pathology. Rats that were found dead during the study and all rats that survived to the end of the study were also subjected to pathological examinations.

In rats fed the diets fumigated with methyl bromide there were no marked toxic changes, except for a slight depression of body weight gain from week 60 onwards in males at 500

ppm, and tumour incidence was unaffected. Rats given a diet containing potassium bromide did not show any treatment-related changes.

It was concluded that residues of up to 500 ppm total bromine in diets fumigated with methyl bromide are not carcinogenic in F344 rats of either sex and that the maximum no-effect level is 200 ppm (6.77 mg total bromine/kg bw/day) in males. The maximum no-effect level was not determined in females.

With respect to potassium bromide, the incidence of prostatitis (20/60) was statistically significantly increased in males as compared to the control group (10/60). The incidence of mononuclear cell leukaemia (11/60) was statistically significantly increased in females treated at 500 ppm potassium bromide, compared to the control group (4/60). As only one dose was applied, no dose-effect relationships can be determined. It was noted that this type of leukaemia was quite similar to that which occurs spontaneously in F344 rats. The historical accumulated incidence of this leukaemia among rats at the test laboratory is 91/839 (10.8%), and its incidence in females 11/60 (18.3%) is not statistically significant when compared to the historical accumulated data.

RAC agrees with the DS that **no classification of ammonium bromide for carcinogenicity is warranted <u>due to inconclusive data</u>**.

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 GLP Rat, Sprague Dawley 10/sex/group	Ammonium bromide Purity 99.94% Oral (feed) 0, 1600, 3200, 6400 ppm Group mean achieved dosages of test material during pre-mating, mating, gestation and lactation: 0, 127, 242 and 503 mg/kg bw/day for males and 0, 228, 454 and 651 mg/kg bw/day for females 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.* **Parental generation – general toxicity** <u>Mortalities and clinical observations</u> *Males and females:* There were no premature deaths during the study. ↑ incidences of transient piloerection starting at 127/228 mg/kg bw/day ↑ incidences of rolling gait at 242/454 mg/kg bw/day: 9/10 males and 6/10 females ↑ 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 ↑ incidences of hyperactivity in females (approx. 50%) at 503/651 mg/kg bw/day <u>Body weight and food consumption</u> *Males* ↓ 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) ↓ food consumption during week 8 at 242 mg/kg bw/day and 503 mg/kg bw/day (88% of control in both groups) *Females* No obvious treatment related effect on bodyweight prior to mating at any dose level. ↓ bodyweight gain gestation days 0-20 (67% of control) at 651 mg/kg bw/day ↓ 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). Food consumption during the pre-mating and gestation periods was similar in all dose groups. **Fertility, parturition and sexual function** ↓ 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 Study report, 2001 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) (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)* ↑ 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) EPA OPPTS 870.3100 (90-Day Oral Toxicity in Rodents) GLP compliant	Ammonium bromide Purity: 99.91% Oral (feed) 0, 100, 225, 500 (males), 750 mg/kg bw/day (nominal in diet) 13 weeks (90-days) Post-exposure period: 4 weeks for the control and high	**Mortality and clinical observations** 3 premature terminations among males at 500 mg/kg bw/day ↑ 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) **Body weight**	A6.4.1/01, Doc. No. 533-001 Study report, 2000a 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 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%) **Food consumption** ↓ food consumption 500 mg/kg bw/day (males 7%) **Reproductive organ weights** ↓ 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) ↓ absolute weight of testes at 225 (10%, p<0.05) and 500 mg/kg bw/day (16%, p< 0.001) **Histopathology** No effects reported.	
Ammonium bromide – non-guideline repeated dose toxicity studies			
Repeated dose toxicity: 4 week dose range-finding study GLP compliant rat (Sprague-Dawley) male/female 5/sex/group	Ammonium bromide Purity: 99.94% Oral (feed) 0, 100, 500, 1000 mg/kg bw/day (nominal in diet) 4 weeks (28 days)	*Mortality and clinical observations* ↑ clinical signs of neurotoxicity and subdued behaviour at 500 (all males) and 1000 mg/kg bw/day (all males and 4/5 females) *Body weight* ↓ body weight gain (males 49% and females 31%) at 1000 mg/kg bw/day *Food consumption* ↓ food consumption (males 29%) at 1000 mg/kg bw/day **Reproductive organ weights** ↓ 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) ↓ mean absolute epididymis weight at 500 and 1000 mg/kg bw/day (-14% (p<0.05) and -16% (p<0.01), respectively) **Histopathology** Not performed	A6.3.1/01, Doc. No. 532-001 Study report, 1999 Reliability 1
Sodium bromide – test guideline reproductive toxicity studies			
Two-generation reproductive	Sodium bromide Purity: 99.5%	**P generation – general toxicity** Mortalities and clinical observations	Study report, 2016a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
toxicity study OECD TG 416 GLP compliant 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). The offspring from the second pairing of the P generation rats formed the F1b generation, terminated at day 40 postpartum. Rat, Crl:CD(SD)	Oral (gavage) 0, 50, 175, 350/500 (M/F) mg/kg bw/day 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). 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. Any dam in the process of parturition was not given the test or control substance formulations until the following work day.	*Males:* ↑ 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. ↑ incidences of adverse clinical signs (p≤ 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). *Females:* ↑ 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. ↑ 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. <u>Body weights</u> *Males:* ↓ 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. ↓ body weight at intermediate dose level: >10% lower than control (p≤0.01) from study day 134 (day 8 of second mating). ↓ 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 ≤0.01) study days 1-183. ↓ 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 ≤0.01). During study days 155-176 the body weight gain was 19-35% of control gain. *Females:* 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. Gestational phase, first pairing: ↓ body weight at high dose level: 89.2% of control weights (p ≤	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. 23/sex in control group, 23/24 (M/F) in low dose group, 23/22 (M/F) in intermediate dose group in in P generation mating to produce F1b. 23/sex in control group, 22/sex in low dose group, 15/sex in intermediate dose group in F1a generation mating to produce F2.		0.05) at GD 20. ↓ body weight gain at high dose level: 72.3% of control (p ≤ 0.01) during GD 14-20. Lactational phase, first pairing: ↓ body weight at high dose level: 89.1% and 86.7% of control weights (p ≤ 0.05, p ≤ 0.01) at lactation day 7 and 21, respectively. ↓ overall body weight gain lactation day 0-21 at high dose level: only 1% of control gains (p ≤ 0.05). Food consumption *Males:* ↓ 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. *Females:* ↓ 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). **P generation - Organ weights and Histopathology** No gross lesions related to treatment with sodium bromide occurred in males or females in any dose group *Males:* ↓ 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. ↑ 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). ↑ spermatid retention in Sertoli cells at 175 mg/kg bw/day (11/23) and 350 mg/kg bw/day (all males) ↑ tubular spermatid retention at 175 mg/kg bw/day (9/23) and 350 mg/kg bw/day (17/20) *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). ↑ pituitary weight, absolute (30%, p≤ 0.05) and relative to body and brain weight (33%, p≤ 0.01) in high dose group. ↑ 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. **P to produce F1a - Fertility, parturition and sexual function** ↓ 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 ↓ 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). ([b]) *percent of pairing that resulted in matings)* ([c]) *percent of matings that resulted in pregnancies)* **P to produce F1b - Fertility, parturition and sexual function** ↓ 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% ↓ 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). **F1a generation (post weaning) – general toxicity** Mortalities and clinical observations 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. Body weights *Males:* ↓ 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.	
		↓ 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).	
		Females:	
		No stat. sign effects on body weight or body weigh gain during premating phase and mating phase.	
		Gestational phase:	
		↓ body weight (p≤ 0.05) on GD 7 (-7%) and 10 (-6%) in the 175 mg/kg/day dose group	
		↓ body weight gain (-20% compared to control group, p≤ 0.05) GDs 0 to 7	
		Lactational phase:	
		↓ body weight (p≤ 0.01) on LDs 0 (-8%), 4 (-8%), 7 (-8%) and 14 (-6%) in the 175 mg/kg/day dose group	
		Food consumption	
		Males:	
		↓ food consumption (-11%) p≤ 0.01) in the 175 mg/kg/day dose group over the entire dosing period (PNDs 22 to 147).	
		Females:	
		↓ 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).	
		F1a generation (post weaning) - Organ weights and Histopathology	
		Males:	
		↓ 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.	
		↑ weight (adjusted to body weight) of the left epididymis (7%, (p≤ 0.05 compared to control)	
		↓ 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.	
		Females:	
		There were no adverse effects on ovary, uterus or pituitary	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		weights, absolute or relative (to brain or to body weight), in the 50 and 175 mg/kg/day dose groups **F1a generation to produce F2 - Fertility, parturition and sexual function** No effect on mating or pregnancy rate in any dose group. ↓ 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)	

Sodium 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
Three-generation reproduction study Not GLP-compliant Deviations including no food consumption, pup body weights, litter size determination. Rat, strain not specified Males and females 7-19/sex/ group	Sodium bromide Purity: not reported Oral (feed) Breeding study and thyroid hormone investigation: 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) Cross-mating experiment: Untreated males and females were mated with females and males of the 19200 ppm group.	Reproduction study No adverse effects on body weights recorded. No information on clinical condition available. ↓ 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). Cross-mating study ↓ 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%). Thyroid hormone investigation: Results are presented in section 10.12	A6.8.2/02, Doc. No. 592-002 Van Leeuwen, F. X. R. et al, 1983 Reliability 2

Fertility (%)

	0	75 ppm	300 ppm	1200 ppm	4800 ppm	19200 ppm
F1	70	70	72	65	25	0
F2	62	54	44	53	-	-
F3	52	67	80	45	-	-

-    = no breeding

Sodium bromide – test guideline studies repeated dose toxicity studies

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD TG 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents) with	Sodium Bromide Purity: 100.0%	**Mortality and clinical observations** *Males* ↑ mortality at 500 mg/kg bw/day (4 animals euthanasied) ↓ motor activity postdose in 6/10 males in week 2 at 175	Study report, 2016b

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 OECD TG 416 (Two-Generation Reproductive Toxicity Study) Rat, Crl:CD(SD) Male/female 10/sex/group and an additional 10/sex/group for control and high dose groups were included for the recovery assessment.	Oral (gavage) 0, 60, 175, 500 mg/kg/day 90 days	mg/kg/day, but all animals recovered before the end of the working day. ↑ 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. *Females* ↓ 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. ↑ 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. **Body weight** *Males* ↓ body weight at the end of dosing period, 81.2 % of control, (p≤ 0.01) at 500 mg/kg/day ↓ 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 *Females* ↓ 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) **Food consumption** *Males* ↓ 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. ↓ 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). ↑ overall intake during the recovery period (study days 90 to 147, p≤ 0.01). *Females* ↓ 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 **Organ weights** ↓ 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. **Histopathology** ↓ 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) ↓ 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) ↑ 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). ↑ 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). ↑ 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 Rat, Wistar male/female 10/sex/group	Sodium bromide Oral (feed) 0, 75, 300, 1200, 4800, 19200 ppm (corresponding to 0, 6.75, 27, 108, 432, 1728 mg/kg bw/day) 90 days	**Mortality and clinical observations** 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. ↑ clinical signs of neurotoxic effects (motor incoordination of legs) at 19200 ppm (unclear if both males and females) **Body weight and food consumption** ↓ body weight gain (23% in males) at 19200 ppm **Organ weights** ↓ relative prostate weight at 4800 (33%, p≤0.01) and 19200 ppm (50%, p≤0.001)	A6.4.1/04, Doc. No. 592-005 Van Logten, M. J. et al., 1974 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	
		Histopathology	
		(Note: numbers, severity, or incidences not reported in the publication)	
		↓ number of corpora lutea at 19200 ppm	
		↓ size of tubuli at 19200 ppm	
		↑ pituitary cysts (males) at 19200 ppm	
		↓ spermatogenesis (tendency) at 19200 ppm	
		↓ 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 Rat , Wistar male/female 10/sex/group	Sodium bromide Oral (feed) 0, 8, 31, 125, 500 and 2000 ppm (corresponding to 0, 0.72, 2.8, 11, 45, 180 mg/kg bw/day) 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.* **Mortality and clinical observations** 3 males and 3 females at 2000 ppm (low chloride diet) died during the study period ↑ motor incoordination of hind legs (no incidences given and unclear if both males and females) at 2000 ppm (low chlrodie intake) **Body weight and food consumption** ↓ body weight gain after 12 weeks at 2000 ppm (males 31% and females 35%) **Organ weights** ↓ relative (to heart weight) pituitary weight in females at 2000 ppm ↑ relative (to heart weight) adrenal weight in males at 2000 ppm **Histopathology** *(Note: numbers, severity, or incidences not reported in the publication)* ↓ spermatogenesis at 2000 ppm ↓ corpora lutea at 2000 ppm ↓ maturation of uterus at 2000 ppm	A6.4.1/05, Doc. No. 592-006 Van Logten M.J. et al., 1976 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 4 or 12 weeks feeding study, alterations in the endocrine system were investigated 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 males 10/group Oral (diet) Duration: 4 or 12 weeks 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: No treatment related effects. 1200 ppm: ↑ thyroid weight (at 4 weeks) ↓ T4 level (at 4 weeks) ↑ FSH level (at 12 weeks) 19200 ppm: ↓ body weight (9% at 4 weeks; 21% at 12 weeks) ↑ thyroid weight (at 4 and 12 weeks) ↑ incidences of histopathological changes in thyroidea at 4 and 12 weeks (increase of follicles and a decrease in their size) ↑ incidences of histopathological changes in testes at 12 weeks (reduction of tubule diameter) ↓ spermatogenesis (at 12 weeks) Changes in immunoreactivity in pituitary gland: ↓ GH at 4 and 12 weeks, ↑ TSH at 12 weeks, ↑ ACTH at 12 weeks 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 Loeber et al., 1983 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 $\geq$ 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 $\geq$ 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.

<u>P generation – fertility, parturition and sexual function</u>

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≤ 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≤ 0.05 to p≤ 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≤ 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≤ 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≤ 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≤ 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≤ 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≤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] N	24	24	24	22
Number of mated females[b] N/N (%)	24/24 (100.0)	24/24 (100.0)	22/24 (91.7)	10/22 (45.5)
Fertility index [c] N/N (%)	24/24 (100.0)	22/24 (91.7)	16/22 (72.7)**	6/10 (60)**
Number of dams that delivered a litter N (%)	24 (100.0)	22 (100.0)	16 (100.0)	6 (100.0)
Duration of gestation mean±SD	22.8 ± 0.4	22.6 ± 0.5	22.9 ± 0.4	22.8 ± 0.4
Number of dams with stillborn pups N (%)	2 (8.3)	4 (18.2)	0 (0.0)	3 (50.0)**
Number of dams with no liveborn pups N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gestation index [d] N/N (%)	24/24 (100.0)	22/22 (100.0)	16/16 (100.0)	6/6 (100.0)
Dams with all pups dying Days 0-4 post partum N (%)	0 (0.0)	0 (0.0)	0 (0.0)	5 (83.3)**
Second cohabitation (P generation to produce F1b)				
	Control	**50 mg/kg bw/d**	**175 mg/kg bw/d**	**-**
Number of females in cohabitation N	23	24	22	-
Number of mated females N (%)	23 (100.0)	23 (95.8)	19 (86.4)	-
Fertility index N/N (%)	23/23 (100.0)	23/23 (100.0)	14/19 (73.7)**	-
Number of dams that delivered a litter N (%)	22 (100.0) (excludes values for rats that were found dead during gestation)	22 (100.0) (excludes values for rats that were found dead during gestation)	14 (100.0)	-
Duration of gestation mean±SD	22.7 ± 0.6	22.7 ± 0.5	22.8 ± 0.4	-
Number of dams with stillborn pups N (%)	0 (0.0) [e]	4 (18.2)**	0 (0.0)	-
Number of dams with no liveborn pups N (%)	0 (0.0) [e]	0 (0.0)	0 (0.0)	-
Gestation index N/N (%)	21/21 (100) [e]	22/22 (100.0)	14/14 (100.0)	-
Dams with all pups dying Days 0-4 post partum N (%)	0 (0.0) [e]	0 (0.0)	0 (0.0)	-

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

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

* Significantly different from the control group value (p≤ 0.05).
** Significantly different from the control group value (p≤ 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≤ 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≤ 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≤ 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≤ 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.

<u>F1 generation – reproductive organ weights and histopathology</u>

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≤ 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≤ 0.01) and the total count of sperm (p≤ 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 Min-Max
No. Rats per Group	23	22	15	
Vas Deferens				
Number Motile	521.8	504.7	389.2**	481.0 308.4 - 821.1
%	90.4	89.2	88.8	95.6 80.3 - 96.0
Static Count	56.7	65.6	52.8	50.5 15.6 - 101.8
Total Count	578.5	570.3	442.0*	523.5 348.4 – 852.6
Caudal Epididymal				
Sperm Count	323.0	347.7	370.9	281.8 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 27-227
Morphology (epididymal)				
Normal	189.2	190.5	191.7	190.4 168.8-202.1
% Abnormal	5.6	5.5	4.3	4.9 0.5 – 15.8

* Significantly different from the control group value (p≤ 0.05).
** Significantly different from the control group value (p≤ 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≤ 0.01) after week 9, and mean body weights were 87.9% (p≤ 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 adminsterd 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≤ 0.01) in the number of normal sperm, a reduction in the percent motile sperm from the vas deferens (75.3% of control, p≤ 0.05) and increases in

mean non-motile sperm (110.4% of control, $p \leq 0.01$), percent abnormal sperm (11.9%, $p \leq 0.01$) and mean number of sperm with detached head (20.6%, $p \leq 0.01$) or no head (3.2%, $p \leq 0.01$) 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, $p \leq 0.05$) and the percent abnormal sperm was increased (3.0% compared to 0.6% in control, $p \leq 0.01$). 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 / Min - Max
Vas Deferens							
Number Motile	10	386.5	345.1	379.7	375.3	265.8 (9)	481.0 / 308.4-821.1
%	10	91.2	88.4	91.7	87.1	68.7* (9)	95.6 / 80.3- 437.8
Static Count	10	41.0	40.6	37.3	52.7	110.4* (9)	50.5 / 15.6- 101.8
Total Count	10	427	385	417	428	376 (9)	531.1 / 348.4- 852.6
Caudal Epididymal							
Sperm Count	10	270.8	241.0	259.9	219.6	185.6 (9)	299.4 / 230.0-410.5
Sperm Concentration[b]	10	1015	922.3	987.9	872.2	912.0 (9)	1150.6 / 599.6-1713.08
Testicular							
Spermatid Concentration[c]	10	85.0	103.4	76.4	69.3	85.0 (9)	66.8 / 28.4-100.6
Morphology (epididymal)							
Normal	10	198.9	195.9	198.9	194.1	176.2** (9)	190.4 / 168.8-202.1
% Abnormal	10	0.6 [0-1.5]	2.0 [0-6.5]	0.6 [0-1.5]	3.0** [0-7.5]	11.9** (9) [6-21]	4.9 / 0.5-15.8
Detached Head	10	0.8 [0-3]	3.2 [0-9]	1.0 [0-2]	5.0* [0-15]	20.6** (9) [7-40]	6.0 / 1.0-19.4
No Head	10	0.3 [0-1]	0.9 [0-4]	0.1 [0-1]	0.9 [0-3]	3.2** (9) [1-8]	3.2 / 0.1-12.3

( ) = number of values averaged

[a.] Historical Control Data for the vas deferens, caudal epididymal, and testicular generated from 203 studies from 1998 to 2012. Historical Control Data for morphology generated from 54 studies from 1996 to 2012.

[b.] million sperm/mL.

[c.] million spermatid/mL.

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≤ 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≤ 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≤ 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≤ 0.05). At **500 mg/kg/day**, average and relative food consumption in males were significantly reduced (p≤ 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≤0.01 and p ≤0.05) and the percentage of sperm with abnormal morphology in epididymis was increased (7.6% and 21.3%, p≤0.01 and p≤0.05 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 toxicity 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 epididymides (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 OECD TG 414 GLP Rat, Sprague Dawley (Charles River CD) Females 24/group	Ammonium bromide Vehicle: water Oral (gavage) Dose: 0, 100, 300, 1000 mg/kg bw/day Days 6-19 of gestation	*Maternal toxicity* ↑ mortality (one dam was sacrificed on day 10 of gestation due to the severity of clinical signs) at 1000 mg/kg bw/day ↑ maternal clinical signs and neurotoxic effects at 1000 mg/kg bw/day in all animals generally throughout the study ↓ 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 *Offspring* ↓ mean foetal weight (15% less than control, p<0.001) at 1000 mg/kg bw/day ↑ incidence of small foetus at 1000 mg/kg bw/day, foetal incidence: 24% compared to 2% in controls ↑ incidence of skeletal abnormalities/variants: - 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 Study report, 2000b 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	
		- curved scapula at 1000 mg/kg bw/day, foetal incidence: 8.7% compared to 0.5% in controls	
		- slightly kinked ribs at 1000 mg/kg bw/day, foetal incidence: 4% compared to 1% in controls	
		- incomplete ossification of ribs, foetal incidence: 2, 9 and 16% at 100, 300 and 1000 mg/kg, respectively compared to 0% in controls.	
		↑ incidence of visceral abnormalities at 1000 mg/kg bw/day:	
		- 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	
		- narrowing of the left uterine horn with flattened ovarian end and displaced from ovary, foetal incidence: 7% compared to 0% in controls	
		- spleen abnormalities, flattened and/or reduced in size, foetal incidence: 9% compared to 0% in controls	
		- reduced/absent thyroid, foetal incidence: 3.8% compared to 0.5% in controls	
		↑ 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 OECD TG 414 GLP 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 Vehicle: water Oral (gavage) Doses: 0, 50, 300, 600, 800 mg/kg bw/day Days 6-19 of gestation	*No statistical analysis of the data performed in this study* *Prenatal study:* **Maternal toxicity** ↑ mortality (one dam was sacrificed GD 11 due to the severity of clinical signs) at 600 mg/kg bw/day ↑ incidence of maternal clinical signs (neurotoxic effects) at 600 and 800 mg/kg bw/day ↓ maternal bodyweight gain (9%) at 800 mg/kg bw/day **Offspring** ↑ 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, ↑ 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, ↑ incidence of foetuses with 13 complete ribs: 96%, 87% and	A.6.8.1/04, Doc. No. 551-002 Study report, 2007a 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. Rat, Sprague Dawley (Crt:CD(SD)) Females 22/group		76% at 300, 600 and 800 mg/kg bw/day respectively, compared to 92% in controls ↑ 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 *Postnatal study:* ↓ 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 GLP Rat Sprague Dawley 10/sex/group	Ammonium bromide Oral (feed) 0, 1600, 3200, 6400 ppm 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 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.* *Maternal toxicity* There were no premature deaths during the study. ↑ incidences of transient piloerection starting at 228 mg/kg bw/day ↑ incidences of rolling gait (6/10) at 454 mg/kg bw/day ↑ 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 ↑ incidences of hyperactivity in females (approx. 50%) at 651 mg/kg bw/day *Offspring - F1 generation* ↑ 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) ↓ 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 Study report, 2001 Reliability 1
Sodium bromide			
Pre-natal developmental toxicity study OECD TG 414	Sodium bromide Purity 99.84% Vehicle: distilled water	*Maternal toxicity* ↑ mortality (one dam was sacrificed on Day 11 of gestation due to the severity of clinical signs) at 1000 mg/kg bw/day ↑ incidence of maternal clinical signs (neurotoxic effects in all animals) at 1000 mg/kg bw/day	A6.8.1/05, Doc. No. 551-003 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 Females 25/group	Oral (gavage) 0, 100, 300, 1000 mg/kg bw/day 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) ↓ food consumption (9%, days 18-19, p≤0.01) at 1000 mg/kg bw/day ***Offspring*** ↑ 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 ↑ 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. ↑ 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) ↑ 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 Rabbit, New Zealand White Females 6/dosage group and 5 control animals;	Sodium bromide Vehicle: water Oral (gavage) Day 3-28 of gestation 0, 100, 200, 400 mg/kg bw/day Pre-study (non-	*Pre- study:* ↑ incidence of clinical signs (ataxia and decreased respiration) at 500 and 1000 mg/kg bw/day *Pregnant phase of study:* ↑ incidence of clinical signs (ataxia) at 400 mg/kg bw/day No embryotoxic or teratogenic effects recorded	A6.8.1/01, Doc. No. 551-004 Study report, 2008a 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 125, 250, 500, 1000 mg/kg bw/day (escalating dosing regimen) 500 mg/kg bw/day (fixed dose level)		
Pre-natal developmental toxicity study OECD TG 414 Rabbit, New Zealand White Females 30/group	Sodium bromide Vehicle: water Oral (gavage) 0, 25, 75, 250 mg/kg bw/day 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 Study report, 2008b Reliability 1
Two-generation reproductive toxicity study 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) Rat, Crl:CD(SD) 24/sex/group in P	Sodium bromide Purity: 99.5% Oral (gavage) 0, 50, 175, 350/500 (M/F) mg/kg bw/day 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). F1a generation (Subset A, rearing	***Maternal toxicity - P generation females*** <u>Mortalities and clinical observations</u> ↑ 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. ↑ 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. <u>Body weights</u> 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. Gestational phase, first pairing: ↓ body weight at high dose level: 89.2% of control weights (p ≤ 0.05) at GD 20. ↓ body weight gain at high dose level: 72.3% of control (p ≤	Study report, 2016a 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. 23/sex in control group, 23/24 (M/F) in low dose group, 23/22 (M/F) in intermediate dose group in in P generation mating to produce F1b. 23/sex in control group, 22/sex in low dose group, 15/sex in intermediate dose group in F1a generation mating to produce F2.	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. 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. Lactational phase, first pairing: ↓ body weight at high dose level: 89.1% and 86.7% of control weights (p ≤ 0.05, p ≤ 0.01) at lactation day 7 and 21, respectively. ↓ overall body weight gain lactation day 0-21 at high dose level: only 1% of control gains (p ≤ 0.05). <u>Food consumption</u> ↓ 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). *Maternal toxicity – F1 generation females* <u>Mortalities and clinical observations</u> 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. <u>Body weights</u> *Females:* No stat. sign effects on body weight or body weigh gain during premating phase and mating phase. Gestational phase: ↓ body weight (p≤ 0.05) on GD 7 (-7%) and 10 (-6%) in the 175 mg/kg/day dose group ↓ body weight gain (-20% compared to control group, p≤ 0.05) GDs 0 to 7 Lactational phase: ↓ body weight (p≤ 0.01) on LDs 0 (-8%), 4 (-8%), 7 (-8%) and 14 (-6%) in the 175 mg/kg/day dose group <u>Food consumption</u> *Females:* ↓ 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). *Offspring* **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). ↓ viability index PND 0-4 in 500 mg/kg bw/day dose group, 0% compared to 96.6% in control (p≤ 0.01). ↑ 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. **F1b** ↓ live birth index in 50 mg/kg bw/day dose group (97.5% compared to 100% in control, p≤ 0.01). ↑ 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. 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. **F1b** (post weaning to PND 40) 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. **F2** ↓ 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 Rat (strain not specified) Male/female	Sodium bromide Oral (feed) 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)	*Maternal toxicity* No adverse effects on body weights recorded. No information on clinical condition available. *Offspring* ↓ 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) ↓ 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 Van Leeuwen et al., 1983 Reliability 2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results							Reference

	0 ppm	75 ppm	300 ppm	1200 ppm	4800 ppm	19200 ppm
Viability index**						
F0	90	98	96	92	32/61¥	-
F1	92	88	80	97	-	-
F2	96	98	93	98	-	-
Lactation index***						
F0	95	96	95	94	0/100¥	-
F1	93	85	72	80	-	-
F2	99	99	99	99	-	-

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

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

¥ data are given separately for first and second litter

- no breeding

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) Rat, Wistar Male and female 16-20 offspring	Sodium bromide Vehicle: water	Oral (gavage) 0, 40, 80, 120 mg/kg bw/day Offspring to dams exposed day 3 to day 20 of gestation Postexposure period: 85 days after pups were born	*Maternal toxicity* No information available *Offspring* At 40 mg/kg bw/day: ↑ pup mortality (pups died before 20 days of age: 27%, compared to 2.3% in controls)	A6.9.2/02, Doc. No. 592-017 Harned et al., 1944

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

<u>Adverse effects on the offspring</u>

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%, $p \le 0.01$ 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.

<u>Maternal toxicity</u>

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.

<u>Conclusion</u>

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.

<u>Maternal toxicity</u>

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

<u>Adverse effects on the offspring</u>

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.

<u>Adverse effects on the offspring</u>

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≤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≤ 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≤ 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≤ 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. Bromide transfer through mother's milk and its impact	Sodium bromide Potassium bromide Vehicle: distilled water	*Maternal toxicity* ↑ mortality at 300 mg bromide/kg bw/day (one dam) and at 900 mg bromide/kg bw/day (one dam) ↓ maternal mean bodyweight (18%) at 900 mg bromide/kg bw/day ↓ food consumption at 900 mg bromide/kg bw/day	A6.8.1/07, Doc. No. 592-015 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 Exp. 1: bw of each dam and the corresponding litter and the consumption of diet and drinking water were recorded Exp. 2: determination of production rate of mother´s milk and its elemental composition Exp. 3: measurement of bromide transfer through mother´s milk Rat, Wistar 5 dams/group 8 pups/dam	Exp. 1 and 2: 1 or 5 g bromide/L (corresponding to 300 and 900 mg bromide/kg bw/day) Oral (drinking water) Whole lactation period Exp. 3: 1 or 5 g bromide/L (corresponding to 300 and 900 mg bromide/kg bw/day) Single exposure day 12 of lactation	↓ drinking water at 900 mg bromide/kg bw/day No clinical observations were reported for any dose group. *Effects on or via lactation* ↓ milk production -elemental composition of milk changed (about 54% of the chloride in mother´s milk was replaced by bromide) *Offspring toxicity* ↓ 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) ↓ mean bodyweight in pups (65%) at 900 mg bromide/kg bw/day	and Babický A. 2005 Reliability 2
No guideline study, no GLP. Determination of - the production rate of milk in the dams on lacation day 10 an 15. - iodine transfer to sucklings from the mothers on lactation day	Testmaterial unknown, only 'bromide' stated Vehicle: distilled water Oral (drinking water) Group A: 0, 1, 5 g bromide/L during the whole lactation period or Group B: 1 g	*Maternal toxicity* No significant differences in the body weight among the lactating dams. No examination of clinical signs during the study. *Effetcs on or via lactation* ↓ milk production rate on days 10 and 15 (-67% and -70% respectively at at 5 g bromide/L, unclear if stat. sign.) *Offspring toxicity* ↓ (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) ↑ (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 Pavelka et al., 2002. Reliability 2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
16. - thyroid hormones in sucklings and mothers. Rat, Wistar 5-10 dams/group 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 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).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

One dose-range finding reproductive toxicity study with ammonium bromide and two-generation studies with sodium bromide (one two-generation and one three-generation reproductive toxicity study) in rats were available for the DS. In addition, data from oral 90-day repeated dose toxicity studies with sodium bromide and ammonium bromide and one four-week dose-range finding study with ammonium bromide were used.

Significant alterations to the male and female reproductive system, gamete production and transport as well as clear evidence of effects on impaired male and female fertility were reported in these studies.

For females, a decreased number of corpora lutea was reported in the sub-chronic and the two-generation reproductive toxicity studies with sodium bromide. In the P females of the two-generation study, depletion of corpora lutea was observed in the ovary of 10/24 at 500 mg/kg bw/day in presence of excessive toxicity, and in 3/24 females at 175 mg/kg bw/day in absence of general toxicity. Depletion of corpora lutea in absence of severe general toxicity was also reported in 3/10 females at 500 mg/kg bw/day in a 90-day repeat dose toxicity study with sodium bromide. Further, a decreased number of corpora lutea in the ovaries of rats was found in a non-guideline 90-day repeated dose toxicity study of sodium bromide, 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 DS considered them as supportive evidence for classification.

In males, decreased reproductive organ weights, histopathological changes, and adverse effects on sperm count, morphology, and motility were reported. All P males at 350 mg/kg bw/day of the two-generation reproductive toxicity study showed minimal to moderate cellular debris in the epididymis and/or spermatid head retention in the testes. Similar, although mostly minimal changes were seen in 11/23 males at 175 mg/kg bw/day. Compared to control, lower count of motile sperm in vas deferens and increased percentage of sperm with abnormal morphology in epididymis was observed in both mid and high dose groups. The total sperm count and the number of motile sperm in vas deferens was also lower in F1 males at 175 mg/kg bw/day. In the 90-day repeated dose toxicity study with sodium bromide, retained spermatids in testes were seen in 2/10 and in 9/9 male rats at 175 and 500 mg/kg bw/day, respectively. Related findings such as minimal to moderate spermatid retention in the seminiferous tubule epithelium and in Sertoli cells were found during examination of four early deaths in the 500 mg/kg bw/day dose groups. Reduction in the number of normal sperm (88.6% of control, p ≤ 0.01) and in the percent motile sperm from vas deferens (75.3% of control, p ≤ 0.05) was reported at 500 mg/kg bw/day. At high and mid dose, the mean numbers of sperm with detached head or no head were increased compared to the control.

The DS noted that the adverse histopathological changes in the gonads observed in the high dose groups of both studies occurred in the presence of severe general toxicity, and thus were only used as supportive information in the weight of evidence approach. Several non-guideline toxicity studies with sodium bromide in the rat were considered to provide further support for classification. Effects on testes (decreased spermatogenesis, reduction of tubules, decreased serum testosterone) and epididymis (reduced weight) were reported at very high doses (Van Logten et al., 1974; Loeber et al., 1983). Reduced weight of testes and epididymis without correlated histopathology were also seen in the 90-day repeated dose toxicity study on ammonium bromide (Study report, 2000a) and the dose range finding reproductive toxicity study on ammonium bromide (Study report, 2001) at ≥ 100 mg/kg bw/day.

Some indications of fewer oestrous stages were seen in P females at the top dose of the two-generation study with sodium bromide, however accompanied with severe general toxicity. Statistically reduced average number of oestrous stages in the absence of significant toxicity was observed in F1 generation at 175 mg/kg/day, however without effect on mating or pregnancy rate. Impaired mating performance was observed at

350/500 mg/kg bw/day of the above study and at 503/651 mg/kg bw/day in the dose-range finding reproductive study with ammonium bromide. The decreased mating index was considered due to the neurotoxic effects observed in high dose animals.

Clear signs of impaired fertility were noted in the rat studies with ammonium bromide and sodium bromide. The dose-range finding study on ammonium bromide reported slightly (80/90% of control, m/f) and markedly (10%) reduced fertility indices at 242/454 and 503/651 mg/kg bw/day, respectively. This effect was considered not to be a consequence of the observed neurotoxicity at the high dose. In the two-generation reproductive toxicity study with sodium bromide, fertility was significantly reduced at 175 mg/kg bw/day in absence of severe general toxicity, and severely reduced at 500 mg/kg bw/day in presence of excessive general toxicity (mortality, adverse clinical signs and effects on body weights). In the three-generation reproductive toxicity study of sodium bromide, impaired reproductive capacity was reported for males and females of all three-generations. Results from cross-mating indicated that the observed effects were due to infertility of males as well as females, and there were indications of some recovery from the effects on fertility. The DS noted that the fertility index in the controls was unusually low (70, 62 and 52% in P, F1 and F2, respectively), and that the lack of clinical observations for any of the animals and the lack of body weight recordings at the top dose may question the quality of the study and its relevance for classification.

In a weight of evidence approach, the DS considered that the available data provided clear evidence of an adverse effect on both male and female sexual function and fertility. Since there was no mechanistic information indicating that the observed effects were not relevant for humans, classification as Repr. 1B, H360F was proposed.

Developmental toxicity

Information from two pre-natal developmental toxicity studies (OECD TG 414) on ammonium bromide in rat, two pre-natal developmental toxicity studies (OECD TG 414) on sodium bromide in rabbit and rat, and one dose range finding study on sodium bromide in rabbit were provided. In addition, results from one dose-range finding study for reproductive toxicity of ammonium bromide in rat, a two-generation reproductive toxicity study (OECD TG 416) and one non-guideline multi-generation reproductive toxicity study on sodium bromide in rat were available. Two developmental neurotoxicity studies on sodium bromide in rat and several case reports of infants exposed to bromide during the pregnancy were considered as well.

A dose-related increase in the incidence of displaced testis was seen in a prenatal developmental study in rats treated with ammonium bromide up to 1000 mg/kg bw/day (Study report, 2000b). Clinical signs of neurotoxicity and reduced body weight gain were reported only at the top dose level, where also major visceral malformations (left kidney, left uterine horn, spleen, thyroid) and skeletal abnormalities/variants (reduction in size of the 13th ribs) were observed. At lower doses (100 and 300 mg/kg bw/day), skeletal abnormalities/variants and incomplete ossification of ribs were seen in the absence of maternal toxicity. Similar findings were reported in another prenatal developmental toxicity study with rats exposed to ammonium bromide at doses up to 800 mg/kg bw/day (Study report, 2007a). Visceral (kidney, ureter, uterine horn) and skeletal (ribs) malformations were seen also in the pre-natal developmental study on sodium bromide at 1000 mg/kg bw/day in the presence of maternal toxicity (Study report, 1995). Skeletal anomalies/variants (ribs, sternebrae, cranial centres) occurred at lower doses (300

mg/kg bw/day) in absence of maternal toxicity. No teratogenic effects were noted in two pre-natal developmental studies with rabbits administered sodium bromide at dose levels up to 400 mg/kg bw/day (Study report, 2008a and 2008b).

Adverse effects on pup viability and survival were seen in the range finding reproductive toxicity study on ammonium bromide at doses of 454 and 651 mg/kg bw/day in the presence of severe maternal toxicity (Study report, 2001). The single litter produced at high dose did not survive to day 4 of lactation, and 4/9 litters in the mid dose group died before day 21. Pup viability was also markedly decreased at 500 mg/kg bw/day in the two-generation reproduction toxicity study on sodium bromide in rat, however in the presence of excessive maternal mortality (Study report, 2016a). No effects on litter size and pup viability were reported at lower doses (175 mg/kg bw/day). In the three-generation reproduction toxicity study (Van Leeuwen *et al*., 1983), viability of pups was significantly reduced at 432 mg/kg bw/day for the first litter (32%) and increased in the second litter (61%). A dose-dependent increase in pup mortality was also reported at 40-120 mg/kg bw/day in a pre-natal developmental toxicity study by Harned *et al*. (1944).

Considering all the data in a weight of evidence approach, the DS proposed classification as Repr. 1B, H360D based on clear evidence of structural abnormalities and some evidence of pup mortality and retarded growth derived from the above studies. The effects were considered of high concern, dose related and evident also at dose levels where there was no severe maternal toxicity.

Adverse effects on or via lactation

Classification proposal for effects on or via lactation was based on results from a non-guideline study with sodium bromide or potassium bromide showing transfer of bromide to the pups via mother's milk. Wistar rats (5 dams/group, 8 pups/dam) were orally exposed to doses of 300 and 900 mg bromide/kg bw/day during the whole lactation period, or only once on day 12 of lactation (Vobecký, 2005). Milk production in dams of the high and low dose was significantly reduced at lactation days 10 and 15. Analysis of mother's milk composition showed that about 54% of its chloride content was replaced by bromide. Decreased pup survival was observed at low (94.8%) and high dose (56.3%) compared to 100% in control. Mean body weight of pups at high dose was less than 40% of control, and their general condition was described as very poor. After single administration of radiolabelled potassium bromide on day 12 of lactation, approximately 10% and 17% of the applied dose was found in the offspring at 3 and 25 hours post dosing, respectively. Clinical observations were not available from the study, however mortality of one dam in each group was reported. DS concluded that the reduced body weight and survival of the pups in the high dose group was likely a consequence of reduced milk production in the dams. Reduced milk production on days 10 and 15 (-67% and -70%, respectively) and decreased body weight in pups (ca. -30%) were also reported in an earlier study by Pavelka *et al*. (2002), where dams were given 1 or 5 g bromide/L during lactation. Apart from some indications of reduced levels of T3 and T4 hormones in both dams and pups, a decreased transfer of iodine through mother's milk was observed, possibly due to an enhanced intake by the dam.

Several human case reports provided also some weak indications on possible effects on the central nervous system in infants following maternal intake of bromide during lactation.

In an overall assessment of the available evidence, DS concluded that bromide may cause harm due to its effects on and via lactation, and additional category for effects on or via lactation with a hazard statement H362 "May cause harm to breast-fed children" was therefore proposed.

Specific concentration limits for reproductive toxicity and adverse effects on or via lactation were not proposed by DS since the estimated ED_{10} values were within the medium potency group (4 mg/kg bw/day < ED_{10} < 400 mg/kg bw/day).

Comments received during consultation

Comments on the classification proposal for reproductive toxicity were provided by 1 MSCA and one industry organisation.

In their comments, the MSCA supported the proposed classification as Repr. 1B for both sexual function and fertility, and for developmental toxicity (H360FD), but disagreed with the classification proposal for adverse effects on or via lactation (H362) pointing out that the effects observed during lactation were more likely secondary to maternal effects such as poor maternal care because of the observed clinical signs of neurotoxicity. With respect to adverse effects on sexual function and fertility, the MSCA discussed thyroid dysfunction as one plausible mode of action for the observed effects. The MSCA concluded that while the exact mechanism of bromide action was unresolved and a direct action on the reproductive organs could not be excluded, thyroid dysfunction was one of the possible explanations for these effects.

The industry association provided detailed comments on all reproductive toxicity studies disagreeing with the interpretation and some of the conclusions of the DS leading to the proposed classification. These concerned mainly the assessment of maternal toxicity and its impact on the study outcome, the quality of the studies and their weight of evidence for the overall assessment, as well as the use of historical control data. In addition, industry provided data on bromide levels from employees working in different workplaces demonstrating that over a 5-year period (2013-2017) the measured mean annual bromide serum levels of 0.042-0.050 mmol/L were within the range reported for the general population (0.04-0.09 mmol/L).

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

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

In a dose-range finding study for reproductive toxicity (Study report, 2001), SD rats (10/sex/group) were treated with ammonium bromide via food at concentrations of 0, 1600, 3200 and 6400 ppm (corresponding to 0, 127/228, 242/454 and 503/651 mg/kg bw/day for males/females, respectively), from two weeks prior to mating until weaning. The study is GLP-compliant, but no specific test guideline was followed. Oestrous cyclicity and sperm parameters were not examined, and statistical analysis was not performed due to the small group size.

In males, body weight gain was reduced at 3200 ppm (13%, week 0-8) and 6400 ppm

(16%, only for the first week of treatment). Food consumption was also lower at ≥ 3200 ppm. In females, body weight gain was reduced by 33% only during gestation at 6400 ppm. However, there was only one animal in this group due to the poor pregnancy rate. Clinical signs such as rolling gait (all animals), piloerection, unkempt coat and hunched posture were noted at 6400 ppm at the beginning and throughout the treatment period. Approximately half of the females in this dose group showed hyperactivity. The clinical effects observed at 3200 ppm were the same (except for unkempt coat), but of lower severity. First signs of rolling gait were observed around the fifth week of treatment and persisted throughout the treatment. At 1600 ppm, transient piloerection was noted in three animals only.

Mating performance was reduced at ≥ 3200 ppm, and fertility index was 80% in males and 90% in females at 3200 ppm, and 10% in both sexes at 6400 ppm. 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 (Table below).

Table: *Effects reported in Study report (2001)*

Dose level [ppm]	0	1600	3200	6400
F0 males				
Clinical observation				
Rolling gait	0/10	0/10	9/10	10/10
Piloerection	1/10	0/10	2/10	2/10
Hunched posture	0/10	0/10	0/10	1/10
Unkempt coat	0/10	0/10	0/10	6/10
Staining of coat (head/nasal/dorsal/abdominal)	1/10	2/10	5/10	8/10
Staining around eye(s)	0/10	0/10	3/10	9/10
Food consumption			↓	↓
Body weight at week 8		↑(4%)	↓(7%)	↓(7%)
Reproductive performance				
Fertility index	100%	90%	80%	10%
F0 females				
Clinical observation (No premature deaths)				
Rolling gait	0/10	0/10	6/10	10/10
Piloerection	2/10	3/10	2/10	5/10
Hunched posture	0/10	0/10	3/10	8/10
Unkempt coat	0/10	0/10	0/10	8/10

Staining of coat (head/nasal/dorsal/abdominal)	4/10	5/10	6/10	8/10
Staining around eye(s)	0/10	0/10	0/10	4/10
Hyperactive behaviour	0/10	0/10	0/10	4/10
Food consumption during lactation		Slight effect		
Body weight		No effect		
Reproductive performance				
Mating index	100%	100%	90%	70%
Fertility index	100%	100%	90%	10%
Duration of gestation (days)	21.6	21.4	22.1	22.0

Mating index: % of pairing that resulted in mating; Fertility index: % of matings that resulted in pregnancies.

During the general consultation, the Industry representative disagreed with the DS assessment of parental toxicity and questioned the suitability of the study for classification due to lack of specific test guideline compliance and small group size preventing proper statistical analysis. RAC agrees with the DS, that the slightly reduced (mid dose) and markedly decreased (high dose) fertility index cannot be considered as being secondary to general toxicity. Effects on body weight and body weight gain were not severe, but clinical signs of neurotoxicity (including rolling gait) persisted in high dose group throughout the treatment period. Thus, it can be expected that mating performance in the high dose group was affected, however, impact on fertility is considered by RAC rather unlikely. As discussed by DS, hyperactive behaviour was noted in 4/10 females and 10/10 females showed rolling gait (weeks 2-11). Nevertheless, 7/10 females had signs of mating from which only one became pregnant. At 3200 ppm, rolling gait was reported in 6/10 females and only one was not pregnant. In the same group, 9 out of 10 males demonstrated rolling gait, but only two did not sire. Consequently, the inability to conceive cannot be explained as secondary to the observed clinical signs. The effects on fertility are therefore considered by RAC as being treatment related and not secondary to parental neurotoxicity.

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

An oral (feed) repeated dose toxicity study (OECD TG 408) on ammonium bromide in rat is relevant for the assessment of reproductive toxicity (Study report, 2000a). In the study, 25 SD rats/sex/group (control and high doses) and 15 SD rats/sex/group (low and intermediate dose) were exposed to ammonium bromide for 90 days. Dose levels were 100 and 225 mg/kg bw/day for both sexes, and 500/750 mg/kg bw/day for males/females at the top dose. In males, body weight was reduced on day 91 of treatment by 10% at 225 mg/kg bw/day ($p \leq 0.01$) and by 22% at 500 mg/kg bw/day ($p \leq 0.001$) as compared to control. Food consumption was lower at 500 mg/kg bw/day (7%), and reduced body weight gain in males was noted at ≥ 225 mg/kg bw/day. Clinical

signs of neurotoxicity (subdued behaviour, abnormalities of gait) were noted at ≥ 225 (males) and 750 mg/kg bw/day (females). Hunched posture, unkempt coat and claws that were longer than normal were also observed. Three premature terminations among the males in the 500 mg/kg bw/day group were rated as unrelated to treatment.

In males, dose-related decreases in the absolute weights of epididymis

- 100 mg/kg bw/day   -10%, $p \leq 0.05$

- 225 mg/kg bw/day   -12%, $p \leq 0.01$

- 500 mg/kg bw/day   -22%, $p \leq 0.001$

and testes

- 225 mg/kg bw/day   -10%, $p \leq 0.05$

- 500 mg/kg bw/day   -16%, $p \leq 0.001$

were reported at the end of the treatment period, without corresponding histopathology.

When adjusted to body weight, these changes were not statistically different from control at the end of exposure period. Epididymis weights (absolute and adjusted) were significantly lower than control at the end of the 4-week recovery period at 500 mg/kg bw/day. Absolute prostate and testes weights were also reduced during recovery, although without statistical significance. Assessment of these organ weight changes is complicated by the marked effect on body weight. At 500 mg/kg bw/day, there was a slight increase in body weight gain to day 6; from day 6-13, gain was similar to control, but by day 28 mean weights at this level were lower than control, with differences from day 35 attaining statistical significance (-23% on day 91 of treatment). During the 4-week recovery period, there was an increase in weight gain compared to control, although absolute weights remained significantly lower. Overall, epididymis weight was reduced at the end of exposure and throughout the recovery period. Thus, reduced epididymis weights in the high dose group are considered by RAC as treatment-related and not a consequence of lower body weights.

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

Reduced absolute epididymis and testes weights were also reported in a preceding dose-range finding study for the above 90-day repeated dose toxicity test (Study report, 1999). SD rats (5/sex/group) were exposed for 4 weeks to ammonium bromide via the oral route (feed) at dose levels of 0, 100, 500, 1000 mg/kg bw/day. In high dose males, mean body weight (26% lower than in control on day 28) and body weight gain during days 1-28 (49% lower than in control) was significantly decreased as compared to control ($p<0.001$). No histopathological examination was performed.

Decreased absolute weight of epididymis compared to control was observed at doses of:

- 500 mg/kg bw/day   -11%, $p<0.05$

- 1000 mg/kg bw/day -16%, $p<0.01$

and in absolute testes weight at doses of:

- 100 mg/kg bw/day   -11%, $p<0.05$

- 500 mg/kg bw/day   -11%, $p<0.05$

- 1000 mg/kg bw/day -16%, $p<0.01$

Male reproductive organs at 100 and 500 mg/kg bw/day were affected without marked body weight changes. At 1000 mg/kg bw/day, body weight and body weight changes were severely reduced. Due to the decreased mean body weight of the intermediate and high dose males, these effects were not statistically significant after analysis of covariance. These findings are of unclear toxicological significance because only the absolute weights were affected (see above), and a correlation with potential histopathological effects was not possible.

Two-generation reproductive toxicity study on sodium bromide
(Study report 2016):

In a two-generation reproduction study (similar to OECD TG 416) sodium bromide was administered via oral gavage to Crl:CD(SD) rats at dose levels of 0, 50, 175, 350/500 (male/female) mg/kg bw/day. The treatment started 10 weeks premating, and due to reduced pregnancy rate in the mid and high dose groups, a second cohabitation was conducted for all but the high dose group. Males that did not mate during the first 10 days were re-paired with untreated female rats (selected from retained spare females) for 7 days. Females that did not mate during the first 14 days of cohabitation were re-paired with untreated males and remained in cohabitation for up to 10 additional days. The offspring from the first pairing formed the F1a generation, which was dosed from day 21 postpartum and was selected for production of the F2a litters. The offspring from the second pairing formed the F1b generation and was terminated at day 40 postpartum. Pups in the high dose groups (350/500 mg/kg bw/day, male/female) were terminated at the end of the P generation owing to poor condition in parental animals and low viability of the F1a pups. Thus, exposure duration for P generation was approximately 183 days; male and female rats, selected from the F1a litters, were exposed in utero, via lactation, and via oral gavage after weaning at 0, 50 or 175 mg/kg/day for approximately 131 days.

P generation – general toxicity

Severe toxicity was reported in both males and females at 350/500 mg/kg bw/day. Clinical observations in this dose group included dehydration, ungroomed coat, chromodacryorrhea, hunched posture, ptosis, urine-stained abdominal fur, decreased motor activity, chromorhinorrhea, ataxia, piloerection, low carriage, thin body condition, and bradypnea (Table below). Adversity was more severe in males, and 4 males and 9 females died or were terminated earlier resulting in mortality rates of > 10%. According to the OECD TG 416 and CLP Annex I (3.7.2.4.4), such general toxicity is considered as excessive and results from this dose group would normally not be acceptable for further evaluation. Reduced body weight gain and food consumption was observed in both males and females at different stages.

In the 175 mg/kg bw/day dose group, similar clinical signs of lesser severity and lower incidence were reported (not statistically significantly different from control). Food intake and terminal body weight (86.8% of control, $p \leq 0.01$) were reduced in males only. Two out of 24 females died during gestation and lactation, and food intake was reduced only

during the early lactation period. No adverse effect on body weight gain or food intake in males or females was reported at 50 mg/kg bw/day.

Table: General toxicity in males and females of P generation

Dose [mg/kg bw/d]	0	50	175	350/500 (m/f)
P males				
Mortality	0/24	1/24	1/24	4/24 (17%)
Clinical observation during pre-mating				
Chromodacryorrhea		2/24	5/24	9/24**
Urine-stained abdominal fur		1/24	3/24	15/24**
Piloerection		1/24		7/24**
Dehydration			6/24 (mild)	24/24** (11 moderate)
Ungroomed coat			6/24	21/24**
Hunched posture			2/24	19/24**
Ptosis			2/24	19/24**
Chromorhinorrhea			2/24	10/24**
Decreased motor activity				12/24**
Ataxia				8/24**
Low carriage				5/24**
Thin body condition				4/24**
Bradypnea				3/24**
Food consumption			↓11%**	↓17%**
Body weight SD 183			↓13%**	↓25%**
P females				
Mortalitiy	2/24	1/24	2/24	9/24
Clinical observation during pre-mating				
Chromodacryorrhea				6/24**
Dehydration				6/24** (mild)
Ungroomed coat				3/24**

Clinical observation during gestation				
Chromodacryorrhea				2/10**
Urine-stained abdominal fur				4/10**
Dehydration				9/10**
Ungroomed coat				2/10**
Hunched posture				3/10**
Ptosis				2/10**
Chromorhinorrhea				3/10**
Ataxia				2/10**
Thin body condition				3/10**
Clinical observation during lactation				
Dehydration				5/6**
Ungroomed coat				4/6**
Hunched posture				4/6**
Decreased motor activity				1/6**
Ataxia				1/6**
Whole body: pale				1/6**
Food consumption				
Pre-mating day 1-71		Not affected by treatment		
Gestation day 0-20		Not affected by treatment		
Lactation day 1-14			↓ (14%)*	↓ (64%)**
Body weight				
Body weight pre-mating day 71 (last value before mating)		Not affected by treatment		
Body weight GD 20				↓ (11%)**
Body weight PND 21				↓ (13%)**

Excluded are female rats that did not have a confirmed mating with treated male rat and therefore were paired with untreated male rats. Significant difference between control and treated group *p ≤ 0.05, **p ≤ 0.01.

P generation – fertility, parturition and sexual function

In the first cohabitation period, there was no effect 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 ≤ 0.05).

In high dose males, the mating index was 89.5% with all females, and 42.1% with treated females. The fertility index was 64.7% with all females and 62.5% with treated females, both significantly lower than control values (p ≤ 0.01-0.05, Tables 40 and 41 from the CLP report). In high dose females, the fertility index was 60% (6/10) for females mated with treated males (p ≤ 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.

Table: Male and female reproductive performance

Dose [mg/kg bw/d]	0	50	175	350/500 (m/f)	
Reproductive performance (P males)					**HCD**
Mating index to produce F1a (%)	95.8	100	91.7%	42.1	75-100
Mating index to produce F1b (%)	100	95.6	86.4	-	75-100
Fertility index to produce F1a (%)	100	91.3	72.7*	62.5*	75-100
Fertility index to produce F1b (%)	100	100	73.7**	-	75-100
Reproductive performance (P females)					
Females to produce F1a					
Mating index (%)	100 24/24	100 24/24	91.7 22/24	45.5 10/22	75-100
Fertility index (%)	100	91.7	72.7*	60**	76-100
Duration of gestation (days)	22.8	22.6	22.9	22.8	n.a.
Number of dams that delivered a litter	24/24	22/24	16/22	6/10	n.a.
Number of dams with stillborn pups	2/24 (8.3%)	4/22 (18.2%)	0/16	3/6 (50%)	n.a.

Dams with all pups dying PND0-4	0/24	0/22	0/16	5/6 (83.3%)	n.a.
Females to produce F1b					
Mating index (%)	100	95.8	86.4	-	n.a.
Fertility index (%)	100	100	73.7**	-	n.a.
Duration of gestation (days)	22.7	22.7	22.8	-	n.a.
Number of dams that delivered a litter	22/23	22/23	14/19	-	n.a.
Number of dams with stillborn pups	0/22	4/22 (18.2%)**	0/14	-	n.a.
Dams with all pups dying PND 0-4	0/22	0/22	0/14	-	n.a.

* Significantly different from control value (p ≤ 0.05); ** Significantly different from control value (p ≤ 0.01). Mating index: % of pairing that resulted in mating; Fertility index: % of matings that resulted in pregnancies; - animals were not re-paired for a second cohabitation; n.a. not available.

Results from cross-mating with untreated animals indicate that mating performance and fertility are decreased irrespective if males or females are treated. The higher fertility index when treated females are paired with untreated males compared to treated males paired with untreated females (90% versus 60%) suggests that males may be more severely affected.

No effects on the oestrous cycles at 50 or 175 mg/kg bw/day were reported. In the 500 mg/kg bw/day dose group, the number of oestrous stages per 14-day assessment period was significantly reduced (2.3 versus 3.2 in control, p ≤ 0.01).

In the second cohabitation period, there was no effect on male or female mating performance or fertility at 50 mg/kg bw/day. At 175 mg/kg bw/day, the difference in mating index (86.4% compared to 100% in control) was not statistically significant, however, the fertility index was significantly lower (73.7%) than in controls (100%, p ≤ 0.01). According to the study authors, both parameters were within the historical control range of 75-100%. RAC notes that the fertility index of 72.7% (first cohabitation) and 73.7% (second cohabitation) are still outside the HCD. RAC further agrees with the DS that the confidence in the HCD data is low since its collection period spans 8 years (2008-2016) instead of the recommended +/-2 years, and no detailed information on the source data is available (i.e., median value and variation, laboratory, strain etc.). It is further noted that the effect in the second pairing is in line with the observations at the same dose level in the first pairing. Due to declining clinical condition, poor reproductive performance and a marked effect on pup viability, animals treated at 350/500 mg/kg bw/day were not re-paired for a second cohabitation and they were terminated at the end of the P generation.

Gestation index and duration of gestation was not affected at any dose level compared to control in either the first or the second cohabitation.

P generation – reproductive organ weights and histopathology

Absolute or relative weights of the female reproductive organs to the terminal body/brain weight were not affected at 50 or 175 mg/kg bw/day of sodium bromide. At 500 mg/kg bw/day, an increase in pituitary weight and a decrease ovary weight was noted, expressed both as absolute and relative to body/brain values. In males, the absolute weights of all examined reproductive organs were reduced at 175 and 500 mg/kg bw/day. According to the study author, these changes reflected the reduced terminal body weights (86.3% and 75% of control body weight, respectively). The relative to terminal body weight values were comparable or higher than control, while relative to brain weight values remained lower than control ($p \leq 0.05$ to $p \leq 0.01$).

Depletion of corpora lutea was present in the ovaries of 3 females at 175 mg/kg bw/day and 10 females at 500 mg/kg bw/day sodium bromide surviving to terminal kill. In its written comments, Industry representative argued that *"Determination of the number of corpora lutea was variable and depends on the oestrous stage at termination. Ovarian follicle counts seem to be a more reliable endpoint. We recommend to not draw conclusions on any possible substance-related effect from these variable counts of corpora lutea, as variation in numbers is expected within a normal cycle (Yoshida, 2009)".* In response, the DS agreed that in line with OECD GD 43 "*multiple litters produced by a single dam will compromise corpora lutea count; in dams after multiple pregnancies, corpora lutea counts are not likely to be reliable, since corpora lutea remnants from previous pregnancies may be included in the count.*" However, DS noted that due to the design of this study with two cohabitations, the number of corpora lutea may have been an overestimation possibly leading to underestimation of toxicity to the female gonads. The DS noted further that similar findings were observed in a recent 90-day dose toxicity study on sodium bromide (Study report, 2016b) where 3/20 females treated at 500 mg/kg bw/day had no corpora lutea present. The number of corpora lutea per female was also reduced at 19200 ppm sodium bromide/kg diet (in excess of 1000 mg/kg body weight/day, Van Logten *et al*. 1974). Thus, since depletion of corpora lutea was seen in more than one study, the DS considered this effect as treatment related, and RAC agrees with this assessment.

Table: *Selected reproductive organ weights and histopathology in male and female P generation rats*

Dose [mg/kg bw/d]	0	50	175	350/500 (m/f)
Males				
Reproductive organs weights			↓abs. weights reflected ↓terminal body weights and is considered not adverse	
Histopathology				
Spermatid retention in Sertoli cells			11/23	20/20
Tubular spermatid			9/23	17/20

retention				
Debris in the epididymis			4/23 (minimal)	19/20 (mild to moderate)
Sperm evaluation parameters				
[%] Motile sperm in vas deferens (HCD)	92.3 (80.3-96.0)	91.7	89.2*	80.7**
Static count in vas deferens (HCD)	43.4 (15.5-101.8)	45.3	63.0	111.2**
Morphology (epididymal)				
% abnormal (HCD)	4.0 (0.5-15.8)	5.1	7.6**	21.3**
Detached head (HCD)	4.4 (1.0-19.4)	6.0	7.3*	23.7**
No head (HCD)	3.2 (0.1-12.3)	3.0	5.4	17.8**
Organ weights (females)				
Ovary (rel.)				↓ 33%**
Pituitary (rel.)				↑ 34%**
Histopathology				
Depletion of corpora lutea			3/22 (marked)	10/15 (1 mild, 9 marked)
Number of primordial follicles		Not significantly different to control		

* Significantly different from control value (p ≤ 0.05); ** Significantly different from control value (p ≤ 0.01). HCD generated from 65 studies from 1998 to 2013.

A dose-related increase in the incidence and severity of microscopic findings was noted in the reproductive tract of males treated at 175 or 350 mg/kg bw/day sodium bromide. All males (20) at 350 mg/kg bw/day showed retained spermatid heads of minimal to moderate severity, and 19/20 showed associated cellular debris in the epididymis. These findings were also observed in males which died or were killed in week 12. At 175 mg/kg bw/day, 11/23 males were affected, with the majority showing only minimal changes and only 4 showing epididymal debris.

Effects on sperm parameters were noted in males of the mid and high dose groups (Table 43 in CLP report). At 350 mg/kg bw/day, the percentage of motile sperm in 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, $p \leq 0.01$). At 175 mg/kg bw/day, the percentage of motile sperm in the vas deferens was also significantly reduced (89.2%, $p \leq 0.05$).

F1 generation – general toxicity

Only minimal, sporadic and transient clinical observations were reported in the F1 generation. No adverse effect on body weight gain or food intake was reported at 50 mg/kg bw/day. In males at 175 mg/kg bw/day, mean body weights were significantly reduced at the end of the dosing period (87.9% of the control values, $p \leq 0.01$). Female body weights were not affected. Food intake was reduced from day 50 onwards (males) and during late gestation and early lactation (females).

F1 generation - fertility, parturition and sexual function

No adverse effects on mating performance or fertility were reported in males and females of F1 generation at any dose. Significantly reduced average number of oestrous stages (2.7) was noted at 175 mg/kg bw/day (3.3 in controls, $p \leq 0.05$). However, there was no effect on pregnancy since 22/23, 22/22 and 14/15 of the F1 females were pregnant and delivered a litter in the 0, 50 and 175 mg/kg bw/day dose groups, respectively. Duration of gestation, gestation index and mean number of implantation sites per dam was not affected.

F1 generation – reproductive organ weights and histopathology

In females, there were no adverse effects on ovary, uterus or pituitary weights, both absolute and relative (to brain or to body weight). In males, the absolute weight of the left cauda epididymis (88%), left testis (91%), seminal vesicles with fluid (85%), and prostate (82%) were all significantly reduced at 175 mg/kg bw/day as compared to controls. These organ weight changes can be linked to the reduced terminal body weight in this group (87% of control) and were therefore considered as less adverse.

Females in the 175 mg/kg bw/day dose group appeared to have fewer atretic follicles and the follicular types were well represented. According to the study author, the possibility of an effect of sodium bromide treatment cannot be completely excluded.

In males, there was no significant effect on percent motile sperm or static sperm count from the vas deferens, cauda epididymal sperm count/density and testicular spermatid count (Table 44 in CLP report). Reduced numbers of motile sperm ($p \leq 0.01$) and the total sperm count ($p \leq 0.05$) in vas deferens were recorded at 175 mg/kg bw/day. Three males demonstrated a minimal to mild spermatid head retention, however the relevance of this effect is unclear since it was observed also in one control animal.

Three-generation reproductive toxicity study on sodium bromide
(Van Leeuwen et al., 1983):

In a three-generation reproductive toxicity study sodium bromide was administered to rats (no strain specified) via diet 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. The study is not GLP- or guideline-compliant, and no information on substance purity, food consumption, oestrous cyclicity, sperm parameters, pup body weights and litter size was reported. At least two litters per female were raised in three successive generations. A cross-mating

with untreated males and females was performed in the 19200 ppm group.

Some indications of impaired fertility were found in F1 and F2 at 1200 ppm, and in F0 at 4800 ppm and 19200 ppm (Table below). Since no clinical observations were reported, and information on body weight changes at the top dose is lacking, the relevance of these findings for classification is questionable. Some statistically significant changes in the relative weights of uterus, ovary, testis, pituitary and adrenals were reported, however without consistency and dose relation.

Table: *Fertility index* in females from the three-generation reproduction toxicity study*

Dose level (ppm)	0	75	300	1200	4800	19200
P	70	70	72	65	25	0
F1	62	54	44	53	-	-
F2	52	67	30	45	-	-

* Fertility index: No. of pregnancies x 100/No. of matings; - no breeding

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

A recent subchronic study on sodium bromide in Crl:CD(SD) rats performed according to OECD TG 408 provides relevant information on oestrous cycles, sperm evaluation and histopathology. The test substance was administered daily via oral gavage at doses of 0, 60, 175, 500 mg/kg bw/day. A comparative sodium chloride group was included to determine the effect of a sodium dose of equivalent osmolarity to that of the high dose sodium bromide group (284 mg/kg bw/day).

General toxicity

Doses of 500 mg/kg bw/day sodium bromide produced severe general toxicity, characterized by adverse clinical effects and reductions in body weight gain, food and water consumption, with effects generally more severe in males than females. Clinical signs included ataxia and decreased motor activity, prostration or breathing abnormalities (tachypnea/dyspnoea/hyperpnea), limb abnormalities and poor general condition (dehydration, ungroomed coat, chromodacryorrhea, chromorhinorrhea, fur staining). In four males the signs were so severe that euthanasia on study days 52, 55, 86 or 107 was required. Histopathology at necropsy confirmed the presence of bacterial infections in the lungs of these animals, possibly related to mis-intubation or aspiration of the dosing solution. Clinical observations in females were similar but they appeared later in the treatment period and recovery was faster.

No mortalities were reported at 175 mg/kg bw/day. Decreased motor activity were observed in 6/10 males (week 2) and in 6/8 females (study days 11-13). All animals recovered before the end of the working day. Other clinical signs such as chromodacryorrhea, mild dehydration, swollen ear and/or periorbital area and hunched posture were infrequent and transient.

No significant changes in body weight or body weight gain were reported at 60 or 175 mg/kg bw/day, both in males and females. At 500 mg/kg bw/day, body weight and body weight gain in males were significantly lower at the end of the dosing period (81.2% and 68.8% of control, respectively, $p \leq 0.01$). Body weight and weight gain in females were not affected. In general, reduced food intake paralleled the changes in body weight.

In high dose males, effects on sperm motility, morphology and sperm count, and statistically significant decreases in absolute reproductive organ weight were reported. The changes in relative weights of the left and right epididymis, left caudal epididymis, left and right testes, seminal vesicles with/without fluid and prostate were not statistically significant. There was a reduction in the number of normal sperm (88.6% of control, $p \leq 0.01$) and in the percent motile sperm from the vas deferens (75.3% of control, $p \leq 0.05$). The mean non-motile sperm (110.4% of control, $p \leq 0.01$), percent abnormal sperm (11.9%, $p \leq 0.01$) and mean number of sperm with detached head (20.6%, $p \leq 0.01$) or no head (3.2%, $p \leq 0.01$) were increased compared to the control group values (Table 45 in CLP report).

There was no effect on sperm count, motility or morphology at 60 mg/kg bw/day or 175 mg/kg bw/day. The number of sperm with detached/no head was higher than the concurrent control (5.0 compared to 0.8, $p \leq 0.05$) and the percent abnormal sperm was increased (3.0% compared to 0.6% in control, $p \leq 0.01$) at 175 mg/kg bw/day.

In females, three out of 10 animals in the 500 mg/kg bw/day dose group had no corpora lutea in the ovary, but overall follicle counts were not affected. No other effects on reproductive organ weight, histopathology or oestrous cycle were recorded in females in this study.

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

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

At 19200 ppm, clinical signs of neurotoxicity (motor incoordination of the hind legs, depressed grooming) were observed in both sexes, and in males, body weight gain was reduced by 23% ($p < 0.01$). No treatment-related mortality was reported in any dose group.

In males, reduced adjusted prostate weight (33-50%) and secretory activity was recorded at doses starting from 4800 ppm, and decreased spermatogenesis at 19200 ppm. In females, a tendency to decreased number of corpora lutea was observed at 19200 ppm. It is noted that the histopathological findings of decreased spermatogenesis, decreased number of corpora lutea and reduced size of tubules occurred only at a very high dose level (1728 mg/kg bw/day), and details on numbers, severity, or incidences were not reported in the publication.

Industry commented during the general consultation that the study should not be included in the assessment since the histopathological findings relevant for reproductive toxicity were observed only at dose level well in excess of the limit dose of 1000 mg/kg bw/day defined OECD guidance. In line with DS response, RAC considers the histopathological findings observed at the top dose level of 1728 mg/kg bw/day only as a

supportive information.

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

In a non-guideline 90-day oral repeated dose toxicity study rats fed a low-chloride diet were administrated sodium bromide at 8, 31, 125, 500 and 2000 ppm (corresponding to 0.72, 2.8, 11, 45, 180 mg/kg bw/day). Mortality (3/10 animals), clinical signs of neurotoxicity (motor incoordination of the hind legs, depressed grooming), and reduced body weight gain (31/35% for males/females, $p \leq 0.001$) were reported for both sexes at 2000 ppm. At this dose level, reduced spermatogenesis and increased adrenal weight in males, and a decreased number of corpora lutea and retardation of uterus maturation in females were observed. Histopathological findings in the adrenals (decreased vacuolization of the zona fasciculate) were seen in both sexes at 500 ppm.

The DS and RAC consider the relevance of this study as unclear since a low chloride diet was used, and effects on reproductive organs were seen at doses of excessive toxicity (30% mortality). However, it is noted that while low sodium intake enhanced toxicity of bromide, the target organs were the same as reported in the 90-day rat study with normal diet (Van Logten *et al*., 1974).

RAC conclusion on sexual function and fertility

In summary, majority of adverse effects on sexual function and fertility were observed in several guideline and non-guideline repeated dose and reproduction toxicity studies, both with ammonium bromide and sodium bromide. RAC considers the bromide ion as the toxicologically relevant species in repeat dose toxicity studies, while cations such as potassium, sodium or ammonium have little or no influence on its toxicological profile. Thus, for the assessment of the endpoint reproductive toxicity, the use of read-across data from these bromide compounds is justified.

Fertility was slightly reduced at 242/454 mg/kg bw/day (80/90% of control in male/female) and markedly affected at 503/651 mg/kg bw/day (10%) in the dose-range finding study of ammonium bromide. The severe effects on fertility index at the top dose cannot be solely explained with the co-occurring neurotoxicity (rolling gate). Fertility was also reduced at 500 mg/kg bw/day (60%) in the two-generation study on sodium bromide, but in presence of excessive general toxicity (mortality, adverse clinical signs and effects on body weights). Notably, significantly lower fertility (approx. 73% of control) was also reported at 175 mg/kg bw/day, but only in the P parents (1st and 2nd pairing), in absence of severe general toxicity. No fertility effects were seen in F1 generation at the same dose. Findings from the multi-generation study on sodium bromide demonstrate also reduced fertility in all three generations, however, the relevance of the findings may be questioned due to deficiencies in the study.

Adverse changes in the reproductive system, gamete production and transport were reported in both sexes. In the two-generation study on sodium bromide, depletion of corpora lutea was observed in the ovaries of the P generation females at 500 mg/kg bw/day in presence of excessive systemic toxicity, and at 175 mg/kg bw/day where no severe general toxicity was recorded. Depletion of corpora lutea was also observed in the 90-day repeated dose toxicity studies on sodium bromide at 500 mg/kg bw/day (no mortalities, ataxia, body weights unaffected during treatment), and at very high doses of

1728 mg/kg bw/day in presence of general toxicity (some signs of neurotoxicity, reduced body weight gain of 23%, but no mortalities). No effects on corpora lutea were seen in F1 generation at 175 mg/kg bw/day, however, lower number of oestrous stages and some differences in the follicle types (fewer atretic follicles) with possible association with treatment were reported. Effects on oestrous cycle were also seen in the P generation at 500 mg/kg bw/day in the presence of severe general toxicity.

In males, decreased reproductive organ weights, histopathological changes, and adverse effects on sperm count, morphology, and motility were reported. Minimal to moderate cellular debris in the epididymis and/or spermatid head retention in the testis were seen in all males at 350 mg/kg bw/day, and in 11/23 at 175 mg/kg bw/day (mostly minimal changes) in the P generation of the two-generation study on sodium bromide. The count of motile sperm in vas deferens was lower and the percentage of sperm with abnormal morphology in epididymis was increased in both dose groups of the P generation males. The total count and number of motile sperm in the vas deferens was also lower than controls in F1 males of the high dose group (175 mg/kg bw/day). Retained spermatids in testes and increased mean number of sperm with detached head or no head were reported at 175 and 500 mg/kg bw/day in the 90-day repeated dose toxicity study of sodium bromide. A significant reduction in the number of normal sperm and percent motile sperm from the vas deferens was seen at the top dose. These histopathological changes in the high dose groups of both studies occurred in the presence of severe general toxicity, however less adverse changes were noted at doses without significant general toxicity (175 mg/kg bw/kg) indicating a dose-dependency. In another 90-day oral study on rats (non-guideline), statistically significant reduction in the adjusted prostate weight (33-50%) and secretory activity was recorded at doses starting from 432 mg/kg bw/day. Effects on testes (decreased spermatogenesis, reduction of tubules, decreased serum testosterone) and reduced epididymides weight were observed at very high doses (1728 mg/kg bw/day) and these effects are regarded only as supportive evidence.

In a weight of evidence approach, RAC considers that there is clear evidence of an adverse effect on both male and female sexual function and fertility, also in the absence of severe general toxicity. No mechanistic information is available to indicate that the observed effects are not relevant for humans. RAC concludes that **classification in Repr. 1B, H360F is warranted**.

Developmental toxicity

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

In a developmental toxicity study according to OECD TG 414, pregnant SD rats received ammonium bromide once daily by oral gavage at dose levels of 0, 100, 300 and 1000 mg/kg bw/day, during days 6-19 of gestation (Study report, 2000b). Neurotoxicity (rolling gait, animal limp when handled, hunched posture, subdued behaviour, piloerection, eyes dark, abnormal respiration) and reduced body weight gain during gestation were recorded in dams at 1000 mg/kg bw/day. One animal at this dose was sacrificed on day 10 of gestation due to the severity of these effects. Corrected maternal body weight gain was 65% of control. Foetal effects were noted at all dose levels (Table below).

Table: *Maternal and developmental effects reported in Study report, 2000b*

Dose level (mg/kg bw/day)	0	100	300	1000
Maternal toxicity				
Mortality	0/24	0/24	0/24	1/24
Corrected body weight gain [g] (GD 6-20)	52	50	59	34 (-35%)
Clinical signs (neurotoxic effects)		0/24	1/24	24/24
Developmental toxicity				
Total live foetuses	191	247	188	208
Foetal body weight [g]	3.92	3.63	3.7	3.35 (-15%)
Small foetuses	2%			24%
Visceral examination [no. foetuses/ no. foetuses examined (litter)]				
Left kidney (absent/small/displaced), with/without absent left adrenal and absent left ureter	0/191 (0/22)	0/247 (0/22)	0/188 (0/22)	26/208 [12.5%] (7/22)
Spleen flattened and/or reduced in size	0/191 (0/22)	1/247 (1/22)	0/188 (0/22)	19/208 [9%] (7/22)
Narrow left uterine horn with flattened ovarian and displaced from ovary	0/191 (0/22)	0/247 (0/22)	0/188 (0/22)	14/208 [7%] (5/22)
Reduced/absent thyroid	1/191 (1/22)	1/247 (1/22)	0/188 (0/22)	8/208 [4%] (6/22)
Displaced testis HCD (0-4.1%)	3/191 [2%] (3/22)	11/247 [4%] (7/22)	15/188 [8%] (10/22)	20/208 [10%] (10/22)
Skeletal examination [no. foetuses/ no. foetuses examined (litter)]				
Curved scapula	1/191 (1/22)	2/247 (2/22)	2/188 [1%] (2/22)	18/208 [9%] (8/22)
Kinked rib(s)	3/191 (3/22)	11/247 (7/22)	17/188 [9%] (9/22)	52/208 [25%] (18/22)
Slightly kinked rib(s)	2/191	2/247	4/188 [2%]	9/208 [4%]

	(2/22)	(1/22)	(4/22)	(8/22)
Incomplete ossification of rib(s)	0/191 (0/22)	5/247 [2%] (3/22)	17/188 [9%] (4/22)	34/208 [16%] (14/22)
Reduction in size of the 13th ribs	0%			6.8%

At 1000 mg/kg bw/day, 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 observed. Kinked ribs were associated with dose-dependent incomplete ossification of ribs (2%, 9% and 16% at 100, 300 and 1000 mg/kg bw/day, respectively), and together with curved scapula were considered as being reversible (Study report, 2007a). Major abnormalities of the left kidney (reduced/absent/displaced/cystic), often associated with absence of the left adrenal and/or left ureter (12.5%), were noted at 1000 mg/kg bw/day. Reduced/absent thyroid (3.8% compared to 0.5% in controls), narrow left uterine horn (7% compared to 0% in controls), and flattened/small spleen (9% compared to 0% in controls) were also seen at this dose.

Overall, the major malformations at the high dose and the dose-dependent increase in incidences of minor abnormalities/variants starting from lower dose levels are considered as direct effects of the test substance and not secondary non-specific consequences of maternal toxicity.

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

In a second prenatal developmental toxicity study (Study report, 2007a), 22 pregnant SD rats were exposed once daily by gavage to ammonium bromide at 0, 50, 300, 600 and 800 mg/kg bw/day during GD 6-19. Additionally, two further groups were assigned to control and 300 mg/kg bw/day groups as recovery animals (littering phase). The study was specifically designed to supplement the information from the previous developmental toxicity study (Study report, 2000b), and did not provide statistical analysis nor historical control data.

Maternal neurotoxicity (staggering, rolling gait, subdued behaviour, slow/irregular respiration, body held low, hunched posture, piloerection) was reported in all animals at 600 and 800 mg/kg bw/day, and one animal at 600 mg/kg bw/day was sacrificed on GD 11 due to the severity of these signs. Maternal body weight gain was reduced at 800 mg/kg bw/day, while body weight gain at 300 and 600 mg/kg bw/day was increased by 11% and 28%, respectively (no dose-response, and statistical evaluation).

Table: *Maternal and developmental effects reported in Study report, 2007a*

Dose level (mg/kg bw/day)	0	50	300	600	800
Maternal toxicity					

Mortality	0/22	0/22	0/22	1/22	0/22
Corrected body weight gain [g], GD 6-20	46	49	51 (+11%)	59 (+28%)	42 (-9%)
Clinical signs (neurotoxic effects)		0/22	2/22	22/22	22/22
Developmental toxicity					
Total live foetuses	234	242	277	224	255
Foetal body weight [g]	3.62	3.88	3.87	3.85	3.7
Visceral examination [no. foetuses/ no. foetuses examined (litter)]					
Thyroid(s) markedly reduced (in size)	0/234 (0/21)	0/242 (0/21)	0/277 (0/22)	1/224 (1/19)	1/255 (1/21)
Testicle(s) medially displaced	0/234 (0/21)	1/242 (1/21)	1/277 (1/22)	3/224 (3/19)	4/255 [2%] (4/21)
Skeletal examination [no. foetuses/ no. foetuses examined (litter)]					
Curved scapula	0/234 (0/21)	0/242 (0/21)	5/277 (3/22)	5/224 (3/19)	14/255 [5%] (5/21)
Kinked rib(s)	1/234 (1/21)	0/242 (0/21)	15/277 [5%] (9/22)	19/224 [8%] (10/19)	17/255 [7%] (10/21)
Incomplete ossification of rib(s)	4/118 (4/21)	0/121 (0/21)	27/139 [19%] (11/22)	33/112 [29%] (13/19)	30/127 [24%] (15/21)
Fewer than 13 complete ribs (13th ribs vestigial or reduced or absent)	10/118 [8%]	9/121 [7%]	6/139 [4%]	15/112 [13%]	30/127 [24%]
Litter toxicity (recovery animals)					
Mean total litter weight on PND 21 [g]	591	n.a.	529 (-10%)	n.a.	n.a.
Skeletal examination [no. pups/ no. pups examined (litter)] (recovery animals)					
Curved scapula		n.a.	0/213 (0/18)	n.a.	n.a.
Minimally kinked rib(s)	0/249	n.a.	1/213	n.a.	n.a.

	(0/20)		(1/18)		
Fewer than 13 complete ribs	4/249	n.a.	4/213 [2%]	n.a.	n.a.

n.a. not applicable (only two groups, control and 300 mg/kg bw/day, were assigned to serve as recovery animals)

In the prenatal phase of the study, no adverse effects on foetal weights or mortality were seen at any tested dose. Increased incidences of foetuses with kinked ribs (5.4%, 8.5% and 6.7%), curved scapulae (1.8%, 2.2% and 5.5%) and incompletely ossified ribs (19%, 29% and 24%) were reported at 300, 600 and 800 mg/kg bw/day, respectively. Incidences of foetuses with fewer than 13 complete ribs were higher at 600 and 800 mg/kg bw/day than in the control group (13% and 24%, respectively vs. 8% in control). Maternal toxicity (clinical signs of neurotoxicity, one mortality) was present at 600 and 800 ppm, however skeletal variations and retardations were also evident at 300 mg/kg bw/day where no severe maternal effects were reported. Thus, systemic (neuro)toxicity cannot be used to completely disregard these effects on development.

Postnatal evaluation revealed no effect on litter size and survival at 300 mg/kg bw/day. Mean litter weights on day PND 21 were slightly lower (ca. 10%) than in the control group. Incidences of curved scapula were not reported which indicates that the kinked ribs and curved scapulae seen in the previous study may be transient in nature.

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 (Study report, 1995), Crl:CD BR VAF/Plus rats (25/dose) were exposed by oral gavage to 0, 100, 300 or 1000 mg sodium bromide/kg bw/day at GD 6-15.

Clinical signs of neurotoxicity (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) were noted in dams at 1000 mg/kg bw/day. Due to the severity of the clinical signs, one animal was sacrificed on day 11 of gestation. Reduced food consumption during GD 18-19 was noted in dams of the top dose (9%), and body weight gain at 300 and 1000 mg/kg bw/day was reduced by 15% and 16%, respectively, when compared to controls (GD 16-20). No foetal deaths or effects on foetal weight or sex ratio were reported at any dose level.

Table: Maternal and developmental effects reported in Study report, 1995

Dose level (mg/kg bw/day)	0	100	300	1000

Maternal toxicity				
Mortality	0/25	0/25	0/25	1/25
Clinical signs (neurotoxic effects)		0/25	0/25	25/25
Corrected body weight gain [g]	63.7	68	58.5	46.5 ($\downarrow$ 27%)
Developmental toxicity				
No. of live young	14.1	14.0	13.1	13.1
Foetal body weight [g]	3.81	3.92	3.84	3.75
Visceral examination [no. foetuses/ no. foetuses examined (litter)]				
Absent kidney	0/324 (0/23)	0/295 (0/21)	0/315 (0/24)	9/289 (3/22)
Absent ureter	0/324 (0/23)	0/295 (0/21)	0/315 (0/24)	10/289 (4/22)
Absent uterine horn	0/324 (0/23)	0/295 (0/21)	0/315 (0/24)	2/289 (2/22)
Narrow uterine horn	0/324 (0/23)	0/295 (0/21)	0/315 (0/24)	5/289 (3/22)
Displaced testis(es)	1/160 (1/23)	2/146 (2/21)	0/156 (0/24)	4/136 (4/22)
Skeletal examination [no. foetuses/ no. foetuses examined (litter)]				
Distorted/minimally distorted/ossification irregularities ribs	0/324 (0/23)	0/295 (0/21)	0/315 (0/24)	5/289 (4/22)
Distorted rib(s), minimal	0/159 (0/23)	0/149 (0/21)	0/154 (0/24)	8/137 (7/22)
Irregular ossification vertebral centra	5/159 (3/23)	6/149 (5/21)	6/154 (5/24)	12/137 (8/22)
Reduced ossification of one or more cranial centres	7/159 (4/23)	5/149 (2/21)	22/154 (13/24)	28/138 (13/22)
Shortened/absent 13th rib(s)	0/159	1/149	1/154	8/137

	(0/23)	(1/21)	(1/24)	(6/22)

At 1000 mg/kg bw/day, visceral malformations affecting the urogenital system consisted of foetuses with absent kidney (3%), absent ureter (3%), absent uterine horn (0.7%), and narrow uterine horn (2%) compared to 0% in controls (Table above). Skeletal malformations were manifested as distorted/minimally distorted/ossification irregularities in the ribs: 1.7% compared to 0% in controls. Skeletal anomalies such as distorted ribs minimal (6%), shortened/absent 13th ribs (6%), irregular ossification thoracic vertebral centra (9%) and reduced ossification of one or more cranial centres (20%) were seen at increased rate compared to control.

At 300 mg/kg bw/day, an increased rate of skeletal anomalies (reduced ossification of one or more cranial centres, 14% compared to 4% in controls) and of skeletal variants (total variant sternebrae, 57.1% compared to 41.4% in controls, unossified sternebrae, 40% compared to 28% in controls) were reported.

Overall, the observed malformations in the high dose group cannot be considered as secondary to the observed maternal toxicity. Since foetal weights were not affected at any dose level, the skeletal variants and anomalies observed also at lower dose cannot be explained with retarded growth.

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

In a pre-natal developmental toxicity study according to OECD TG 414, New Zealand White rabbits were exposed by oral gavage to sodium bromide at dose levels of 0, 25, 75 and 250 mg/kg bw/day during GD 6-28 (Study report, 2008b). No treatment-related maternal toxicity was noted at any dose. Water intake was higher at 75 and 250 mg/kg bw/day, possibly due to higher salinity of the dose formulations. No significant developmental toxicity was reported in this study. One total litter loss occurred *in utero* at 25 mg/kg bw/day. Since other dose groups were not affected, this single incidence is considered by RAC of no toxicological relevance. At 75 mg/kg bw/day, a significant increase in irregular ossification of more than one cranial bone (53.2% versus 25.3%, p ≤ 0.01) was observed, however without dose-response. The absence of maternal toxicity at the high dose level indicates that this dose level might have been too low.

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

In a preceding developmental dose range finding study (Study report, 2008a), time-mated New Zealand White rabbits (6 per dose group, 5 in control) were exposed to sodium bromide at dose levels of 100, 200 and 400 mg/kg bw/day during GD 3-28. No adverse maternal effects were observed at 100 and 200 mg/kg bw/day. Ataxia was seen in 2/6 animals at 400 mg/kg bw/day during days 25 and 28 of gestation, resulting in early termination of the first affected animal. The study provides no indications of adverse effects on the offspring at any dose investigated.

Dose-range finding study for reproduction toxicity on ammonium bromide
(Study report, 2001):

Information of effects on developing foetus can be found also in the dose-range finding reproductive toxicity study with ammonium bromide and the generation studies with sodium bromide (one two-generation and one three-generation reproductive toxicity study) discussed earlier in terms of adverse effects on sexual function and fertility.

In the dose-range finding study for reproduction toxicity (Study report, 2001), rats (10/sex/group) were administered ammonium bromide via food at concentrations of 0, 1600, 3200 and 6400 ppm (corresponding to 0, 127/228, 242/454 and 503/651 mg/kg bw/day in males/females).

At 6400 ppm, fertility was markedly decreased (10% of control), and the single litter produced did not survive to day 4 of lactation. Pup viability was also decreased at 3200 ppm, where all pups in 4 out of 9 litters died before day 21 of lactation. Decreased mean weights of litter and pups (> 10%) were seen from day 7 of lactation. Three pups from two litters at 3200 ppm, and one pup at 1600 ppm were terminated on or before day 12 of lactation due to poor condition (cold, subdued behaviour, abnormal breathing). Litter size and survival were not adversely affected at 1600 ppm. The effects in the high dose group were observed in the presence of severe clinical observations in dams including rolling gait, piloerection, hunched posture and hyperactivity. Body weight gain during gestation was 33% less than in the control, however only one female was available for this assessment. Similar, but less severe clinical signs were seen at 3200 ppm. Individual body weights of the dams losing their litters were not severely affected.

The interpretation of this study in terms of developmental toxicity is difficult. Maternal toxicity at the top dose was excessive, and decreased mean litter/pup weights and decreased pup viability during lactation at 3200 ppm could possibly be due to poor maternal care (neurotoxicity). Unusually low viability indices in the control group complicate further the evaluation of pup viability.

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

In a two-generation reproduction toxicity study (similar to OECD TG 416), sodium bromide was administered via oral gavage to Crl:CD(SD) rats at dose levels of 0, 50, 175, 350/500 (male/female) mg/kg bw/day (Study report, 2016a). Rats in control, low and intermediate dose groups of the P generation were paired twice due to reduced pregnancy rate at the mid dose. Maternal toxicity at 500 mg/kg bw/day was severe including mortality (> 10%; 9 females died or were terminated early) and clinical observations such as dehydration, ungroomed coat, chromodacryorrhea, hunched posture, ptosis, urine-stained abdominal fur, decreased motor activity, chromorhinorrhea, ataxia, piloerection, low carriage, thin body condition, and bradypnea. At 175 mg/kg bw/day, clinical signs were similar although at lower incidence and severity.

Severe adverse effects on the offspring were reported at 500 mg/kg bw/day where no litters survived after day 5 post-partum. Since the mortality rate in dams was > 10%, results from this dose group are not considered for further evaluation. No effects on embryo-foetal survival, growth or development were noted at any generation at dose levels with no overt maternal toxicity.

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

In a three-generation reproductive toxicity study (not guideline- or GLP-compliant), sodium bromide was administered to rats (no strain specified) via diet 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 (Van Leeuwen *et al*., 1983). Due to low fertility in both high dose groups, the second and third generations were bred only from the groups up to 1200 ppm. A cross-mating with untreated animals was performed in the 19200 ppm group.

No information on gestation index, litter size at birth, altered growth or functional deficiency is available. Viability index of the F1 pups on PND 5 was markedly reduced at 4800 ppm (32% compared to 90% in control). During lactation, all pups that that were alive on day 5 died before day 21. Maternal body weights were not affected at this dose level. Since clinical conditions of the dams were not reported, it is not clear if the pup mortality was due to poor maternal care or to a direct effect by the substance.

Studies from open literature:

Several experimental studies from the open literature indicating developmental neurotoxic effects of sodium bromide were included in this assessment.

In a postnatal growth and brain development study (no guideline) (Disse *et al*., 1996), rats were treated with sodium bromide via the drinking water at 0 and 200 mg/kg bw/day during days 5-15 of gestation. Pups of the treatment group showed reduced body weight (statistically significant from day 19 onwards, adult values differed by 15%) and brain weight (statistically significant from day 8 onwards, adult values about 10% lower). The study shows that administration of bromide during gestation causes changes in the brain (reduced protein content) and olfactory tract (increased size of olfactory glomeruli) in the offspring of the dams dosed with 200 mg/kg bw/day (156 mg bromide/kg bw/day). These effects persisted in the offspring after completed excretion of bromide and showed periods of partial compensation and decompensation.

The effect of prenatal administration of sodium bromide on the central nervous system of the offspring in rats was studied by Harned *et al*. (1944). Pregnant rats were treated with sodium bromide at 40, 80 or 120 mg/kg bw/day from day 3 to 20 of gestation. Pups received bromide only via the milk of their mothers and were weaned until day 20. Each animal was tested in a five cul-de-sac u-maze at the age of 61-85 days.

Pup mortality before day 20 was dose-dependently increased in all dose groups (27%, 42% and 58% compared with 2.3% in control). The study authors concluded that learning ability in offspring of rats treated with sodium bromide was reduced at 80 and 120 mg/kg bw/day (62 and 93 mg bromide/kg bw/day).

There are also several human case reports indicating developmental effects in infants exposed to bromide during the entire pregnancy.

Finken and Robertsson (1963) reported on transplacental passage of bromide to the foetus causing bromism in a 7-day old female infant given birth by a mother severely intoxicated with bromide. The condition of the infant was described as "simply sedated" in spite of a serum bromide concentration rated as "potentially lethal". Treatment consisted of extra salt added to the diet, and follow-up examination one month later revealed a healthy infant.

Blackburn and Pleasure (1975) also described a transplacental passage of bromide to the

foetus leading to central nervous system depression in a female infant born to a mother severely intoxicated with bromide. The neonatal bromide intoxication was characterized by marked hypoactivity, reduced cry and suck. Infant's treatment included antibiotics for seven days, and an intravenous application of 5% glucose and 0.45% sodium chloride. Development of the infant was normal by 3.5 months of age, and no long-term damages were reported.

Mangurten and Ban (1974) described a case of bromism in infant from a mother receiving bromide-containing drugs for psychiatric treatment during 4 days prior to delivery (6 g/day). Neurological signs in the infant were a weak, high-pitched cry, poor suck, partial Moro reflex and diminished tone; deep tendon reflexes were absent. The study confirmed the known slow clearance and increased renal tubular reabsorption of bromide ion. No residual manifestations of bromism were seen after 5 months.

Mangurten and Kaye (1982) reported a case of a pregnant mother using photographic chemicals, containing sodium and potassium bromide during the entire pregnancy. The infant was born by caesarean section after 43-44 weeks gestation and was transferred to intensive care because of cyanosis. 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. The authors cannot exclude that also other chemicals in combination with the bromide salts may have contributed for the symptoms observed in the new-born.

Opitz *et al*. (1972) described growth retardation and the bromism in two children born from a mother who regularly ingested Bromo-Seltzer. However, the authors acknowledged that the growth retardation and the bromism might be entirely coincidental.

Similarly, Rossiter and Rendle-Short (1972) reported about possible relation between bromide ingestion during pregnancy and infant developmental retardation, however not excluding that these observations could be only circumstantial.

Overall, the above human case reports provide only weak indications of developmental growth retardation (height, weight and skull circumference) in infants exposed to bromide during the entire pregnancy.

RAC conclusion on adverse effects on development

In summary, severe developmental effects such as foetal structural abnormalities and death of the developing organism were observed in studies with ammonium bromide and sodium bromide. RAC considers the use of read-across data from these bromide compounds as justified for the assessment of the endpoint reproductive toxicity.

Effects on pup viability and survival were reported in several (multi-)generation studies. In the dose range finding reproductive toxicity study of ammonium bromide, both the viability and the survival index were severely reduced at 651 mg/kg bw/day (the single litter produced did not survive to day 4 of lactation) and at 454 mg/kg bw/day (all pups in 4/9 litters died before day 21 of lactation). Maternal toxicity at the top dose was severe, and unusually low viability in the control group complicates the assessment of the mid dose group. In addition, is not clear if the lower pup viability during lactation was due to poor maternal care or was a direct substance effect on the pups. In the two-generation reproduction toxicity study of sodium bromide, the litter size and pup viability

were markedly decreased at 500 mg/kg bw/day, however in the presence of excessive maternal mortality of > 10%. In the three-generation reproduction toxicity study of sodium bromide, viability index of the F1 pups was significantly reduced at 432 mg/kg bw/day. Maternal body weights were not affected, however no clinical observations were reported. Pup mortality was also increased in an older pre-natal developmental toxicity study of sodium bromide in rat (Harned *et al.*, 1944), however, due to pup-killing/cannibalism and poor reporting, the interpretation of these findings is difficult.

A dose-related increased incidence of displaced testis was noted in the range of 100-1000 mg/kg bw/day in the pre-natal developmental toxicity study on ammonium bromide in rats. Visceral malformations reflecting defects in the urogenital system, uterine, spleen and thyroid occurred in rats at doses of 1000 mg/kg bw/day after treatment with both ammonium bromide and sodium bromide. Skeletal malformations (ribs) in rats were reported at 1000 mg/kg bw/day, while skeletal anomalies (ribs, cranial centres and sternebrae) were recorded at lower doses. Maternal neurotoxicity and reduced body weight gain was present at the top dose level in all prenatal studies, however the effects at lower doses (i.e. at 300 mg/kg bw/day) did not co-occur with severe maternal toxicity. Specifically in Study report (2000b), mortality of 1/24 dams (i.e. < 10%) does not automatically justify discounting the developmental effects in this dose group, and a reduction in maternal body weight gain (43% of control) during the first six days could be largely attributed to marked weight loss in two of the animals. Thereafter, body weight gain was similar to control (94%). Thus, irreversible effects such as structural malformations in foetuses in this study and in Study report (1995), i.e. absence of organs (kidney, ureter, adrenal, thyroid) cannot be seen as a consequence of maternal toxicity.

Considering the weight of evidence of all available information, RAC concludes that there is clear evidence of severe structural abnormalities and some evidence of both death of the organism and retarded growth not secondary to maternal toxicity. These effects are relevant for humans, and therefore **RAC concludes that classification in Repr. 1B, H360D is warranted**.

There are no robust human studies on developmental toxicity of bromide demonstrating adverse effect on human foetal development. The few reported cases provide only indications of developmental growth retardation that at most do not seem to contradict the animal data. Therefore, classification in Repr. 1A, H360D is not justified.

Adverse effects on or via lactation

The transfer of bromide through the milk to the pups was investigated in a non-guideline postnatal study of sodium bromide and potassium bromide. In this study, Wistar rats (5 dams/group, 8 pups/dam) were exposed to sodium bromide and potassium bromide orally at a doses of 300 and 900 mg/kg bw/day during the whole lactation period or only once on day 12 of lactation (Vobecky *et al.*, 2005).

Mortality of one dam was observed in both dose groups. At high dose, food consumption and water intake were reduced during the entire lactation, and mean maternal body weight was 18% lower than control. It is noted that a marked effect on body weight was seen in only two of the five rats while the weights of the remaining 3 were comparable to control group. No clinical observations were reported in any dose group. Offspring toxicity consisted of decreased pup survival at low (94.8%) and high dose (56.3%) compared to 100% in control, and decreased mean pup body weight to less than 40% of control at

high dose. Pups general condition in this group was described as very poor.

Milk production at lactation days 10 and 15 was significantly reduced in dams of the high (-65% and -61%) and low dose (-58% and -64%), respectively. In terms of milk composition, about 54% of its chloride content was replaced by bromide. After a single administration of radiolabelled potassium bromide on day 12 of lactation, less than 10% and approximately 17% of the applied dose was found in the offspring at 3 and 25 hours post dosing, respectively.

Reduced milk production on days 10 and 15 (-67% and -70%, respectively), and decreased body weight in pups (ca. -30%) were also reported in dams given 1 or 5 g bromide/L in distilled water during lactation (Pavelka *et al*., 2002). No clinical signs were reported, and the body weight among the lactating dams was not affected. The study reports an increase in the relative thyroid weights of the pups (no change in dams), and a decreased iodine transfer through milk to the pups.

In humans, irritability, drowsiness, sleepiness, absence of cry and rash on the face were noted in babies of 10 patients on the sixth day after delivery, following maternal intake of sodium bromide at 5.4 g/day (Tyson *et al*., 1938).

In comments provided during the general consultation, the industry representative considered the above animal studies not suitable for classification due to the high dose levels, small group size and non-standard procedures applied. Further, they emphasized that the American Academy of Paediatrics classifies bromides as compatible with breast feeding (AAP, 2001). In addition, one MSCA disagreed with the proposed classification arguing that the effects observed during lactation in Vobecky *et al*. (2005) were more likely secondary to maternal toxicity, noting that feed restriction during lactation can result in a clear reduction in pup weight as demonstrated by Carney *et al*. (2004).

In line with the DS assessment, RAC considers that the two-generation study of sodium bromide and the dose range finding studies of ammonium bromide and sodium bromide provide no clear evidence of adverse effects on or via lactation. However, milk production was affected in dams of the low dose group in Vobecky *et al*. (2005) and at both doses in Pavelka *et al*. (2002), without significant maternal body weight changes reported. Overall, it has been demonstrated that bromide can be transferred via milk to the pups, and that milk production and its elementary composition was changed in dams receiving bromide during lactation. There are also some weak indications on possible effects on the central nervous system in infants following maternal intake of bromide during lactation. Therefore, RAC concludes that **classification for effects on or via lactation, H362 is justified**.

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) EPA OPP 81-1 (Acute Oral Toxicity) Rat, CD strain (remote Sprague Dawley origin) male/female 5/sex/group	Ammonium bromide Oral: gavage 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. LD50 males 2868 mg/kg bw LD50 females: 2566 mg/kg bw LD50 males and females combined: 2714 mg/kg bw	A6.1.1/01, Doc. No. 521-001 Study report, 1986a Reliability 1
Sodium bromide			
equivalent or similar to OECD TG 401 (Acute Oral Toxicity) EPA FIFRA 81-1 Rat, CD [Crl: CD(SD)BR] male/female 5/sex/group	Sodium bromide Oral: gavage 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. LD50 males: 4500 mg/kg bw LD50 females: 3900 mg/kg bw LD50 males and females combined: 4200 mg/kg bw	A6.1.1/02, Doc. No. 521-002 Study report, 1988a Reliability 1

Table 53: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Bromide				
Study type: prognosis following poisoning 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. 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. 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. Other effects include skin rashes, neurological signs, sensory disturbances, and increased spinal fluid pressures.	Gosselin R.E., 1976 Reported as supporting study in REACH Registration dossier
Study type: prognosis following poisoning 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 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.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS proposed to classify ammonium bromide as STOT SE 3, H336 based on transient lethargy, lack of coordination, loss of righting reflex and ataxia at 2000 mg/kg bw in all rats after administration of ammonium bromide in an acute toxicity study.

Comments received during consultation

Classification proposal for this endpoint was not commented during consultation.

Assessment and comparison with the classification criteria

For the assessment of STOT SE endpoint the DS included one rat oral acute toxicity study on ammonium bromide, one rat oral acute toxicity study on sodium bromide as well as human studies describing overdosing of bromides (reported as supporting studies in REACH Registration dossier).

In the animal oral acute toxicity study on ammonium bromide performed according to

---

OECD TG 401, the test substance was administered to groups of five male and five female rats (CD strain) as a single oral dose of 2000, 2714, 3684 and 5000 mg/kg bw at a constant volume of 20 mL/kg bw 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 the LD$_{50}$ 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 even after oral administration of the lowest dose of 2000 mg ammonium bromide/kg bw. At the lowest dose the duration of signs varied from 15 minutes to 2 days and none of the animals died. These signs were also apparent in all animals at higher dose levels also causing death of some animals. At 5000 mg/kg bw all animals were dead within 15 minutes to 1 hour.

In the animal oral acute toxicity study on sodium bromide performed similarly to OECD TG 401, the test substance was administered to groups of five male and five female rats (CD strain) as a single oral dose of 3200, 4000 and 5000 mg/kg bw (Study report, 1988a). 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 after dosing of the lowest dose of 3200 mg/kg bw (Study report, 1988a). The mortality rate was zero at this dose level but started to appear at higher doses.

As regards the human evidence, RAC has taken into consideration the known acute sedating effects by bromine sedatives, three studies reporting poisoning with bromine medications (bromism) of mothers and their new-born infants resulting in central nervous system depression both in mothers and in infants (Finken and Robertsson, 1963; Mangurten and Ban, 1974; Blackburn and Pleasure, 1975) and a study, that was provided during the general consultation in relation to a comment on STOT RE, describing recent bromism case in the mother and infant (Lugassy and Nelson, 2009).

RAC considers that the acute effects by bromine sedatives are relevant for STOT SE, whereas the three studies reporting poisoning with bromine medications (bromism) of mothers and their new-born infants resulting in central nervous system (CNS) depression (Finken and Robertsson, 1963; Mangurten and Ban, 1974; Blackburn and Pleasure, 1975) and the study describing a recent bromism case in the mother and infant (Lugassy and Nelson, 2009) are relevant for STOT RE and reproductive toxicity as well.

RAC concludes that the CNS effects in humans after a single exposure are transient and justify classification as STOT SE 3, H336. Narcotic effects were also observed at 2000 mg/kg bw in the oral acute toxicity study of ammonium bromide in rats and were considered to support classification for STOT SE 3, H336 since the effects (lethargy, lack of coordination, loss of righting reflex and ataxia) were considered by RAC to be transient in nature.

RAC supports the **classification of ammonium bromide as STOT SE 3, H336 (May cause drowsiness or dizziness).**

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 GLP compliant Rat, Sprague-Dawley male/female 5/sex/group	Ammonium bromide Oral (feed) 0, 100, 500, 1000 mg/kg bw/day (nominal in diet) 4 weeks (28 days)	**Mortality and clinical observations** ↑ clinical signs of neurotoxicity and subdued behaviour at 500 (all males) and 1000 mg/kg bw/day (all males and 4/5 females) **Body weight** ↓ body weight gain (males 49% and females 31%) at 1000 mg/kg bw/day **Food consumption** ↓ food consumption (males 29%) at 1000 mg/kg bw/day **Organ weights** ↓ mean absolute testes weight starting from 100 mg/kg bw/day (-11%, p<0.05; -14%, p<0.05; -16%, p<0.01) ↓ mean absolute epididymis weights at 500 and 1000 mg/kg bw/day (-14%, p<0.05; -16%, p<0.01) ↓ mean absolute kidneys (males) weights at 500 and 1000 mg/kg bw/day ↓ mean absolute heart, liver and lungs weights at 1000 mg/kg bw/day ↑ relative liver weight (23%, p<0.01; females) at 1000 mg/kg bw/day No histopathological examination performed to confirm changes in organ weights.	A6.3.1/01, Doc. No. 532-001 Study report, 1999 Reliability 1

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

	Ammonium bromide	**Parental generation – general toxicity**	A6.8.2/01, Doc. No.553-001
Dose range finding study of a reproduction toxicity study		**Mortalities and clinical observations**	
	Oral (feed)	*Males and females*	
GLP	0, 1600, 3200, 6400 ppm	↑ 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	Study report, 2001
Rat, Sprague Dawley	Group mean achieved dosages of test material during treatment, gestation and lactation:		Reliability 1
10/sex/group			
	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	**Body weight and food consumption**	
		Males	
		↓ 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	
		↓ food consumption, 88% of control at 242 mg/kg bw/day and 503 mg/kg bw/day	
	Exposure: 2 weeks prior mating until the first generation had been weaned	*Females*	
		↓ bodyweight gain gestation days 0-20 (67% of control or 33% less than control) at 651 mg/kg bw/day	
		Organ weights	
		↓ 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	
		↓ 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%)	
		No effects on relative organ weights and no histopathological examination performed to confirm changes in organ weights	
Sodium bromide			

Oral Repeat Dose 90-Day Toxicity Study in Rats, Including Recovery Assessments 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) Rat, Crl:CD(SD) Male/female 10/sex/group and an additional 10/sex/group for control and high dose groups were included for the recovery assessment.	Sodium Bromide CAS Number: 7647-15-6 Purity: 100.0% Oral (gavage) 0, 60, 175, 500 mg/kg/day A Sodium Chloride comparator group (284 mg/kg/day) was also included in the assessment. 90 days	**Mortality and clinical observations** *Males* ↑ mortality at 500 mg/kg bw/day (4 animals euthanasied) ↓ 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. ↑ 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. *Females* ↓ 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. ↑ 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. **Functional observation battery and motor activity** *Males* ↑ ambulations (p≤ 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. ↓ incidence of rears at 500 mg/kg/day ↑ 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. *Females* ↑ motor activity (ambulation) (p≤ 0.05) and lack of righting reflex in 5 females and a reduction in hindlimb grip strength at 175 mg/kg/day. ↑ incidences of ataxia and abnormal gait (6/10 females) week 9 at 500 mg/kg/day **Body weight** *Males*	Study report, 2016b Reliability 1

		↓ body weight at the end of dosing period, 81.2 % of control, (p≤ 0.01) at 500 mg/kg/day ↓ 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 *Females* ↓ 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) **Food consumption** *Males* ↓ 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. ↓ 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). ↑ overall intake during the recovery period (study days 90 to 147, p≤ 0.01). *Females* ↓ 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 days 43 to 50, p≤ 0.05) at 500 mg/kg/day **Thyroid Hormone Analysis** ↓ T3 levels in males at 60 mg/kg/day (27%, p≤ 0.05), 175 mg/kg/day (28%, p≤ 0.05) and 500 mg/kg/day (37%, p≤ 0.01). ↓ T4 levels in males at 175 mg/kg/day (28%, p≤ 0.01) and at 500 mg/kg/day (52%, p≤ 0.01) and in females at 60 mg/kg/day (26%, p≤ 0.05), 175 mg/kg/day (34%, p≤ 0.01) and at 500 mg/kg/day (47%, p≤ 0.01). ↑ 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.).	

		Organ weights ↑ relative to body thyroidea weight (27% increase compared to control, p≤ 0.05) in females at 500 mg/kg/day **Histopathology** 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. **Reproductive toxicity** Please refer to table 37 and section 10.10. Only effects relevant for STOT RE are discussed here.	
Three-generation reproduction study (no guideline) Not GLP-compliant Deviations: no food consumption, pup body weights and litter size determination Rat, strain not specified Males and females 7-19/sex/ group	Sodium bromide Purity: not reported Oral (feed) Breeding study and thyroid hormone investigation: 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** No information on clinical condition available **Body weights** No adverse effects on body weights recorded **Food consumption** No information on food consumption available **Thyroid Hormone Analysis** ↓ 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 **Organ weights** ↓ relative adrenal weight in F0 females at 1200 and 4800 ppm (approx. 15%, p< 0.05 and p<0.01 **Histopathology** No histopathological examination performed **Reproductive toxicity** 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 Van Leeuwen et al., 1983 Reliability 2

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

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

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

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.) Dog, Mongrel Male/female 4 dogs/group	Sodium bromide Oral (gelatine capsules) 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 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. 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. 44-185 days	100 mg/kg bw/day: ↑ clinical signs indicating gastrointestinal toxicity ↓ 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)) 200 mg/kg bw/day: ↑ mortalities ↑ clinical signs of neurotoxicity ↑ clinical signs indicating gastrointestinal toxicity ↓ 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)) ↑ skin lesions 400 mg/kg bw/day: ↑ mortalities ↑ clinical signs of neurotoxicity ↑ clinical signs indicating gastrointestinal toxicity ↓ body weight (body weight loss: 15.4% in week 10 of administration ↑ skin lesions	A6.4.1/07. Doc. No. 592-027 Rosenblum I. 1958. Reliability 3
Potassium bromide			

No guideline study: sub-chronic toxicity, oral GLP compliance: no Dog, Beagle male/female 3/sex/group	Potassium Bromide Oral (feed) 30 mg/kg bw (equivalent to 20 mg Br-/kg bw) No control animals 115 days (daily). Plus 5 days for an additional test. (No post-exposure period)	Mean steady-state serum bromide concentration: 245 mg/dL: Electrodiagnostic test revealed only subtle changes over time. No clinical neurotoxic signs. 397 mg bromide/dL: 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 March P.A., Podell M. and Sams R.A. 2002. 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 Relevant information about the study (as applicable)	Observations	Reference
Sodium bromide				

Type of effects studied: endocrine system modulation (and effects on central nervous system) (in vivo) Human female	Sodium bromide	Oral (capsule) 0, 4, 9 mg/kg bw/day (actual ingested) Exposure: The experiment covered six menstrual cycles. Bromide administration during the first three cycles	**General signs/observations:** 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). **Endocrine system:** 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). **Neurophysiology (EEG):** 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 Van Gelderen C.E.M. et al., 1993 Reliability 2
Type of effects studied: endocrine system modulation (in vivo) Human male/female	Sodium bromide	oral: capsule 1 mg/kg bw (actual ingested) Exposure: 8 weeks or two full cycles (daily)	**General signs/observations:** In two females a short lasting itching dermatosis with small vesicles was observed at the beginning of the experiment (reversible effect). **Endocrine system:** 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 Sangster B., et al., 1982 Reliability 2

| Type of effects studied: endocrine system modulation (and effects on central nervous system) (in vivo)

Human male/female | Sodium bromide | oral: capsule

0, 4 and 9 mg bromide/kg bw/day (actual ingested)

Exposure: three month or three full cycles (daily) | **General signs/observations:**

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).

Endocrine system:

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.

Neurophysiology (EEG and visual evoked response):

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

Sangster B., et al., 1983

Reliability 2 |
| Study type: prognosis following poisoning

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

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
Sodium bromide				

Endocrinology 4 or 12 weeks feeding study, alterations in the endocrine system were investigated Rat, Wistar male 10/group	Sodium bromide	Oral (diet) 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) Exposure: 4 or 12 weeks 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: No treatment related effects. 1200 ppm: ↑ thyroid weight (38%, p<0.01 at 4 weeks) ↓ T4 level (23%, p<0.01 at 4 weeks) ↑ FSH level (21%, p<0.05 at 12 weeks) 19200 ppm: ↓ bw (9%, p<0.05 at 4 weeks; 21%, p<0.001 at 12 weeks) ↑ thyroid weight (71%, p<0.05 at 4 weeks and 82%, p<0.001 at 12 weeks) ↑ incidences of histopathological changes in thyroidea at 4 and 12 weeks (increase of follicles and a decrease in their size) ↑ incidences of histopathological changes in testes at 12 weeks (7/10 males with reduction of tubule diameter) ↓ spermatogenesis (at 12 weeks) - changes in immunoreactivity in pituitary gland (↓ GH at 4 and 12 weeks, ↑ TSH at 12 weeks, ↑ ACTH at 12 weeks) - 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 Loeber et al., 1983 Reliability: 2

Endocrinology 4 week (dietary), effects on the thyroid were investigated Low iodine diet Rat, Sprague-Dawley male/female 12/sex/group.	Sodium bromide	Oral (feed) 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) Exposure: 4 weeks (daily) Animals underwent a 2-week pre-tre-treatment period and a 4-week treatment period.	16 g/kg, low-iodine: 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. 8 g/kg, low-iodine: 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. 4 g/kg, low-iodine: Iodine deficiency, decreased free T4 and total T4 levels, increased thyroid weight, decreased T4, T3 and reverse T3 in thyroid gland. Low-iodine: 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 Buchberger W., Holler W. and Winsauer K., 1990 Reliability: 2

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

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

Neurotoxicity Mouse (murine C1300 neuroblastoma cells) in vitro study	Sodium bromide	0, 10^{-4}, 10^{-5} or 10^{-6} M sodium bromide 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 Reported as supporting study in REACH Registration dossier

Neurotoxicity in vitro study	Sodium bromide	Mouse (C1300 mouse neuroblastoma cells, clone Neuro-2a) 0, 10-4, 10-5 and 10-6 M 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 Reported as supporting study in REACH Registration dossier
Neurotoxicity The effects of sodium bromide on the bullfrog sympathetic ganglion were studied by extracellular and intracellular recording techniques. in vitro study	Sodium bromide	Bullfrog, Rana catesbiana 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 Reported as supporting study in REACH Registration dossier
Potassium bromide				

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. 16 or 66 day administration of potassium bromide in drinking water Rat, Wistar Male 6 animals/group	Potassium bromide	Oral (drinking water) The following dose levels were used in the study: 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: Histopathological changes in thyroidea at all dose levels, with a dose-related increase in extent. 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. 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). 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 Velický et al., 1997a Reliability: 2

Endocrinology 16, 66 or 133 days of administration, effects on the thyroid were investigated Rat, Wistar Male 10 animals/group	Potassium bromide	Oral (drinking water) 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) 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.	10, 50, 100, 200, 400 mg Br⁻/L in drinking water: Increased mitotic activity of follicular cells (increased values of mitotic index with increasing bromide concentrations) in thyroids. Increase in bromine content and a concomitant decrease of the I/Br molar ratio in the thyroid tissue . Histopathological changes in thyroidea (microfollicular rearrangement of the follicular epithelium, reduction of the amount of colloid). LOAEL: 0.5 mg bromide/kg bw/day	A6.10/10, Doc. No. 592-039 Velický J. et al., 1997b Reliability: 2

Endocrinology 133 day administration of potassium bromide in drinking water, effects on the thyroid were investigated Rat, Wistar male 10 animals/group	Potassium bromide	Oral (drinking water) 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). Exposure: 133 days (daily)	100 mg Br-/L drinking water: 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. 200 mg Br-/L drinking water: 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. 400 mg Br-/L drinking water: 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. 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. LOAEL: 100 mg Br-/L drinking water (3.3 mg bromide/kg bw/day)	A6.10/13, Doc. No. 592-034 Velický, J., 1998 Reliability: 2

| Endocrinology 16, 66 or 133 day administration of potassium in drinking water, effects on the thyroid were investigated

Rat, Wistar
male
10 animals/group | Potassium bromide | Oral (drinking water)

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.

Exposure: 16, 66 or 133 days (daily) | 10 mg Br-/L drinking water:

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.

50 mg Br-/L drinking water:

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.

100 mg Br-/L drinking water:

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.

200 mg Br-/L drinking water:

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.

400 mg Br-/L drinking water:

Small follicles with reduced lumina, dilated cisterns of ER, increased number of spherical structures and granules. | A6.10/12; Doc. No. 592-038)

Velický, J. et al 2004

Reliability: 2 |

Endocrinology	Potassium bromide	Oral (drinking water)	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
The purpose of the study was to evaluate the effects of potassium bromide on the canine thyroid gland		Loading dose of 100 mg/kg bw/day for two days and a maintaining dose of 30 mg/kg bw/day for 180 days.		
		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.		Paull et al., 2003
Dog, Laboratory Hound dog				Reliability: 2
2 males and 3 females per group (control and treatment)		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.		

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≤ 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≤ 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≤ 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≤ 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≤0.01) males and in 6/10 (p≤0.01) females of the high dose groups, respectively. Five females also showed unusual posture. Increased ambulations (p≤ 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≤0.01), but 5 (p≤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≤0.01) females and abnormal gait and unusual posture was slightly increased. 8/10 (p≤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≤ 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≤ 0.05). At **500 mg/kg/day**, average and relative food consumption in males were significantly reduced (p≤ 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≤ 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≤ 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≤ 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≤ 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≤ 0.01) and in relative food consumption (study days 8 to 15, p≤ 0.01 and study days 43 to 50, p≤ 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≤ 0.01) observed in the first 2 weeks were followed by increases (p≤ 0.05 to p≤ 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≤ 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≤ 0.05, males) and T4 (p≤ 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≤ 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≤ 0.05 and p≤ 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≤ 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≤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 $\geq$ 1200 ppm (108 mg/kg bw/day) and reduced thyroid hormone (T4) noted in males at $\geq$1200 ppm and in females at $\geq$ 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 δ_1 and δ_2-activities, an increase in β-activities and an increase in mean frequency, expressed in the mobility. Increased α_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 amministration 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 δ_1-, δ_2- activities but significant changes in α_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 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 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 Nervous system
Study report, 2007a	600 mg/kg bw/day ammonium bromide 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 Nervous system
Study report, 2001	242/454 mg/kg bw/day ammonium bromide 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 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 Nervous system
Study report, 2016b	500 mg/kg bw/day sodium bromide 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 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 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 Nervous system
Thyroidea				
Loeber et al., 1983	108 mg/ kg bw/day sodium bromide 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 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 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 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 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 $\delta 1$- and $\delta 2$-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 $\alpha 1$-activity) in a 3-month study by Sangster et al., 1982.

- Significant changes in $\alpha 1$-band and β activity at 4 and 9 mg/kg bw/day but no changes in $\delta 1$-, $\delta 2$-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.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed to classify ammonium bromide as STOT RE 2, H373 (nervous system, thyroid). This proposal was based on an overall weight of evidence evaluation and expert judgement taking into account histopathological findings and changes in hormone levels

in thyroid in non-guideline studies at dose levels within the guidance value range for STOT RE 1 or 2, and effects on nervous system seen in a guideline and non-guideline studies within the guidance value range for category 2.

Nervous system as a target organ was justified by the following evidence:

- Slight limpness in 3 males at 100 mg/kg bw/day in a 90-day feeding study of ammonium bromide in rats.

- Maternal neurotoxic effects including staggering, rolling gait, and subdued behaviour at 600 mg/kg bw/day in a pre-natal developmental toxicity study (exposure on gestation days (GD) 6-19) of ammonium bromide in rat.

- Decrease in evasion time at 240 mg/kg bw/day sodium bromide (corresponding to 228 mg ammonium bromide/kg bw/day) (26-day exposure).

- Clinical signs of neurotoxicity in dogs at 200 mg/kg bw/day potassium bromide (corresponding to 243 mg ammonium bromide/kg bw/day) (42-day exposure).

- Neurophysiological effects in humans was considered as supporting evidence.

Thyroid as a target organ was considered justified by the following evidence:

- Statistically significant reduction in triiodothyronine (T3) (males only) and in thyroxine (T4) (males and females) from 175 mg sodium bromide/kg bw/day (corresponding to 167 mg ammonium bromide/kg bw/day) (week 4) and depletion (mild/moderate) of colloid in the thyroid at histopathology in the 90-day repeated dose toxicity study in rat.

- Marked growth activation of the follicular epithelial component, frequent mitoses in the follicular cells, microfollicular tissue rearrangement, decrease of colloid in the thyroid tissue, slight to moderate thyroglobulin-positivity of colloid tissue, decreased plasma T4 and T3 levels in rats at 0.5 mg bromide/kg bw/day corresponding to 0.673 mg ammonium bromide/kg bw/day (16- or 66-day exposure).

- Changes in the thyroid gland and slightly increased thyroid-stimulating hormone (TSH) level and decreased T4 level in rats that were administered potassium bromide at 100 mg/L in drinking water up to 133 days, corresponding to 4.90-6.13 mg ammonium bromide/kg bw/day.

- Statistically significant increase in thyroid weight at ≥108 mg sodium bromide/kg bw/day (corresponding to 103 mg ammonium bromide/kg bw/day) and decreased thyroxin levels in serum noted at ≥ 108 mg/kg bw/day after 4-week administration of sodium bromide in rat.

- Thyroid effects in humans were considered as supporting evidence.

Comments received during consultation

One industry organization agreed with classification STOT RE 2, H373 (nervous system), but disagreed including thyroid as a target organ.

One MSCA was of the opinion that classification as STOT RE 1, H372 (nervous system)

was warranted. They claimed that during a prolonged exposure, bromide accumulation may occur, giving rise to bromide intoxication or bromism. Symptoms could include nausea and vomiting, anorexia, confusion, behavioural disturbances, slurred speech, memory impairment, drowsiness, irritability, ataxia, tremors, hallucinations, mania, delirium, psychoses, stupor, coma and other manifestations of CNS depression. However, the MSCA disagreed including thyroid as a target organ because in the 90-day rat study reduction of serum thyroxine levels and increase in FSH levels and thyroid weight were not accompanied by histopathological changes.

Justification for read-across from other bromide salts

In addition, data on other bromide salts was used via read-across to assess the potential systemic toxicity of ammonium bromide. 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 on the ammonium ion (ammonium sulphate), no systemic toxicity was reported, whereas a comparable toxicity profile has been demonstrated in teratogenicity studies with ammonium bromide and sodium bromide. Therefore, RAC concludes that read-across from other inorganic bromide salts to ammonium bromide to assess systemic toxicity, including STOT RE, is justified.

Considerations on bromism

Bromism is a syndrome which results from the long-term consumption of bromine, usually through bromide-based sedatives such as potassium bromide, lithium bromide, etc. Bromide intoxicated patients can show one or more of the following signs and symptoms: drowsiness, lethargy, dysarthria, weakness, ataxia, various skin disorders, memory loss, disorientation, psychosis with delirium and hallucinations. During the general consultation, it was commented that serum bromide concentration above 6.3 mmol/L in humans is confirmatory for bromism (Carney, 1973), and levels of 21.5 mmol/L (Frances, 2003) and 39.8 mmol/L (Horowitz, 1997) in human case studies demonstrated neuropsychological manifestations of confusion, disorientation, auditory and visual hallucinations and ataxia.

The DS provided a number of publications dealing with bromine poisoning cases in mothers and new-born infants to document transplacental passage of bromide to the foetus with resultant central nervous system depression in infants as supportive information for reproductive toxicity.

In a publication by Finken and Robertsson (1963), the transplacental passage of bromide to the foetus resulting in bromism in a 7-day old female infant born to a mother severely intoxicated by bromide following her 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). The mother experienced about ten days of toxic delirium as a consequence of her ingestion. However, the internal dose in the infant was much higher (serum bromide concentration 365% corresponding to about 1460 mg/kg bw), the infant remained "simply sedated". The infant responded minimally to painful

stimuli and her physical reflexes were poor. Pupils reacted very slowly to light. Otherwise the physical examination was normal, and there was no rash. Follow-up examination after medical treatment one month later revealed a healthy infant without obvious signs of her "neonatal bromism", and an apparently well-adjusted mother.

Another case report also documented transplacental passage of bromide to the foetus resulting in central nervous system depression in a female infant born to a mother severely intoxicated by bromide following her ingestion of Nervine (a sedative consisting of sodium bromide, potassium bromide and ammonium bromide) (Blackburn and Pleasure, 1975). The mother was semiconscious and hyperreflexic on admission in the 34th week of pregnancy. 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 about 22 mg/kg bw, while the infant's blood level on the fifth day of life was about 120 mg/kg bw. After medical treatment and 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.

A report by Mangurten and Ban (1974) described a case of a pregnant mother who was prescribed bromide-containing drugs 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 of gestation and was 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 as 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 serum 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. After 5 months the patient showed no residual manifestations of bromism.

In controlled investigations with human volunteers (both males and females) exposed to sodium bromide by daily intake up to 9 mg/kg bw/day for up to three months or three full menstrual cycles no signs of bromism were detected (Van Gelderen *et al*., 1993; Sangster, *et al*., 1982; Sangster *et al*., 1983).
Furthermore, no signs of bromism were detected in hound dogs receiving potassium bromide via drinking water **-** loading dose of 100 mg/kg bw/day for two days and a maintaining dose of 30 mg/kg bw/day for 180 days. 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 exposure period of the study (Paull *et al*., 2003).

Assessment and comparison with the classification criteria

For the assessment of STOT RE, the DS provided three animal oral toxicity studies performed with ammonium bromide and considered as Klimisch 1. In addition, the DS assessed five non-guideline oral toxicity studies performed with sodium bromide (28-day, 90-day and 90-day (low chloride diet) oral rat studies, a three-generation reproduction rat study and an oral dog study), one non-guideline 115-day oral dog study performed with potassium bromide and one study with sodium bromide performed in accordance with OECD TG 408 and characterised with "reliability 1". The DS provided also eight non-

guideline studies on endocrinological (thyroid) investigations (three studies with sodium bromide and five studies with potassium bromide) characterised with "reliability 2" and six non-guideline neurotoxicological studies on sodium bromide (only one had a reliability score of 2). Furthermore, the DS included three human studies with sodium bromide investigating endocrine system modulation. No repeated dose toxicity studies performed with ammonium bromide or related substances in animals by the dermal and inhalation routes were available.

CLH report included also non-guideline neurotoxicological studies on sodium bromide. Two of them investigated behavioural effects - disturbance of the normal nocturnal rhythm of motility and evasion time of mice (Hansen and Hübner, 1983) or development (learning ability) of offspring of dams exposed to sodium bromide during gestation (Harned *et al*., 1944). One study investigated effects of sodium bromide on the superior cervical ganglion of adult rats by long-lasting micro-infusion into the ganglion (Joo *et al*., 1979). The three other studies were *in vitro* studies on mouse neuroblastoma cells (Spoerri and Wolff, 1982; Eins *et al.*, 1983) or on bullfrog sympathetic ganglion cells (Montoya and Riker, 1982). RAC considers that the reliability and relevance of these studies is impossible to interpret in relation to STOT RE classification criteria and does not give weight to these studies in the WoE assessment.

Guideline studies on ammonium bromide

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

A 90-day feeding study according to OECD TG 408 which included also a neurotoxicity screening battery was performed with ammonium bromide in SD rats (25/sex/group for control and high doses, 15/sex/group for low and intermediate dose) following administration of 100 and 225 mg/kg bw/day in both sexes and 500 mg/kg bw/day for males and 750 mg/kg bw/day for females (nominal dose in diet). All test-substance exposed animals were treated continuously for at least 13 consecutive weeks. Recovery animals were fed with untreated diet for a period of at least 4 weeks after the exposure period.

Three premature terminations occurred among males at 500 mg/kg bw/day. The principal clinical signs observed were rolling gait, intermittent staggering, subdued behaviour, nasal bleeding, unkempt coat, hunched posture, discharge from the eyes and splayed hind limbs. They are summarised in the table below.

Table: Clinical signs in a 90-day oral study on ammonium bromide in rats

Clinical observations	0 mg/kg bw/day	100 mg/kg bw/day	225 mg/kg bw/day	500 mg/kg bw/day	750 mg/kg bw/day
Subdued behavior	0/25 (M) 0/25 (F)	0/15 (M) 0/15 (F)	11/15 (M) 0/15 (F)	14/15 (M)	9/15 (F)
Rolling gait	0/25 (M) 0/25 (F)	0/15 (M) 0/15 (F)	4/15 (M) 0/15 (F)	15/15 (M)	13/15 (F)
Staggering	0/25 (M)	0/25 (M)	2/15 (M)	12/15 (M)	11/15 (F)

	0/25 (F)	0/25 (F)	0/15 (F)		
Nasal bleeding	0/25 (M)	0/25 (M)	0/25 (M)	5/15 (M)	8/15 (F)
	0/25 (F)	0/25 (F)	0/25 (F)		

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). These included increased limpness and 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 animal showed the finding on more than one occasion. There were also minor changes in biochemical parameters (decreased cholesterol levels in females at 100 mg/kg bw/day).

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. Reduced body weight gain was 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.

Minor changes in haematology parameters (increases in haemoglobin, haematocrit and total white cell count) in males at 500 mg/kg bw/day and in females at 750 mg/kg bw/day had been recorded. There were also minor changes in biochemical parameters (decreased cholesterol levels in males at ≥ 225 mg/kg bw/day and in females at ≥ 100 mg/kg bw/day) and decreased urine pH (in females at 750 mg/kg bw/day).

4-week repeated dose toxicity dose range-finding study
(Study report, 1999):

The study was conducted based on a previous 4-week repeated dose toxicity dose range-finding study according to GLP with SD rats (Study report, 1999). The animals (5/sex/group) were fed with diet containing 0, 100, 500 and 1000 mg/kg bw/day of ammonium bromide (nominal concentration). Clinical signs of neurotoxicity and subdued behaviour at 500 (all males) and 1000 mg/kg bw/day (all males and 4/5 females) were reported.

Dose range finding study for a reproduction toxicity study on ammonium bromide in rats
(Study report, 2001):

The study was conducted according to GLP and similar to OECD TG 416. SD rats were fed with diet containing ammonium bromide at concentrations of 0, 127, 242 and 503 mg/kg bw/day for males and 0, 228, 454 and 651 mg/kg bw/day for females. The animals were exposed two weeks prior mating until the first generation had been weaned for a total of 56 days. At 242/454 mg/kg bw/day 9/10 males and 6/10 females had rolling gait starting around the fifth week of treatment and lasting throughout the end of the/remaining treatment period. At 503/651 mg/kg bw/day, all animals displayed clinical signs during 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.

Guideline studies on sodium bromide

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

An oral 90-day toxicity study in Crl:CD(SD) rats was performed according to OECD TG 408 and including investigations on oestrous cycle, sperm evaluation and histopathology according to OECD TG 416. Sodium bromide was administered daily via oral gavage at doses of 0, 60, 175, 500 mg/kg bw/day (once daily). There were 10 animals per sex and group and additional 10 animals per sex and group for control and high dose groups for the recovery assessment.

A sodium chloride group (284 mg/kg bw/day) was also included in the assessment for comparison. Administration of 284 mg/kg bw/day of sodium chloride at an equivalent osmolarity as 500 mg/kg bw/day of sodium bromide produced no toxicity, indicating that the toxicity observed at 500 mg/kg bw/day by sodium bromide was due to exposure to bromide.

Reduced motor activity was observed after dosing in 6/10 males at 175 mg/kg bw/day on week 2, but all animals recovered before the end of the working day. With respect to females, reduced motor activity was observed after dosing in 8/10 animals at 175 mg/kg bw/day on study days 11-13, but also all females recovered before the end of the working day. Increased incidences 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) was observed in both sexes at 500 mg/kg bw/day. After approximately 6 weeks of dosing, one or more of these observations persisted throughout the working day, and were still evident before the dosing on the next day in 2 or more males for the remainder of the dosing period. For females the clinical signs appeared later in the treatment period, did not persist until the following day and the recovery was faster, suggesting a higher tolerance for females than for males. At week 9, all males and 6/10 females exhibited ataxia and abnormal gait at 500 mg/kg bw/day.

There were no significant treatment-related effects at 60 or 175 mg/kg bw/day on body weight or body weight gain in males and females, although values at 175 mg/kg bw/day were slightly lower than those in controls. At 500 mg/kg bw/day there was a significant reduction in body weight gain in males after week 2, and at the end of the dosing period body weight and body weight gain were significantly lower than those in control (81.2% and 68.8% of control, respectively, $p \leq 0.01$). Body weight remained significantly lower than that in controls for the first two weeks after the dosing period but increased to 90.4% of control values at the end of the recovery period, owing to weight gain markedly higher than that in control (134.7%). Female body weight and weight gain were unaffected by treatment during the treatment period, but the body weight gain was substantially lower at 500 mg/kg bw/day than that in controls at the end of the recovery period (66.5% of control values).

Thyroid hormone analysis in serum was conducted on a single occasion at week 4.

Interpretation of thyroid hormone data was constrained by the limited amount of historical control data. As compared to controls, there was an apparent reduction (p ≤ 0.01) in T3 (males only, 37%) and in T4 (males, 52%; females, 47%) at 500 mg/kg bw/day. At 175 mg/kg bw/day the differences from control in T3 (males, 28%) and T4 (males, 28%; females, 34 %) were less marked and the values were comparable to historical control values.

In summary, administration of 500 mg/kg bw/day of sodium bromide for 90 days produced severe toxicity, characterized by adverse clinical observations and reductions in body weight gain, food intake and water consumption, with effects generally more severe in males than females. Administration of 175 mg/kg bw/day of sodium bromide produced similar but less severe toxicity including adverse clinical observations and reductions in food and water consumption, occurring more frequently in males than females. Effects observed at 60 mg/kg bw/day were comparable to the effects by sodium chloride or in historical controls and/or the findings were common findings in this rat strain and are regarded as not adverse.

Non-guideline studies on sodium bromide and potassium bromide

Three-generation reproductive toxicity study on rats with sodium bromide
(Van Leeuwen et al., 1983):

Feeding of sodium bromide to male and female rats (7-19 animals per sex and group, strain not specified) for 90 days in concentrations of 0, 75, 300, 1200, 4800 and 19200 mg/kg diet (corresponding to 0, 6.75, 27, 108, 432 and 1728 mg/kg bw/day) led to a decrease in T4 serum concentration in males by 10-16% at 6.75 and 27 mg/kg bw/day, 25% at 108 mg/kg bw/day, 40% at 432 mg/kg bw/day and 60% at 1728 mg/kg bw/day. In females reduced T4 levels were observed at the two highest doses only. The study was characterised as non-GLP study, however, considered as Klimisch 2.

28-day oral repeated dose toxicity study on rats with sodium bromide
(Van Logten et al., 1973):

Feeding of sodium bromide to female Wistar rats (4 animals per group) for 28 days in concentrations of 0, 300, 1200, 4800 or 19200 ppm in diet (corresponding to 0, 36, 144, 576, 2304 mg/kg bw/day) showed neurotoxic effects (incoordination of motor activity of hind legs) at the highest dose of 2304 mg/kg bw/day. The study was considered as Klimisch 2.

90-day oral repeated dose toxicity study on rats with sodium bromide
(Van Logten et al., 1974):

Feeding of sodium bromide to male and female Wistar rats (10 animals per sex and group) for 90 days in concentrations of 0, 75, 300, 1200, 4800 and 19200 ppm in diet (corresponding to 0, 6.75, 27, 108, 432, 1728 mg/kg bw/day) indicated neurotoxic effects (incoordination of motor activity of hind legs) at the highest dose of 1728 mg/kg bw/day. No striking effects on haematological and biochemical parameters were seen except for a doubling of the percentage of neutrophil granulocytes at the top dose. In females at 108, 432 and 1728 mg/kg bw/day and in males at 1728 mg/kg bw/day an increase in relative thyroid weight was found. In male rats an increase in the relative

weight of the adrenals was found at 1728 mg/kg bw/day. The study was considered as Klimisch 2.

90-day oral repeated dose toxicity study on a low chloride diet of rats with sodium bromide (Van Logten et al., 1976):

Feeding of sodium bromide to male and female Wistar rats receiving low chloride diet (10 animals per sex and group) for 90 days in concentrations of 0, 8, 31, 125, 500 and 2000 ppm (corresponding to 0, 0.72, 2.8, 11, 45, 180 mg/kg bw/day) showed motor incoordination of hind legs at 180 mg/kg bw/day. Three males and 3 females at 180 mg/kg bw/day died during the experiment. There was an increase in the percentage and total number of neutrophil granulocytes at 180 mg/kg bw/day. In addition, the total leucocyte count was increased and the relative weights of the adrenals and thyroid were increased. It was concluded that the toxicity of sodium bromide in rats on a low chloride diet is about 10 times higher in comparison with the toxicity for rats on a normal diet due to the competition for uptake between bromide and chloride ions. The study was considered as Klimisch 2.

Sub-chronic oral toxicity no guideline study on dogs with sodium bromide (Rosenblum, 1958):

Mongrel male and female dogs (4 per sex and group) were fed with increasing doses of sodium bromide (100 – 400 mg/kg bw/day) at intervals of six weeks until death resulted between 44 and 185 days. No deaths were observed at the lowest dose but the incidences increased with the dose and treatment time. Three out of four dogs died within 44 days at the top dose. Neurotoxicity was seen in all dogs at ≥ 200 mg/kg bw/day. The severity of neurotoxicity was associated with the blood bromide level and progressed from slight ataxia to stupor, severe ataxia (unable to stand) and finally to coma. The study was considered as Klimisch 3.

Sub-chronic oral toxicity no guideline study on dogs with potassium bromide (March et al., 2002):

Beagle male and female dogs (3 per sex) were fed with potassium bromide at 30 mg/kg bw/day for 115 days. No control group was used, however, the study was considered as Klimisch 2. No clinical neurotoxic signs were observed. Taking into account the conditions of the study, it is not relevant for STOT RE classification assessment of ammonium bromide.

Non-guideline studies on endocrine system with sodium bromide and potassium bromide

Three human studies on endocrine system modulation in vivo caused by sodium bromide (Van Gelderen et al., 1993; Sangster et al., 1982; Sangster et al., 1983):

The DS provided three human (both with males and females) studies about the influence of sodium bromide on levels of T3 and T4 hormones in blood plasma. All three studies were considered as Klimisch 2. Human volunteers (45 females in Van Gelderen *et al.*, 1993; 11 females and 10 males in Sangster *et al.*, 1982 and 7 females and 7 males in

Sangster et al., 1983) were tested by daily intake of sodium bromide up to 9 mg/kg bw/day for up to three months or three full menstrual cycles. Only in one study (Sangster et al., 1983) a slight but significant increase in T4 and T3 in females only was detected (at 9 mg/kg bw/day). However, the number of tested volunteers was low. Individual concentrations of T4 and T3 in this group was within normal limits at the start and the end of the investigation.

Four or 12-week feeding study of sodium bromide in rats to investigate alterations in the endocrine system
(Loeber et al., 1983):

Male Wistar rats (10 per group) 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, 1.8, 6.75, 27, 108 and 1728 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. Histopathological examination revealed an activation of the thyroid in the highest dose group. Histopathological changes in thyroid at 4 and 12 weeks included increase of follicles and decrease in their size. Using immunocytochemical techniques a decrease in the amount of T4 was noted in the thyroid. No effect was found in growth hormone-producing cells in the pituitary gland, while immunoreactivity for thyroid-stimulating hormone and for adrenocorticotropic hormone was increased. The concentration of T4 in the serum appeared to be decreased as well. Due to the feedback regulation, the pituitary gland was stimulated to produce and release thyroid-stimulating hormone, follicle-stimulating hormone, adrenocorticotropic hormone and insulin, whereas the release of growth hormone was suppressed. Most of these changes were restricted to rats at the top dose. At 108 mg/kg bw/day, thyroid weight was increased by 38% at 4 weeks, but the T4 level was decreased by 23%. At 1728 mg/kg bw/day, thyroid weight was increased by 71% at 4 weeks and by 82% at 12 weeks. T4 level in plasma was decreased at 4 and 12 weeks. No treatment-related effects were detected at lower doses. It was concluded that sodium bromide directly disturbs the function of the thyroid at high doses.

4-week dietary study of sodium bromide in rats to investigate effects on the thyroid on low iodine diet
(Buchberger et al., 1990):

The influence of bromide on thyroid function was studied in iodine-deficient SD male and female rats (12 per sex and group), fed a diet containing 4, 8 and 16 g/kg sodium bromide (corresponding to 200, 400 and 800 mg/kg bw/day) for 4 weeks. Measurement of total and free T4 and thyroid-stimulating hormone in blood, as well as the thyroid hormones in the thyroid gland, revealed typical signs of hypothyroidism (increased thyroid weight, decreased levels of T3 and T4) which were significantly enhanced by bromide intake. Special attention was paid to the possible formation of bromo/iodosubstituted thyronines in the thyroid. These measurements were performed by high-performance liquid chromatography with off-line radioimmunoassay detection. Such thyroid hormone analogues could be detected in all groups of animals with additional bromide intake, but the amounts were found to be too low to compensate adequately for the reduced amounts of thyroid hormones. The results of this study also indicate that bromide toxicity is dependent upon the state of the iodine supply.

2-week dietary study in rat to investigate the effect of sodium bromide on thyroid function
(Van Leeuwen *et al*., 1988):

Male Wistar rats (8 per group) were fed a diet containing 19 g NaBr/kg (corresponding to about 950 mg/kg bw/day) or 11 g NaCl/kg for two weeks. Animals were sacrificed after the treatment period and blood was collected for determination of T4 and TSH levels. Thyroid glands were weighed and homogenized. After two-week feeding of a NaBr-containing diet, body weight of animals was lower than in the controls, and the absolute and relative weight of the thyroid gland was significantly higher. In the serum of these rats the concentration of T4 was lower and that of TSH higher than in the serum of control animals. These observations are in accordance with the earlier findings pointing to a disturbance of thyroid hormone biosynthesis, consequently followed by a compensatory increase in TSH. In addition to this, in NaBr-treated animals the uptake of 125-I by the thyroid gland was significantly lower than in control animals.

16- or 66-day study on potassium bromide to investigate effects on the thyroid gland of the rats.
(Velický *et al*., 1997a):

In order to establish the morphological and functional effects of bromine on the thyroid, male Wistar rats (6 animals per group) were fed small quantities of bromide expected to be encountered in the environment (0, 10, 50 and 100 mg of Br-/L in drinking water) for 16 and 66 days. These values correspond 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. Animals were sacrificed at the end of the treatment period. The treatment induced a growth of the follicular epithelial component, a microfollicular tissue rearrangement, a reduction of intrafollicular colloid, an increase in the height of the follicular cells and in the number of mitoses, and an enhanced vascularization. Histopathological changes in thyroid were observed at all dose levels, with a dose-related increase in extent. The concentration of bromine in the thyroid increased with the amount of bromine intake, and at the same time the molar ratio of iodine/bromine decreased. The plasma level of T4 decreased after both 16 and 66 days of treatment, but the T3 level decreased only after 66 days of treatment. The level of TSH did not change significantly.

16-, 66- or 133-day study on potassium bromide to investigate effects on the thyroid gland of the rats
(Velický *et al*., 1997b):

Analysis of expression of the proliferating cell nuclear antigen was used to determine the presumed hyperplastic character of morphological changes in the rat thyroid evoked by bromide administration. Wistar male rats (10 animals per group) fed by a standard diet with determined iodine and bromine content were given potassium bromide. Control animals received no bromide. Experimental animals were given 10, 50 or 100 mg Br$^-$ per 1L drinking water for 16 and 66 days, or 100, 200 or 400 mg Br$^-$/l drinking water for 133 days. These levels correspond to 0.5, 2.5 and 5 mg Br$^-$/kg bw/day and 5, 10 and 20 mg Br$^-$/kg bw/day, respectively (or 0.745, 3.73 and 7.45 mg KBr/kg bw/day and 7.45, 14.90 and 29.81 mg KBr/kg bw/day). Treatment-related increase in mitotic activity of follicular cells in thyroids was observed. In addition, histopathological changes in thyroid (microfollicular rearrangement of the follicular epithelium and reduction of the

amount of colloid) were detected. Increase in bromine content led to a decrease in the I/Br molar ratio in the thyroid tissue. LOAEL for morphological changes is estimated to be 0.5 mg bromide/kg bw/day.

133-day study on potassium bromide to investigate effects on the thyroid gland of the rats
(Velický *et al*., 1998):

Male Wistar rats (10 animals per group) were exposed to a 133-day oral administration of KBr (0, 100, 200, 400 mg Br⁻/L drinking water). These levels correspond to 0, 3.3-5, 6.7-10 and 13-20 mg Br⁻/kg bw/day and 0, 4.92-7.45, 9.99-14.90 and 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). There was a treatment-related increase in bromine concentration in the thyroid, a decrease in iodine content and a decrease in I/Br molar ratio. In the blood plasma of the bromide-exposed animals the T4 concentration was significantly and dose-dependently decreased. The thyroid glands of treated animals showed increased growth of the epithelial cells reflected by a microfollicular rearrangement of the parenchyma due to proliferation of very small follicles with a low or zero content of colloid. In addition, the nuclei of thyrocytes showed an increased number of mitoses. The vascularization was increased as well. LOAEL for morphological changes is estimated to be 3.3 mg bromide/kg bw/day.

16-, 66- or 133-day study on potassium bromide to investigate effects on the thyroid gland of the rats
(Velický *et al*., 2004):

Male Wistar rats (10 animals per group) fed by a standard diet with determined of bromine and iodine content were exposed to a 16-, 66- and 133-day oral administration of KBr (0, 10, 100, 200 and 400 mg Br⁻/L drinking water). These levels correspond to 0, 0.5, 5, 10 and 20 mg Br⁻/kg bw/day and 0, 0.745, 7.45, 14.90 and 29.81 mg KBr/kg bw/day based on an assumed average body weight of 300 g/rat over the study period and taking into account a water consumption of 15 mL/animals/day via the drinking water. Electron microscopic examination of thyroid tissue following administration of bromide to rats showed marked hypertrophy and hyperplasia in the thyrocytes as well as morphological changes including intracellular cavities, dilated endoplasmic reticulum cisterns, enlarged Golgi complex, poor colloid droplets, irregular-shaped nuclei, increased density of nuclear chromatin, etc. LOAEL for morphological changes is estimated to be 0.6 mg bromide/kg bw/day.

Repeated dose toxicity study on potassium bromide to investigate effects on the thyroid gland of the dogs
(Paull *et al*., 2003):

An experiment was performed to evaluate the effect of potassium bromide on the canine thyroid gland. Basal total T4, free T4, TSH and basal thyrotropin serum concentrations were evaluated over a 6-month period in potassium bromide-treated and control hound dogs (2 males and 3 females per group). Potassium bromide was administered to dogs via drinking water - loading dose of 100 mg/kg bw/day for two days and a maintaining dose of 30 mg/kg bw/day for 180 days. 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. Animals were killed on termination

of the study on day 182. Thyroid histopathology was compared between treated and control dogs at the end of the study. No difference was detected in any parameter between the two groups at the end of the study. A decline in thyroid hormone concentrations over the course of the study did occur in both groups of dogs. Potassium bromide did not have a significant effect on canine thyroid function or morphology at the tested exposure levels.

Comparison with the classification criteria

<u>Thyroid</u>

In addition, RAC considers that the severity of the effects on thyroid is not sufficient to include thyroid as a target organ. The DS did not provide any studies investigating effects on thyroid by ammonium bromide. The single study on sodium bromide according to OECD TG 408 showed pronounced effects at the highest dose tested (500 mg/kg bw/day) (Study report, 2016b). There were less significant alterations in thyroid hormone levels also at ≥ 60 mg/kg bw/day (at 60 mg/kg bw/day 27% lower T3 in males, 26% lower T4 in females, and 60% higher TSH in females as compared to controls). However, it is noted that interpretation of thyroid hormone data is constrained by the limited amount of historical control data. Three human studies on influence of sodium bromide on levels of T3 and T4 hormones in blood plasma indicated no increase in general, however, it should be noted that the dose levels tested were low (at therapeutic dose levels).

Three non-guideline studies on rats with sodium bromide showed a decrease in thyroid hormone levels within guidance value range for STOT RE 2 (10 – 100 mg/kg bw/day for 90-day exposure, 30 – 300 mg/kg bw/day for 30-day exposure). Loeber *et al*. (1983) showed an increase in thyroid weight by 38% and a decrease in T4 level by 23% at 108 mg/kg bw/day (corresponding to 103 mg/kg bw/day ammonium bromide) after a 4-week exposure (and increase of TSH by 21% after a 12-week exposure). Increased thyroid weight and decreased levels of T3 and T4 were found in Buchberger *et al*. (1990) 4-week dietary study starting at 200 mg/kg bw/day (corresponding to 190 mg/kg bw/day ammonium bromide). Van Leeuwen *et al*. (1983) showed a slight decrease (10-16%) in T4 serum concentration in males at 6.75 and 27 mg/kg bw/day (corresponding to 6.40 and 25.65 mg/kg bw/day ammonium bromide) after 90 days of exposure. The decrease was higher (20-60%) at 108 – 1728 mg/kg bw/day.

In Van Logten *et al*. (1974) the relative thyroid weight was increased in females by 32% at 108 mg/kg bw/day after a 90-day exposure of sodium bromide.

A series of non-guideline studies (four in total) performed by Velický *et al*. (summarised above) on rats with potassium bromide for different exposure periods (16 - 133 days) showed morphological changes and proliferation potential of thyroid even at very low doses (< 10 mg/kg bw/day; (corresponding to < 8.2 mg/kg bw/day ammonium bromide) being within the guidance value range for STOT RE 1. This potency is in contradiction with the study on sodium bromide on rats performed by Loeber *et al*. (1983) showing histopathological changes in thyroid at > 100 mg/kg bw/day only (corresponding to > 95 mg/kg bw/day ammonium bromide) at 4 and 12 weeks.

The study on dogs (Paull *et al*., 2003) showed that potassium bromide did not appear to have a significant effect on canine thyroid function or morphology at the tested exposure levels thereby not meeting the classification criteria for STOT RE. The applicant under the

Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) discussed the potential reasons why there were no treatment-related effects on thyroid function or morphology in dogs (section 3.12.3.14 in Annex I of the CLP report). During the general consultation, industry also discussed the potential species differences in the physiology and regulation of the thyroid and thyroid hormones. Nevertheless, RAC concludes that the effects on thyroid in rats and the related mode of action can be relevant to humans as well.

RAC concludes not to add thyroid as the target organ because the severity of effects on thyroid is considered not to fulfil the CLP criteria for STOT RE.

Nervous system

The available human data on bromism stemming from poisoning case reports (Finken and Robertsson, 1963; Blackburn and Pleasure, 1975; Mangurten and Ban, 1974) are considered to justify classification for STOT RE 1 with the nervous system as the target organ. Positive human data, regardless of the dose, predominates over animal data (CLP 3.9.1.1, 3.9.2.7.3; 3.9.2.10.2).

In the 90-day ammonium bromide feeding study on rats carried out according to OECD TG 408 the findings within the guidance value range for classification (at 100 mg/kg bw/day) were limited to slight limpness in 3 males and to minor changes in biochemical parameters in females (decreased cholesterol levels). The neurotoxic effects consisted of increased limpness, decreased alertness, increases in landing foot splay and decreases in fore and hind limb grip strength at higher doses.

Neurotoxic findings were also observed in 4-week repeated dose toxicity dose range-finding study (Study report, 1999), but these effects occurred above the guidance value range for classification.

In a dose range finding study for a reproduction toxicity study on ammonium bromide in rats a rolling gait was observed at 242 mg/kg bw/day in males and at 454 mg/kg bw/day in females starting at the fifth week of treatment (between 28 and 35 days) (Study report, 2001). The guidance value range for STOT RE 2 is 257-321 mg/kg bw/day for a 28-35 day exposure. Thereby only the effects in males occurred within the guidance value range for STOT RE.

The 90-day oral repeated dose toxicity study on sodium bromide in rats (Study report, 2016b), no effects were seen within the guidance value range for classification.

In supporting studies by Van Logten *et al*. (1973, 1974 and 1976), no effects were observed within the guidance value range for classification.

In addition, in the old non-guideline study on dogs fed with increasing doses of sodium bromide until the death of animals (between day 44 and 185), Rosenblum (1958) showed that neurotoxicity was seen in all dogs that received 200 mg/kg bw/day or more of sodium bromide. No signs of neurotoxicity and no death cases were observed at the dose of 100 mg/kg bw/day.

RAC concludes that the **classification of ammonium bromide as STOT RE 1, H372 (Causes damage to nervous system through prolonged or repeated exposure) is warranted** based on the human evidence. The animal studies provided supporting

evidence.

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.

14   REFERENCES

Buchberger W., Holler W. and Winsauer K. (1990). Effects of Sodium Bromide on the Biosynthesis of Thyroid Hormones and Brominated/Iodinated Thyronines. J. Trace Elem. Electrolytes Health Dis 4 (1), 25-30.

Disse M., Joó F., Schulz H. and Wolff J. R. Disse M., Joó F., Schulz H. and Wolff J. R. (1995). Prenatal Exposure to Sodium Bromide Affects the Postnatal Growth and Brain Development. J. Brain Res. 37, 127-134.

Dowling, P. M (1994). Management of canine epilepsy with phenobarbital and potassium bromide. Can. Vet. J. 35 (11), 724-725.

Eins S., Spoerri P. E. and Heyder E. (1983). GABA or Sodium-Bromide-Induced Plasticity of Neurites of Mouse Neuroblastoma Cells in Culture. Cell Tissue Res 229:457-460.

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

Gamble J. L. Jr., Robertson J. S., Hannigan C. A., Foster C. G. and Farr L. E. (1953). Chloride, Bromide, Sodium and Sucrose Spaces in Man. J. Clin. Invest., 32, 483.

Gaskill C. L., Cribb A. E. (2000). Pancreatitis associated with potassium bromide/phenobarbital combination therapy in epileptic dogs. Can Vet J 41, 555-558.

Gosselin R. E. (1976). Clinical Toxicology of Commercial Products. 4th ed. Baltimore: Williams and Wilkins, p. II-77.

Grant W. M. (1974). Toxicology of the Eye. 2nd ed. Springfield, Illinois: Charles C. Thomas, p. 199, 200.

Hansen K. and Hübner H. (1983). Effects of Bromide on Behaviour of Mice. Fd. Chem. Toxic. Vol. 21, no. 4, pp. 405-408.

Harned B. K., Hamilton H. C. and Cole V. V. (1944). The Effect of the Administration of Sodium Bromide to Pregnant Rats on the Learning Ability of the Offspring, II. Maze Test. Journal of Pharmacology and Experimental Therapeutics, Vol 82, Issue 3, 215-226.

Joo, F., Dames W., Wolff J. R. (1979). Effect of Prolonged Sodium Bromide Administration on the Fine Structure of Dendrites in the Superior Cervical Ganglion of Adult Rat. Progress in Brain Research, Vol. 51, pp.109-115.

Kast (1994) "Wavy ribs" A reversible pathologic finding in rat fetuses; Exp Toxic Pathol 46, 203-210; Doc. No. 592-047

Loeber J. G., Franken M. A. M. and van Leeuwen F. X. R. (1983). Effect of Sodium Bromide on Endocrine Parameters in the Rat as Studied by Immunocytochemistry and Radioimmunoassay. Fd. Chem. Toxic. 21 (4), 391-404.

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.

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

Mason M.F. (1963). Halide distribution in body fluids in chronic bromide intoxication. J. Biol. Chem., 113, 61.

McDanal C. E., Owens D. and Bolman W. M. (1974). Bromide Abuse: A Continuing Probl. Am. J. Psychiatry 131:8, August 1974.

Mitsumori K., Maita K., Kosaka T., Miyaoka T. and Shirasu Y. (1990). Two-year oral chronic toxicity and carcinogenicity study in rats of diets fumigated with methyl bromide. Fd. Chem. Toxic., Vol. 28, No. 2, p. 109-119.

Montoya G. A. and Riker W. K. (1982). A study of the Actions of Bromide on Frog Sympathetic Ganglion. Neuropharmacology, Vol. 21, pp. 581-585.

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.

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.

Paull, L. C. et al (2003). Effect of Anticonvulsant Dosages of Potassium Bromide on Thyroid Function and Morphology in Dogs. Journal of the American Animal Hospital Association, 39, 193-202.

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

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

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

Pavelka S., Babický A. and Vobecký M. (2005). Biological Half-life of Bromide in the Rat Depends Primarily on the Magnitude of Sodium intake. Physiol. Res., 54: 639-644.

Pollay M. (1967). The Processes Affecting the Distribution of Bromide in Blood, Brain, and Cerebrospinal Fluid. Experimental Neurology 17, 74-85.

Rauws A. G. (1983). Pharmacokinetics of Bromide Ion - an Overview. Fd. Chem. Toxic., Vol. 21, No. 4, 379-382.

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

Rosenblum I (1958). Bromide Intoxication: I. Production of Experimental Intoxication in Dogs. J. Pharmacol Exp. Ther. 122 (3), 379-385.

Rossiter E. J. R. and Rendle-Short T. J. (1972). Congenital Effects of Bromism?. The Lancet, 1972, ii, 705.

Safdari F, Rabbani M, Hosseini-Sharifabad A (2017) Effect of acute and long term potassium bromide administration on spatial working memory in rat. Res Pharm Sci.;12(2):154-159.

Sangster B., Blom J. L., Sekhuis V. M., Loeber J. G., Rauws A. G. and Koedam J. C. (1983). The Influence of Sodium Bromide in Man: A Study in Human Volunteers with Special Emphasis on the Endocrine and the Central Nervous System. Fd. Chem. Toxic., Vol. 21, No. 4, 409-419.

Sangster B., Krajnc E. I., Loeber J. G., Rauws A. G. and van Logten M. J. (1982). Study of Sodium Bromide in Human Volunteers, with Special Emphasis on the Endocrine System. Human Toxicol., 1, 393-402.

Skibsted LH (1981). Acid dissociation constant of the ammonium ion in aqueous ammonium perchlortae, sodium bromide solutions of unit ionic strength. Acta Chem. Scand. Vol A35, 3, 229-230.

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

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

Spoerri P.E. and Wolff J.R. (1982) Morphological Changes Induced by Sodium Bromide in Murine Neuroblastoma Cells in vitro. Cell Tissue Res, 222:379-388.

Study report (1986a) Ammonium Bromide: Acute Oral Toxicity in the Rat.

Study report (1986b) Ammonium Bromide Acute Eye Irritation/Corrosion Test in the Rabbit.

Study report (1988a) Oral Toxicity to Rats of Sodium Bromide, Technical Grade.

Study report (1988b) Acute Dermal Toxicity to Rabbits of Sodium Bromide, Technical Grade.

Study report (1988c) Irritant Effects on Rabbit Skin of Sodium Bromide Technical Grade.

Study report (1988d) Irritant Effects on the Rabbit Eye of Sodium Bromide Technical Grade.

Study report (1988e) Delayed Contact Hypersensitivity in the Guinea-Pig with Sodium Bromide Technical Grade.

Study report (1988f)). Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Sodium Bromide.

Study report (1988g) Sodium Bromide Technical Grade – Metaphase Chromosome Analysis of Human Lymphocytes Cultured in vitro.

Study report (1988h) Assessment of Unscheduled DNA Repair Synthesis in Mammalian Cells after Exposure to Sodium Bromide (technical grade).

Study report (1995) Sodium Bromide – Developmental Toxicity (Embryofoetal Toxicity and Teratogenic Potential) Study in Rats (Gavage Administration).

Study report (1996) Ammonium Bromide Acute Dermal Irritation/Corrosion Test in the Rabbit.

Study report (1998a) Ammonium Bromide Acute Dermal Toxicity (LD50) test in Rats.

Study report (1998b) Ammonium Bromide Acute Inhalation Toxicity Study in Rats (Limit Test).

Study report (1998c) Ammonium Bromide Magnusson-Kligman Maximisation Test in Guinea Pigs.

Study report (1998d) Ammonium Bromide – Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 1537, TA98 and TA 100 and Escherichia coli WP2 uvr A.

Study report (1998e) Ammonium Bromide Mouse Lymphoma Mutation assay.

Study report (1998f) Ammonium Bromide Micronucleus Test in Bone Marrow of CD-1 Mice.

Study report (1999) Ammonium Bromide 4-Week Dose Range Finding Study in Rats with Administration by the Diet.

Study report (2000a) Ammonium Bromide 13-week Toxicity Study in Rats with Administration by the Diet with Neurotoxicity Screening Battery.

Study report (2000b) Ammonium Bromide Developmental Toxicity Study in Rats.

Study report (2001) Ammonium Bromide Dose Range Finding Reproduction Study in Rats.

Study report (2007a). Ammonium Bromide – Developmental Toxicity Study in Rats.

Study report (2008a) Sodium Bromide – Preliminary Oral (gavage) Pre-Natal Developmental Toxicity Study in the Rabbit.

Study report (2008b) Sodium Bromide – Oral Gavage Pre-Natal Developmental Toxicity Study in the Rabbit.

Study report (2016a) Two-Generation (One Litter per Generation) Reproduction Study of Sodium Bromide in Rats.

Study report (2016b) Oral (Gavage) Repeat Dose 90-Day Toxicity Study of Sodium Bromide in Rats, Including Recovery Assessments.

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

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

Takagi, H. et al.(1999) 13-week subchronic oral toxicity study of ammonium sulfate in rats. Bull. Natl. Health Sci., 1999, 117, 108-114

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

Trepanier, L.A. (1995). Use of Bromide as an Anticonvulsant for Dogs with Epilepsy. JAVMA, 207 (2), 163-166

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

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

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.

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

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

Van Gelderen C. E. M., Savelkoul T. J. F., Blom J. L., van Dokkum W. and Kroes R. (1993). The No-Effect Level of Sodium Bromide in Healthy Volunteers. Human and Experimental Toxicology, 12, 9-14.

Van Leeuwen, F. X. R. et al (1983). Toxicity of Sodium Bromide in Rats: Effects on Endocrine System and Reproduction. Fil Chem Toxic 21 (4), 283-399.

Van Leeuwen F. X. R., Hanemaaijer R. and Loeber J. G. (1988). The Effect of Sodium Bromide on Thyroid Function, The Target Organ and the Toxic Process. Arch. Toxicol. Suppl. 12, 93-97.

Van Leeuwen, F. X. R. and Sangster, B. (1987): The Toxicology of Bromide Ion; Critical Reviews in Toxicology, Volume18, Issue 3; Doc. No. 592-004

Van Logten, M. J. et al (1973). Short-term Toxicity Study on Sodium Bromide in Rats. Toxicology 1, 321-327.

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

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

Velický J., Titlbach M., Dusková J., Vobecký M., Strbák V. and Raska I. (1997a). Potassium Bromide and the Thyroid Gland of the Rat: Morphology and Immunohistochemistry, RIA and INAA Analysis. Annals of anatomy = Anatomischer Anzeiger: official organ of the Anatomische Gesellschaft, Vol. 179, No. 5, pp. 421-31.

Velický J., Titlbach M., Lojda Z., Jelinek F., Vobecký M. and Raska I. (1997b). Expression of the Proliferating Cell Nuclear Antigen (PCNA) in the Rat Thyroid Gland After Exposure to Bromide. Acta histochemica, Vol. 99, No. 4, pp. 391-9.

Velický J, Titlbach M, Lojda Z, Dusková J, Vobecký M, Strbák V, Raska I. (1998). Longterm action of potassium bromide on the rat thyroid gland. Acta histochem. 100, 11-23.

Velický J, Titlbach M, Lojda Z, Dusková J, Vobecký M, Raska I. (2004). The effect of bromide on the ultrastructure of rat thyrocytes. Ann Anat 186, 209-216.

Vobecký M., Babický A. and Lener J. (1996). Biological Trace Element Research, Vol. 55, No. 3, pp. 215-219.

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

Wolf R.L. and Eadie G.S. (1951). Reabsorption of Bromide by the Kidney. Am. J. Physiol., Vol. 163, pp. 436-441.

15 ANNEXES

Annex I to the CLH report

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